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Original Article

The effectiveness of *Furcraea* plants in controlling golden apple snail and their effects on the non-target organism at the rice field

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Abstract

Golden Apple Snail (GAS) is regarded as a serious invertebrate pest at the rice field. Most of the farmers prefer synthetic molluscicide, which delivers fast and effective responses, to control this pest. However, the synthetic molluscicide application negatively affects the farmers' health and ecosystem. Therefore, the greener pest management technique is needed to eliminate this pest. In this study, we investigated the effectiveness of *Furcraea* plants in killing GAS, and their effects on the non-target organisms, which were catfish and rice seedlings. The results showed that *F. gigantea* was more effective in controlling GAS compared to *F. foetida* and *F. selloa*. In controlled condition, the application of 27.59 g of *F. gigantea* in 1.2 liter of water killed at least 90% of GAS population within 24 hours. The *F. gigantea* could kill at least 80% of GAS population at most for 3 days after the application. When applied in the field, *F. gigantea* cut leaves resulted in 100% mortality to GAS, but at the same time did not kill the catfish. The application of *F. gigantea* cut leaves did not affect the chlorophyll content, and shoot to root ratio of rice plant, but enhanced the plant height and dry weight compared to the synthetic molluscicide.

Keywords: Golden Apple Snail, Rice field, Furcraea plant, Non-target organism

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Introduction

The Golden Apple Snail (GAS) is recognised as one of the major pests in the rice field in Southeast Asia (Cowie, 2020). A record has shown that since 2002 until 2008, the GAS has infested almost 20,000 ha of the rice areas in Malaysia, thus affecting the income of rice farmers; in 2010 alone, the costs associated with the damage caused by this pest were estimated at USD 28 million (Yahaya et al., 2017). In other parts of Southeast Asia, such as Vietnam, Philippines, and Thailand, the GAS infestation has also caused significant damages to the rice crop; it is estimated that the total annual loss caused by these snails is about USD 806 to USD 2138 million (Schneiker et al., 2016). Therefore, it could cause food insecurity, especially to the countries that depend on the rice grain as their main staple food.

Golden Apple Snail feeds voraciously on the newly sprouted rice seeds. This snail has an ability to damage the direct wet-seeded rice and transplanted rice up to 40 days old of planting (Teo, 2003). It has a wide host range and voracious appetite (Yang et al., 2018). In their favourable environment, GAS can completely destroy 1 m² of rice field overnight (IRRI, 2020). Their success as a major pest at rice field is related to their ability to reproduce rapidly. It can produce thousands of eggs during their life time (Kyle et al., 2013). Moreover, it has a high hatchability rate, which is 80%, with 10-15 days of incubation (Cowie, 2020). This pest also has fast growth, strong stress tolerance and adaptation to a broad range of harsh environmental conditions (Liu et al., 2018; Yang et al., 2018). This pest has an ability to colonize and invade diverse habitats through multiple pathways, such as irrigation canals, natural water distribution pathways, occasional flooding events and human activities (Ravindra and Velez Parera, 2017).

There are various Integrated Pest Management (IPM) techniques which have been applied to control GAS, such as cultural, mechanical, and biological methods, but so far, they are unsuccessful in fully eliminating GAS. The current control measures include quarantine, collection and destruction of eggs and adults. These techniques involve using plant attractants, such as pineapple, papaya, and cassava leaf in order to easily hand pick the snails, install traps at the canal to avoid the dispersion of the snails, and to use ducks to feed the snails (Salleh et al., 2012). However, farmers prefer to use synthetic molluscicides such as metaldehyde, copper sulfate, niclosamide and fetin acetate due to their effectiveness, faster results and low cost (Mokhtar et al., 2019). Although chemical control is included in the IPM package, farmers may secretly prefer to use the banned and unregistered molluscicides (Bernama, 2020). They also tend to overuse those molluscicides because the price is cheap and they yield faster results (Ali et al., 2018).

