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Molluscicidal activity of the aqueous extract of garlic (*Allium sativum* L.) bulb against golden apple snail *(Pomacea canaliculata* L.)

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Abstract

The search for efficient, organic, and environment-friendly molluscicide that could minimize the spread of the invasive Golden Apple Snail (*Pomacea canaliculata*) in the Philippines is still on going. To investigate the molluscicidal activity of garlic (*Allium sativum*) towards the rice pest (*Pomacea canaliculata*), this study employed Complete Randomized Design in 3 trials (n=630 snails) to test the efficacy of Aqueous Garlic Extract [AGE] under five experimental treatments (T1=10ppm AGE; T2=8.75ppm AGE; T3=7.5ppm AGE; T4=6.25ppm AGE and T5=5.0ppm AGE) and two control groups (Positive Control=Niclosamide [Snail Shatter™] and Negative Control= distilled H_2O. After 48h experimental period, mortality data were analyzed using One Way ANOVA ($p \leq 0.05$) and post hoc analysis (Tukey's test) while Probit analysis was employed to determine toxicity level of AGE at LD_{50} and LD_{90}. Results showed that there is a direct relationship between AGE and snail mortality, suggesting that all treatments exhibited molluscicidal properties. However, T1 and T2 were reported to have a comparable molluscicidal effect to that of the commercially-available molluscicide (Niclosamide™). Toxicity level (LD_{50}) was found to be at 4.007ppm while LD_{90} is 7.602ppm. As evidenced by the laboratory experiment results, this study concludes that the best AGE concentration against the target mollusk *(Pomacea canaliculata)* is T3=7.5ppm. For future research, this study recommends the use of similar AGE concentrations to *P. canaliculata* and economically important, non-target organisms (Tilapia, *Oreochromis niloticus*) under field trials.

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Introduction

The Golden Apple Snail (*Pomacea canaliculata*) is one of the worst invasive species that have continued to destroy lowland rice production in the Philippines (Cagauan *et al.,* 1993; Cagauan and Joshi, 2002). Since the introduction of this mollusk to the Philippines in 1982, approximately 40% of the planting regions in Philippines have been adversely affected by the snail according to the Food and Agriculture Organization of the United Nation [FAO–UN] (Pastorino and Darrigan, 2012). The gastropod destroys the young stem and leaves of rice paddy and could approximately consume 7–24 rice seedlings per day (Cagauan and Joshi, 2002). This situation results to a great damage in our rice growing area which threatens rice production, and eventually, food security in the country. Despite the great damage that this invasive pest has caused, few studies have been reported in terms of managing the population organically, as well as reducing infestation rate of this gastropod species (Acosta and Pullin, 1991).

To date, there is still a continuous effort to search for the most potent organic biological agents that could minimize invasion and infestation of golden apple snails (local name "Golden kuhol") in the rice granaries of the Philippines. Recent botanicals used as organic molluscicide include lemongrass, *Cymbopogon citratus* [Demetillo *et al.,* 2015]; santol, *Sandoricum vidalii* ; fruit and barks of Tulipwood tree, *Harpulia arborea* and locust bean, *Parkia* sp. [Taguiling 2015]; tobacco dust [Borja 2012]; bakuwog fruit [Ngaloy and Andrada 2005]); powderpuff tree, *Barringtonia racemose* [Musman *et al.,* 2013]; lion's tail, *Agave attenuata* [Brackenbury and Appleton, 1997]; and honey locust, *Gleditsia* sp. [Crebassa *et al.,* 2012]. The main target of these organic, plant–based molluscicides is to reduce the population of the golden apple snails, but not harm non-target organisms and their environment. Farmers who aspire to use organic molluscicide for health and environment protection (i.e. organic farming) support this initiative with the claim that it is cheaper, locally available and will bring more good than harm. This farmer's initiative supports the Philippines' Organic Agriculture Act of 2010, which promotes the use of organic fertilizer and organic pesticides in organic farming (Manila Bulletin, June 18, 2010).

The bulbs of garlic, *Allium sativum* have been widely used not only in culinary practice but also in traditional herbal medicine. Recent studies have also unveiled the potentiality of garlic as a pesticide. These studies indicate that some of the chemical components of garlic show a very promising repellent activity in the area of pest control. Numerous studies have also proven garlic's nematocidal, rodenticidal, anthelminthics and insecticidal activity (Amonkar and Reeves, 1970; Singh and Singh, 2008; Nwanchukwu and Asawalam, 2014). Despite these claims, very few researches ventured into the molluscicidal potency of *A. sativum*. Initial report of the plants molluscicidal potency was recorded by Singh & Singh (1995) wherein the water extract of *A. sativum* bulb has shown a high toxicity against *Lymanea acuminata* and *Indoplanurbus exustus* which are the intermediate host of liver fluke *F. hepatica* and *F. gigantica* (Singh and Agarwal, 1984; Singh and Agarwal, 1987). The molluscicidal activity of garlic towards the snail *Bromphalaria alexandrina* was also proven in the study of Mantawy and Mahmond (2002). Characterization of *A. sativum's* molluscicidal components has shown that its allicin component is responsible for snail mortality. In addition, it is also a great source of bioactive substances (e.g. saponins, steroid, tannins, alkaloids, triterpenes and cardiac glycosides) which are mostly poisonous against snails (Singh and Singh, 2008).

The golden apple snail, *P. canaliculata* is an alien invasive species that causes severe damage on rice cultivation areas because of its herbivorous mode of nutrition (Halwart, 1994; Joshi, 2007). *P. canaliculata* could consume young rice seedlings in a whole field overnight and the obvious signs of severe damage are characterized by missing hills and floating fragments of rice plants (Massaguni and Latip, 2015). Current strategies for controlling golden apple snail in paddy fields relied heavily on synthetic molluscicides, however, excessive use of synthetic

molluscicides have been found to have numerous drawbacks on the environment and hazard and human health (Cowie, 2002; Joshi *et al*., 2008; Cowie and Hayes, 2012). The hazardous nature of synthetic molluscicides has prompted scientists to determine the least disruptive options of pest control technologies (Massaguni and Latip, 2015). Although these literatures hold promise on the potential of *A. sativum* as a pesticide, there is still a need to verify its molluscicidal and toxicological activity *in vivo*. Thus, this study was conducted to evaluate the molluscicidal activity of the aqueous extracts of *A. sativum* bulb and its toxicological effect to Golden Apple Snail (*P. canaliculata*).

Materials and methods

Research environment

The Golden Apple snails were collected from one of the rice fields in Brgy. Linao Minglanilla, Cebu [coordinates: 10.3158°N, 124.912°E] while the garlic used in this experiment was purchased from Carbon Market, Cebu City [coordinates: 10.2915°N, 123.8992°E]. Confirmation of the snails' species I.D. was done at the Integrated Fisheries Laboratory Unit, Bureau of Fisheries and Aquatic Resources [BFAR], Cebu City. Laboratory trials (i.e. range finding test, protocol optimization and final experimentation in three [3] trials) were conducted at the Biological Science Laboratory, Don Vicente Rama Memorial National High School [DVRMNHS], located at Macopa St., Basak, Cebu City [coordinates: 10.2909°N, 123.8656°E].

Collection and selection of viable snails

The test organism collection procedures were adopted from the study of Joshi (2005), with slight modifications. Representative sample of the snails that were collected from one of the rice paddies in Minglanilla, Cebu were brought to the Bureau of Fisheries and Aquatic Resources [BFAR] for verification. The snails were taxonomically identified as *P. canaliculata* and authenticated by BFAR, Cebu City. After the authentication, the rest of the snails left in the laboratory were measured using an analytical balance and a caliper for the initial weight and height of the snails, respectively. Only those snails with a weight greater than 5 grams and a height ranging from 25mm–35mm were subjected to the acclimatization process. These weight and height dimensions are considered viable for biological assay because these are the most commonly found snails in the locality mentioned.