The overuse of synthetic pesticides will impact human health, pollute the environment and harm the non-target organisms. Furthermore, the repeated applications of the same molluscicide may cause resistance in GAS. Thus, the use of botanical molluscicides and biocontrol agents is a promising alternative to control GAS infestation. Theoretically, toxic plant is organic, and it does not pose any hazard to farmers and the environment (Taguiling, 2015). Previous researches have shown that some plant species have molluscicidal properties and ability to kill snails; the plants include *Furcraea selloa* var. marginata, *Monochoria vaginalis* (Ramli et al., 2017a; 2017b), *Acacia mangium* (Joseph et al., 2016), *Azadirachta indica* (Massaguni and Latip, 2012; 2015), *Ipomoea aquatic*, *Pelthoporum pterocarpum* (Latip et al., 2015), *Cymbopogon citratus* and *Piper bitle* (Ibrahim et al., 2017).

In Malaysia, the Department of Agriculture Malaysia (DOA) has encouraged farmers to apply the raw toxic plants such as *F. selloa* to manage GAS infestation, and promoted this method as the most effective, cheap and eco-friendly solution. However, only few studies have been conducted to investigate the effectiveness and effects of the plant application on non-target organisms. For example, Md Rejab et al. (2020) stated that the application of *F. gigantae* reduced the paddy seeds germination and altered its traits. In addition to that, the exposure of high amount of *F. gigantae* in a controlled condition was also reported to be harmful for catfish (Md Rejab et al., 2020). There are three species of *Furcraea* plants available in Malaysia: *F. selloa, F. foetida* and *F. gigantea*. To date, no research has compared the effectiveness of those three *Furcraea* species in killing GAS.

In this study, we investigated the efficacy of three *Furcaea* species, *F. selloa, F. foetida* and *F. gigantea*, in controlling GAS. We also measured the amount of *Furcraea* cut leaves needed to kill at least 50% and 90% of the GAS population and evaluated the longevity of application effectiveness. Our laboratory findings were then applied to the field to investigate the effects of *F. gigantea* on GAS, catfish and rice plant.

Material and Methods

Study system

Plant: Leaves of *Furcraea* plants, consisted of *F. gigantea*, *F. selloa* and *F. foetida* were collected from the garden area at Universiti Sultan Zainal Abidin, Gong Badak Campus, Terengganu, Malaysia (5°23'50.5"N 103°04'46.4"E). The leaves were harvested, cut into small pieces (2 to 3 cm long) and kept in the chiller at 4°C for 12 hours, a day before the experiment started. The rice seeds variety MR220CL1 were purchased from the Peladang Shop at Besut Terengganu. Only certified rice seeds were used for this experiment.

Animal: Golden Apple Snails (*Pomacea canaliculate*) were collected from the rice field at Kampung Oh,

Besut, Terengganu, Malaysia (5°39'54.3"N 102°31'58.3"E). The snails were kept in the laboratory at the ambient temperature (25-30°C) and humidity (80-90%) for a week before the experiment began to make sure that the snails were in good condition and fit for the experiment. The snails were kept in the aquarium filled with dechlorinated tap water and fed with water spinach as needed. A snail was recorded as 'dead' if it was inactive and immobile for ten minutes after captured, or if it failed to show muscular response upon mechanical stimulus of the head-foot, shell discoloration, and foul odour (Meléndez and Capriles, 2002). Catfishes (2 inches long) were purchased from a catfish breeder at Besut, Terengganu, and kept in the laboratory up to 1 week before the experiment started. The catfishes were kept in the aquarium and fed with fish food pellet (Star Feed, Malaysia) as needed until the day of experiment.
Experimental sites: All laboratory experiments were done at the Toxicology Laboratory, Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Campus, and the field study was done at the rice field plot, Kampong Oh, Besut, Terengganu, Malaysia. Three rice plots with an area of 0.5 ha per plot were ploughed and flattened completely for the experiment. The preview lanes were prepared around the plot to trap the GAS and the water level was controlled at 2 to 3 cm above the ground level.

Laboratory experiment

Screening of Furcraea plant species: Fifty grams of *F. gigantea*, *F. selloa and F. foetida* cut leaves were prepared and put into respective aquariums containing 1200 ml of dechlorinated tap water and 10 GAS. The number of dead and alive GAS was counted every four hours for 24 hours. The treatments were replicated ten times.
Determining the amount of F. gigantea that could kill at least 50% and 90% of GAS population: As the *F. gigantea* showed the best performance in killing GAS, this plant species was chosen for further experimentations. Fresh leaves of *F. gigantea* were weighted to obtain 20 g, 40 g, 60 g, and 100 g and placed into respective aquariums containing 1200 ml of dechlorinated tap water and 10 GAS. The control for this experiment was 1200 ml of dechlorinated tap water and 10 GAS without the addition of *F. gigantea* leaves. The experiments were repeated ten times. After 24 hours, the number of dead and alive snails was counted and recorded.
Determining the longevity of molluscicidal properties of Furcraea in controlling GAS: Thirty grams of *F. gigantea* leaves were immersed in 1200 ml of dechlorinated tap water in the aquarium. After 24 hours, the leaves were discarded and 10 GAS were put into the aquarium. The control for this experiment was 1200 ml of dechlorinated tap water and 10 GAS without the addition of *F. gigantea* leaves. The number of dead and alive GAS was counted after 24 hours. All GAS were then discarded from the aquarium and never used again. The water was filtered to discard any sludge in the container and a new batch of GAS was added.

Field experiment

The experiment was carried out in January 2020. Field plots were marked as Plot 1 (untreated), Plot 2 (treated with 30 kg of *F. gigantea* cut leaves) and Plot 3 (treated with commercial synthetic molluscicide). The commercial molluscicide had metaldehyde as the active ingredient, and 6 kg per hectare was applied as recommended by the manufacturer.
Measuring the effectiveness of F. gigantea in controlling Golden Apple Snail at field condition: The number of GAS in each rice plot was counted before the experiment to determine the density of GAS before the treatment. The number of alive or dead snails was counted again 24 hours after the application. The snails were sampled by using quadrat (1m × 1m). The sampling was replicated ten times per plot on the same day.
Investigating the effect of F. gigantea application on non-target organism:
Catfish survival: Ten plastic cages (20cm × 20cm × 20cm) containing 10 catfish were left in all experimental plots after the application. The number of dead and alive catfish was counted after 24 hours.
Rice plant traits: After 21 days of application, the plant growth data were collected. The parameters observed were plant height, plant dry weight, chlorophyll content and shoot to root ratio of the plant. Ten plants were randomly selected from all the experimental plots for ten replicates. The plants were washed using tap water to remove soil and dirt on their root. The height of the plant was measured using ruler by straightening the plant to its fullest length. The chlorophyll content was measured by using the handheld chlorophyll content meter (SPAD 502 Plus Chlorophyll Meter, Japan). The plants were dried in the drying oven (Memmert U Universal Heating Oven, German) until it reached a constant weight. The

dry weight of the plant was determined by using scientific balance (RADWAG AS 220 R2, Poland). The shoot to root ratio was measured by dividing the dry weight of shoot per the dry weight of root.

Statistical analysis

All data were analysed using R statistical software version 3.4.0 (R Core Team, 2017). The data for response variables were transformed when necessary to meet the assumptions of normality and homoscedasticity. The significance of differences between means values was determined by using LS means and separation by post-hoc Tukey tests using treatments as explanatory variables. The snail mortality and catfish survival data were transformed to percentage. The effects of time and *Furcraea* species in killing GAS were analysed by using GLS function with linear and nonlinear mixed effects model package for repeated measures analysis with time set as a factor. The exact amount of *F. gigantea* cut leaves that could kill 50% and 90% of GAS was calculated by using the GLM function in MASS package (family binomial). The count data on the effectiveness of *F. gigantea* cut leaves in controlling GAS at field condition was analysed using GLM function (family poison).