Collection and extraction of aqueous garlic extract (AGE)

The garlic bulbs (1 kg) were purchased from Carbon market, Cebu. Quality of garlic (i.e. no damage, good quality and from the *hard neck* variety [single layer, 8–10 cloves per bulb) was verified from the Department of Agriculture – 7 prior to purchase. It was brought to DVRMNHS laboratory for extraction. The methods of extraction for the garlic was adopted from the study of Singh and Singh (1995) wherein the bulbs of garlic were unhusked and were placed in a blender containing 100 mL of distilled water. It was blended for 3–5 min (at a high speed) until a sticky paste was obtained. The sticky paste was then squeezed and filtered using cheesecloth. The filtrate serves as the pure aqueous garlic extract (AGE).

Protocol optimization and acclimatization process

The acclimatization is the first step in the protocol optimization. Such optimization procedure aims to pre-test the entire research procedure, verify if the procedure works as stated in the reference methods, and if the ambient laboratory conditions are favorable for the experiment to proceed. During the acclimatization process, the snails were allowed to adapt to the ambient temperature of the DVRMNHS Biology laboratory. The acclimatization process was based from the methods of Joshi *et al.* (2005) wherein 10 snails were placed in a 500 ml container (i.e. rectangular, transparent plastic containers). Each container was filled with 350 ml dechlorinated water (26°C) (Dai *et al.,* 2011; He *et al.,* 2017) and 5g fresh, young, papaya leaves (given *ad libitum*). The acclimatization process lasted for 24 hours, and only those active snails (i.e. moving, rasping using the radula, movement of inhalant siphon, tentacles and visceral foot outside the lip) after 24h were selected,

and subjected to further experimentation. Snails which did not pass the weight and height requirements, and those which did not show an active state described above were removed, crushed and disposed as suggested by the standard protocol for disposal of gastropods (WHO, 1983).

Preliminary screening and range finding test

From the extracted aqueous garlic solution of 1000 ml, a 500 ml solution was aliquoted. From aliquot, all serial dilutions were made and the following concentrations were prepared: 10 ppm, 7.5 ppm, 5.0 ppm, and 2.5 ppm. These concentrations were used during the preliminary screening of concentration ranges (i.e. same experimental design employed, total sample per treatment was 90 snails). Results of the range finding test showed high snail mortality (i.e. 100% mortality after 24h) in the following solutions: 10 ppm, 7.5 ppm and 5 ppm only. Thus, the 2.5ppm was discarded in the final experimental design as it did not exhibit strong molluscicidal activity against snails during the preliminary screening.

Preparation of serial dilutions

From the 500ml aliquot described above, 100 ml was taken, placed in a graduated cylinder and diluted with 900 ml amount of distilled water, resulting to 1,000ml solution. The 1000 ml solution will now serve as the main stock solution. Out from the stock solution, five serial dilutions for final experimentation were made namely; 10ppm, 8.75ppm, 7.5ppm, 6.25ppm, and 5.0ppm. This procedure was done after the range finding test of dilution concentration that best induce mortality of snails.

Experimental design for the final experiment

This research employed Complete Randomized Design (CRD), with equal replications (Table 1). Here, the experimental subjects (i.e. the test organism, *P. canaliculata*) were randomly assigned to treatments and were subjected to three (3) experimental trials. Each trial consists of seven treatments (5 experimental groups; 2 control groups) in triplicates (Table 1). Each replicate contain 10 snails as sample, with a total of 30 snails for every treatment. Therefore, for every treatment, there were 90 snails subjected for observation derived from the 30 snails for each of the trial or repeat experiment.

The negative control was represented by distilled water while the positive control was a powdered form of Niclosamide (Snail Shatter™). According to local farmers, Snail Shatter™ is one of the most effective synthetic molluscicide available in the local agrivet stores in the city. The experimental groups are represented by the following concentrations of AGE: 10ppm, 8.75 ppm, 7.5ppm, 6.25ppm, and 5.0 ppm.

Sampling design

The collected snails were randomly chosen from the qualified population and randomly assigned to various treatments and replicates. The weight and height of the snails were also measured to ensure homogeneity of sample in terms of weight and size based on the following parameters: weight $\geq$ 5 grams and a height ranging from 25mm – 35mm.

In the actual experimentation, 21 transparent containers (i.e. 3 replicates per treatment, 21 total container) were all labeled (e.g. T1R1 = treatment1 (10ppm), replicate 1). Ten golden apple snails were distributed randomly to every container, with a total of 630 golden apple snails used during the experimentation for the three trials. The mortality of the snails was continuously observed for the succeeding 48 hours.

Confirmatory test for mortality

Every after 24h, a mortality confirmation test was conducted, wherein all the snails were gently poked by a needle. A snail is considered dead if it did not exhibit any muscular contractions in the following body parts: inhalant siphon, radula, muscular foot and have already retracted completely into its shell (see protocol of Massaguni and Latip, 2015). A needle was also used as stimulus (i.e. to induce painful sensation) and to initiate response from the snails head section. The snails' death could be furthered ascertained by the complete closing of its operculum

(i.e. the flap found in the shell opening that covers the entire visceral mass).

Data analysis

Data collected include (1) qualitative description of the snail morphology pre- and post mortem (e.g. retraction of anterior head and foot visceral mass, twisting, hyperactivity, and release of white sticky mucous) and (2) mortality rate every after 24h. Prior to data analysis, all results from the three trials were collated and pooled (e.g. now each treatment has 90 observations). For the qualitative description, ocular inspection of alive and dead snails was used. For the mortality rate per hour, differences of the number of death per treatment were compared using one way Analysis of Variance (ANOVA). Significant *p* values ($\leq$0.05) per group comparison were subjected to post hoc analysis (Tukey's Highly Significant Difference Test) to be able to determine via pairwise comparison, which among the specific treatments are significantly different to each other. Similar letter superscripts were added to treatment means to denote the compared treatments which were not statistically significant at $p \leq 0.05$. Lethal doses (LD_{50} and LD_{90}), were evaluated by means of Probit Analysis (Finney, 1971). The plot probit of kill against log of concentration (mg/l) provides a simple graphic representation of the doses to response ratio. In the Probit Analysis, the data utilized was the average of all snails that have died in each of the replicates performed which is by 10. Statistical analyses were performed using Minitab v. 16 and Probt analysis tool under SPSS v.20.

Results

General observation

The data gathered were taken from the three trials of experiment (i.e. performed in three different schedules) to ensure consistency of results. Qualitative observations were also made in terms of the behavior of the snails pre- and postmortem when exposed to various treatment concentrations of AGE at 24h and 48h. Data presented are averages of mortality from the three repeat experiments conducted.

Table 1. Complete Randomized Design (CRD) with equal replications per trial*.

Treatments	Replicates	Sample** per replicate	Total number of snails per treatment
Control groups			
Negative	3	10	30
Positive (Niclosamide) [Snail Shatter™])	3	10	30
Experimental groups			
T1 (10.0 ppm AGE***)	3	10	30
T2 (8.75 ppm AGE)	3	10	30
T3 (7.50 ppm AGE)	3	10	30
T4 (6.25 ppm AGE)	3	10	30
T5 (5.00 ppm AGE)	3	10	30
Total			210 snails for 7 treatments for 1 trial

*For each trial, 210 snails were used. For the three trials (conducted on separate days), the grand total of the snails used is 630 snails.

**Each snail represents 1 sample

*** AGE = aqueous garlic extract.

Results of the range finding test showed molluscicidal activity of the different AGE concentration (Table 2). Across the different treatment concentrations, the comparable mortality vs the positive control (Snail Shatter™) was T1 (10ppm AGE), followed by T2 (7.5ppm), and T3 (5.0ppm), in decreasing order of death reported.

In all the experimental groups, it is remarkable that snail mortality were mostly observed within 24h, similar to the positive control. Since the purpose of the range-finding test was to determine which among the AGE treatments could have a comparable molluscicidal property with the positive control, the results suggest to eliminate T4 (2.50ppm) in the treatments for the final experimental set-up.

Table 2. Mortality rate of *Pomacea canaliculata (*Golden apple snail) due to Aqueous Garlic Extract (AGE) treatment during the range finding test, N=180.