Results and Discussion

Screening of Furcraea plant species

The results obtained showed that *F. gigantea* and *F. foetida* managed to kill 100% of GAS after 24 hours of exposure. On the other hand, *F. selloa* only managed to kill 61.4% of GAS population ($F= 130.70$, $P < 0.001$). However, the time taken to kill GAS differed amongst plant species ($F= 96.28$, $P < 0.001$). Figure 1 shows the mortality of the GAS on four different treatments: control (T1), *F. gigantea* (T2), *F. foetida* (T3), and *F. selloa* (T4). *F gigantea* (T2) took 7 hours and 47 minutes to kill 50% of GAS population in the aquarium. Meanwhile, *F. foetida* (T3) needed at least 10 hours and 31 minutes and *F. selloa* (T4) took 18 hours 49 minutes to kill 50% of GAS. Overall, the application of *F. gigantea* and *F. foetida* took 20 hours to kill all GAS population while *F. selloa* failed to kill 100% of GAS within 24 hours of exposure.

Furcraea plant species contains a steroidal saponin compound (Simmons-Boyce et al., 2004) that can act as a molluscicide agent and be effective in controlling GAS (Ramli et al., 2017a; 2017b). This compound occurs in many plants; however, the types of saponin and their amount differ between plant species. Many species of plants with saponin content were effective in controlling GAS. For example, the application of quinoa saponin caused the snails to close their opercula, killing them in 48 hours, and exhibited ovicidal effects (Joshi et al., 2008); tea saponin stressed the growth of GAS (Xian et al., 2012); and the saponin extract from *F. selloa* acted as feeding deterrent and managed to kill GAS after 48 hours of exposure (Ramli et al., 2017a; Yahaya et al., 2017; Mokhtar et al., 2019).

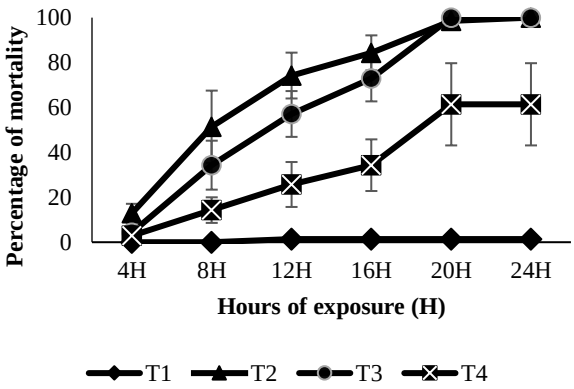

Figure-1. The effectiveness of three species of *Furcraea* plant in controlling Golden Apple Snail in controlled environment. T1: Control, T2: *F. gigantea*, T3: *F. foetida*, T4: *F. selloa*.

Furcraea plants are listed under Agavoideae subfamily. Until 2015, approximately 141 types of steroidal saponins and sapogenins were recorded in the plants of subfamily Agavoidea (Sidana et al., 2016). Research shows that *F. selloa* var. marginata contains furostanol saponin, furcreafurostatin, spirostanol saponins, furcreastatin, yuccaloeside C and cantalasaponin-1 (Simmons-Boyce et al., 2004). Similar to *F. selloa*, *F. foetida* also contains furcreastatin. The furcreastatin in *F. foetida* consists of hecogenin as the aglycone, and a hexasaccharide containing D-galactose, L-rhamnose and four D-glucose residues (Itabashi et al., 1999). Meanwhile, the *F. gigantea* has bidesmosidic furostanol saponin. The structure of furostanol saponin in *F. gigantea* was established as 3-[(O-6-deoxy-α-L-mannopyranosyl-(1→4)-O-β -D-glucopyranos-yl-(1→3)-O-[O-β -D-glucopyranosyl-(1→3)-β -D-glucopyranosyl-(1→2)-O-β -D-glucopyranosyl-(1→4)-β -D-galactopyranosyl)oxy]-(3β ,5α,15α,22α,25R)-26-(β -

D-glucopyranosyloxy)-15,22-di-hydroxy-furost-12-one (Da Silva and Parente, 2006).

As the types of saponin in those three *Furcraea* species differ, the amount of saponin may also differ. Thus, the efficacy those plants in controlling GAS also differs. This was observed in our findings. In this experiment, we did not measure the quality and quantity of saponin in each *Furcraea* plant species since we were focusing on the usage of the raw fresh leaves in controlling GAS. In addition, the existence of saponin that acted as molluscicide in those three *Furcraea* species has been proven by other researches as mentioned above.