Treatment	Replicates						
	1st		2nd		3rd		Total mortality (48h)
	No. of dead snails after		No. of dead snails after		No. of dead snails after		
	24h	48h	24h	48h	24h	48h	
Positive control (Snail Shatter™)	10	0	10	0	10	0	30
Negative control	0	0	0	0	0	0	0
T1-10.0ppm AGE	10	0	9	1	9	1	30
T2-7.50ppm AGE	9	1	8	1	8	1	28
T3-5.00ppm AGE	5	1	6	0	5	1	18
T4-2.50ppm AGE	0	6	1	0	2	0	9

*Mortality refers to the number of dead Golden apple snails.

The average mortality rate of the *P. canaliculata* exposed to the two control and various concentrations of the AGE (aqueous garlic extract) in three trials (repeat experiments) is shown in Table 3. The highest recorded mortality after 24h among the five treatments was that of the 10 ppm and 8.75ppm which are both comparable to the positive control (Snail Shatter™) (Table 3).

Table 3. Mortality rate and post mortem observations of *P. canaliculata (*Golden apple snail) due to Aqueous Garlic Extract (AGE) treatment during the final experimental phase (3 trials), N=630.

	Trials*						
Treatment	1st Trial No. of dead snails after		2nd Trial No. of dead snails after		3rd Trial No. of dead snails after		Post-mortem observation
	24h	48h	24h	48h	24h	48h	48h
Positive control (Snail Shatter™)	30	0	30	0	30	0	Rapid death of snails in 5min
Negative control	0	0	0	1	2	0	Normal behavior in 2days
T1: 10.0 ppm AGE	30	0	28	2	30	0	Secretes white sticky mucus; closing of operculum; retracted visceral foot; no movement of siphon/ proboscis; float in the water
T2: 8.75 ppm AGE	28	2	30	0	30	0	
T3: 7.50 ppm AGE	27	3	25	5	24	6	
T4: 6.25 ppm AGE	24	6	23	7	23	7	
T5: 5.00 ppm AGE	20	10	21	9	20	10	

*No. of snails used per trial is 210. Total no of snails used for 3 trials is 630.

Except for two snails which died on the 2nd day (after 48hrs) of experimentation, all snails in both 10 and 8.75ppm died within the first 24h. Remarkably, snails in all experimental groups manifested similar post mortem behavior (i.e. secretes white sticky mucus; closing of operculum; retracted visceral foot; no movement of siphon/ proboscis; float in the water), but the manifestation of these observable changes did

not happen at the same time (Table 3). There seems to be a concentration – response relationship, where under 10 ppm and 8.75ppm, the more rapid is the manifestation of these observable changes in the snail behavior / physiology.

There was a negligible death of snails observed on the negative control set-up (distilled water). Although there were three (3) recorded deaths, the snails exposed on the negative control were freely moving and did not show any symptoms of intoxication. Most likely, the deaths were due to stress and inability to adapt to the purely distilled water environment, or artificially – contained situation.

Table 4. Mean Mortality Rate (mean±S.D.) of AGE against P. *canaliculata*.

Treatment	Mortality rate per day		Total	% Mortality
	n= 30 per treatment (Mean ± S. D.)			No. of dead snails / N*100
	after 24 hrs.	after 48 hrs.		
+ Control (Snail Shatter™)	30.0 ± 0.00[a]	-	30 ± 0.00	100%
– Control (Distilled water)	0.67 ± 0.33[d]	0.33 ±0.57	1 ± 0.9	10%
T1 (10.0 ppm AGE)	29.33 ± 0.67[a]	0.67±1.15	30 ± 0.00	100%
T2 (8.75 ppm AGE)	29.33 ± 0.67[a]	0.67±1.15	30 ± 0.00	100%
T3 (7.50 ppm AGE)	25.33 ± 0.88[b]	4.67±1.53	30 ± 0.00	100%
T4 (6.25 ppm AGE)	23.33 ± 0.33[b]	6.67±0.57	30 ± 0.00	100%
T5 (5.00 ppm AGE)	20.33±0.33[c]	9.67±0.57	30 ± 0.00	100%
p ≤ 0.05	0.000*			

Significant at p ≤ 0.05

Similar letter superscript of the mean ± S.D. indicates non-significant at Tukey's post hoc test (p ≤ 0.05).

Results of the mean mortality across treatments were compared using ANOVA (Table 4, Table 5) while post-hoc test (Table 4, Table 7) determined which among the specific treatment means were comparable, statistically. ANOVA result showed a p=0.000, indicating a very high significant difference among the treatment means. This further shows that at least one (1) group among the treatments registered a very low mortality (i.e. in this case, the negative control) compared to the rest, thus reflecting a very high difference of mortality number among the test organisms.

Table 5. One way ANOVA on the mortality of *P. canaliculata* under different treatments.

Source	DF	SS	MS	F	*P*
Factor	6	1946.952	324.492	486.74	0.000*
Error	14	9.333	0.667		
Total	20	1956.286			
S = 0.8165	R-Sq = 99.52%	R-Sq(adj) = 99.32%			

**significant at p ≤ 0.05*

Results also showed that positive control, T1 (10.0ppm) and T2 (8.75ppm) are statistically comparable to each other based on the post-Hoc test (Table 4). This result may indicate that the number of deaths among snails in these treatments were comparable (both 100% mortality), although may have vary in the time of death after the addition of Niclosamide [Snail Shutter™] and AGE to the positive and treated groups, respectively. As reflected in the previous table (Table 2 and Table 3), those snails under the positive control died immediately within 5 min, while those of the experimental groups died at different time within the 24h experimental period. Similar trend was also observed in T3

(7.50ppm) and T4 (6.25ppm) where these two experimental groups registered a comparable mortality rate after the experimental period. Although the number of deaths after 24h and 48h vary, the total mortality after the entire experimental period is comparable, statistically. Meanwhile, T5 (5.00ppm) also registered 100% mortality after 48h, but statistically insignificant to the rest of the treatments at 24h, suggesting that T5 is not as effective in killing the snails at 24h, compared to positive control, T1 or T2. The results of table 5 and 6 further supports that all experimental treatment groups are potent molluscicides against *P. canaliculata*.

Table 6. Calculated lethal dosages (in ppm) after the 24 hour of exposure of adult *P. canaliculata* to the varying concentrations of AGE (in ppm).

Lethal doses (LD) of AGE (ppm)		R^2	Chi-square value	Slope value	Fitting
LD50	LD90				
4.007 ppm *95% LCL =2.978 ppm *95% UCL =5.392 ppm	7.602 ppm *95% LCL =5.650 ppm *95% UCL =10.229 ppm	0.868	0.303	3.399	Good fit

*95% Fiducial LCL (Lower Confidence Limit) and UCL (Upper Confidence Limit), values significant at $p<0.05$.

Toxicity of aqueous extract of Allium sativum

The lethal dosages (along with its limits) (Table 6) were calculated and estimated using Probit analysis. Under laboratory conditions, the dosage that can cause 50% snail mortality at a given *P. canaliculata* sample is 4.007 ppm of AGE while 90% snail mortality can be manifested if the solution used is 7.602 ppm AGE.

The analysis significantly gives the lowest LC50/ LC90 values at 95% confidence interval which is 4.007 ppm and 7.602 ppm respectively which indicate the highest potency (Fig.1). Among the treatment groups, T5 (5.00ppm) is the most ideal concentration because it has the lowest toxicity level yet it can induce 100 percent mortality on snails.

Table 7. Pairwise comparison result of different treatment means (Tukey's test; $p \leq 0.05$).

Treatment	Pairwise Comparison	P-value ($p \leq 0.05$)	Interpretation
+C	+C VS -C	0.000	Significant
	+C VS T1	1.000	Not Significant
	+C VS T2	0.946	Not Significant
	+C VS T3	0.000	Significant
	+C VS T4	0.000	Significant
	+C VS T5	0.000	Significant
-C	-C VS T1	0.000	Significant
	-C VS T2	0.000	Significant
	-C VS T3	0.000	Significant
	-C VS 6T4	0.000	Significant
	-C VS T5	0.000	Significant
T1	T1 VS T2	0.946	Not Significant
	T1 VS T3	0.000	Significant
	T1 VS T4	0.000	Significant
	T1 VS T5	0.000	Significant
T2	T2 VS T3	0.001	Significant
	T2 VS T4	0.000	Significant
	T2 VS T5	0.000	Significant
T3	T3 VS T4	0.103	Not Significant
	T3VS T5	0.000	Significant
T4	T4 VS T5	0.107	Not Significant

Legend: +C = positive control; -C = negative control; Treatment 1: (10 ppm); Treatment 2: (8.75 ppm); Treatment 3: (7.50 ppm); Treatment 4: (6.25 ppm); and Treatment 5: (5.0 ppm).

It was also revealed in the Probit analysis that the slope value is positive. In addition, the coefficient of correlation of the treatments and mortality rates is 0.868 which can be interpreted as "very high correlation" (of concentration and mortality). Thus, there is a direct relationship between the concentration of treatment and snail mortality. As observed from the results, as the concentration of the aqueous extract of AGE increases, mortality of *P. canaliculata* also increases, and vice versa.

Discussion

This experiment disclosed the efficacy of *A. sativum* as a potential plant molluscicide that could control the most invasive rice pest *P. canaliculata*. Extracts of garlic bulb (100%) had shown a comparable effect to those of the positive control (i.e. proven synthetic molluscicide, Niclosamide) which brought complete mortality to the snails after 24 hours. Upon the application of the AGE treatments, several behavioral changes were observed in this experiment such as hyperactivity, twisting motion and the release of white sticky saliva-like-substance. The twisting motion was also observed in the study of Ahmed *et al.,* (2014) wherein as the *Solenostemma argel* extract enters the snail's (*Biomphalaria pfeferri*) body, muscular twitching occurred and the snails became spirally twisted, which resulted in ataxia, convulsion, paralysis and finally death. Prior to death, there was complete withdrawal of the body inside the shell that indicated nerve poisoning.

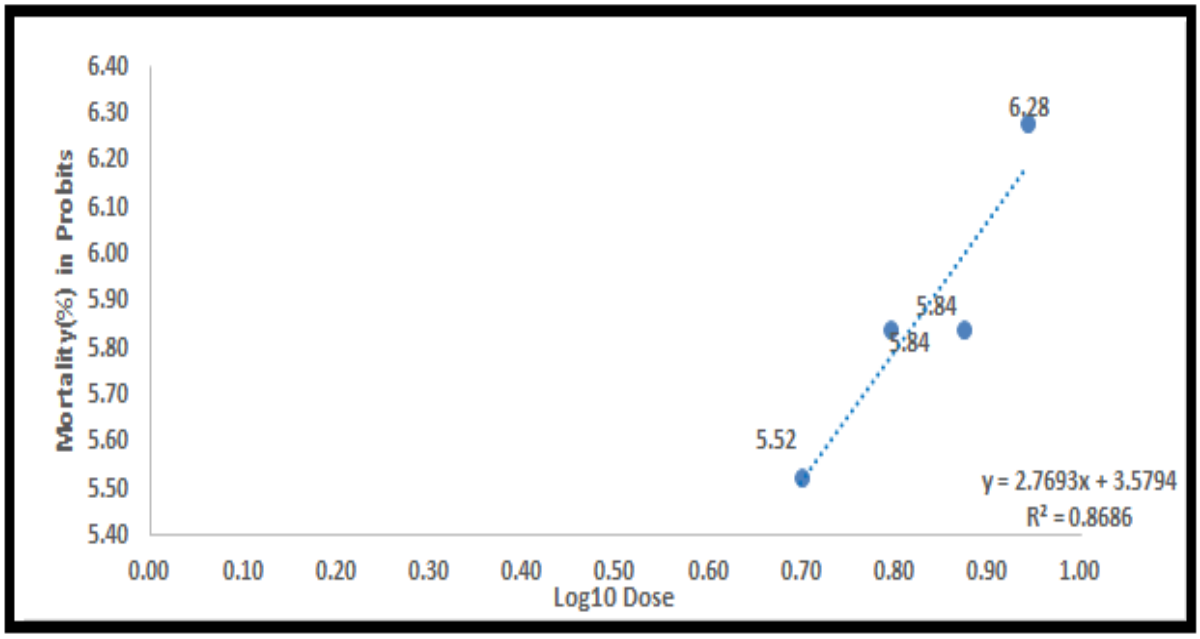

Fig. 1. Calculated LD50/LD90 of the lethal dose of AGE against the *P. canaliculata* as target organisms using Probit.

The presence of secondary metabolites (flavonoids, tannin, alkaloids, glycosides etc.) on the plant of interest, *S. argel* was believed to have caused fatality in the snail's nervous system leading to their mortality. Tripathi and Singh (2000) and Tiwari and Singh (2004) have also observed the same activity on the snail *Indoplanurbus exustus* and *Lymnaea acuminata* when exposed to plant phytochemicals.

In the present study, the white sticky substance was probably triggered upon the reaction of the snails' mucus membrane towards the polar compounds (e.g. eugenol, esters, saponins etc.) of *A. sativum*. Allicin, the major component of *A. sativum,* is a phytochemical that is highly irritant in nature and may have induced the reaction of the snail's mucosal membranes (Aladesanmi, 2007). In other studies, slugs and snails were also observed to produce large

amounts of mucus when come into contact with copper surfaces and the excretion of too much mucus can cause dehydration and could subsequently lead to the death of the snails (Singh and Singh, 2008; Massaguni and Hajjar, 2015).

In support to the result of the present experiment, a study of Mwine and Van Damme (2011) have revealed that the toxicity of *A. sativum* towards the numerous rice pest *P. canaliculata* could be associated to its molluscicidal phytochemicals, namely; saponins, tannins, phorbol, steroids, flavonoids, terpene and esters (Metwally, 2006; Michail, 2010; Meriga *et al.,* 2012). Saponin, for instance, inhibits certain chololinesterase enzymes that have a lethal effect to the nervous system of the snail and also causes formation of cholesterol in the body of the snail (San Martin and Cruz, 2013). Large numbers of saponins also affect the snail's membrane permeability by either forming pores in the membrane, altering sodium-potassium and calcium ATPase activity or insertion of the hydrophobic saponin nucleus into the lipid layer. Ahmed *et al.,* (2014) also indicated that the molluscicidal activity of their plant of interest was due to its high alkaloid content which are heterocyclic compounds having adverse effects in the central nervous system of the snail. Dai *et al.,* (2011) also showed that cardiac glycosides had a significant effect on GAS, resulting to the decrease in the glycogen content of the snail. This resulted to the impairment of the physiological metabolism of the snails and altering the hepatopancreas tissues of *P. canaliculata* which consequently leads to fatality.

In this study, the most effective AGE was 10ppm. This result finds support from the standards of World Health Organization (1983) where an aqueous extract must exhibit lethality at a dose lower than 20 ppm. There is also a direct relationship between the concentrations of AGE and its effect which is on the mortality rate of the snails. Each concentration of AGE has a significant effect to the target organism. A 10 ppm and 8.75 ppm concentrations yielded a comparable result to that of the commercial molluscicide agent, Snail Shatter™. The mortality rate was correlated positively with the extract concentrations as the mortality of snail increased with the increase of AGE concentration. Mott (1987) suggested that for a plant extract to be considered molluscicide, it should register a concentration up to 100 mg/L and be able to kill 90% of the snails, 24 h after contact. Farnsworth *et al.,* (1987) also agreed that experimental treatments could be tested up to 100 mg/L. However, the latter author used percentage scales that allow the discrimination of the molluscicidal activity as weakly active and active, depending on the number of dead snails.

In the case of *A. sativum* used in the present experiment, the required amount that could kill all *P. canaliculata* is 5.0ppm in 48hours, an amount that is less toxic to the snails, but high enough to induce complete mortality. This result may suggest that *Allium sativum* extract could be an effective organic, molluscicide against the invasive snail, *Pomacea canaliculata*. To further understand the toxicity of aqueous garlic extract, this study recommends a separate investigation of its ecotoxicity on non-target organisms like Tilapia (*Oreochromis niloticus*), both under laboratory and field settings.

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