Determining the amount of *F. gigantea* cut leaves that could kill at least 50% and 90% of GAS population

Our findings showed that the application of 7.05 g of *F. gigantea* in 1200 ml water could kill at least 50% of GAS population in the controlled environment 24 hours after exposure. On the other hand, the 90% mortality of GAS was achieved after exposure to 27.59 g of *F. gigantea* leaves (Table 1).

Table-1. The amount of *F.gigantea* cut leaves needed to kill 50% and 90% GAS population

	Amount (g)	LCL	UCL
LD50	7.05 ± 1.10	5.81	8.55
LD90	27.59 ± 1.17	20.26	37.58

*LD50: Lethal dose that can kill 50% of GAS of test population; LD90: Lethal dose that can kill 90% of GAS test population; LCL: Lower confident level; UCL: Upper confident level.

To date, most of the research focused on the effectiveness of *F. selloa* as mollucical agent to control GAS and less attention was given to other species of *Furcraea* such as *F. fioetida* and *F. gigantea*. Previous research found that the application of *F. selloa* var. marginata plant extract on GAS resulted in 90% of mortality (Ramli et al., 2017b). This *Furcrea* species aqueous extract showed a 100% of snail mortality starting from the concentration of 50 mg/ml within 24 hours, with its lethal concentration (LC50) being identified at 24 mg/ml (Joseph et al., 2016). Thus, the results from those studies have confirmed the molluscicide effectiveness of *F. selloa* var. marginata in controlling GAS. However, no research has studied the effectiveness of *F. foetida* and *F. gigantea* before this experiment was conducted. Thus, our findings have yielded an early information on the amount of *F. gigantea* cut leaves needed to control the GAS.

Determining the longevity of molluscicidal properties of *F. gigantea* in controlling GAS

The results obtained showed that the effectiveness of *F. gigantea* in controlling GAS decreased over the time (Figure 2). *F. gigantea* managed to kill 100% of GAS population on the first and second day of application. However, the effectiveness of molluscicidal properties was reduced on the third day of application when it could only kill 82% of the GAS population. The number of dead GAS dropped to 26% on day four, and more than 90% of GAS was observed alive on day five onwards. *F. gigantea* lost its effectiveness on day seven when all GAS were found alive.

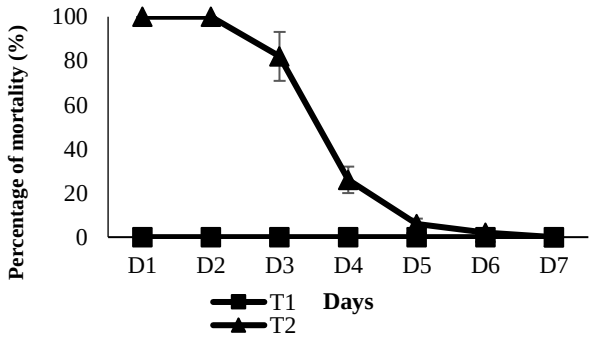

Figure-2. The longevity of molluscicidal properties of *F. gigantea* in controlling GAS. T1 is control (declorinated water) and T2 is *F. gigantea* soaked water.

The saponin derived from *Furcraea* plant is naturally biodegraded (Sparg et al., 2004). This explained our findings since *F. gigantea* was only effective in killing up to 80% GAS within less than 72 hours after application. After three days, the effectiveness of *F. gigantea* decreased, and there was no molluscicidal effect on GAS in day seven. This characteristic is important since GAS can still enter the rice field through the irrigation system. The longevity of the *F. gigantea* bioactivity will guarantee the effectiveness of this method in controlling GAS.

Measuring the effectiveness of *F. gigantea* in controlling Golden Apple Snail at field condition

The application of *F. gigantea* in rice field managed to kill 100% of GAS in the rice plot (Figure 3). No sign of alive snail was observed during the sampling at the

plot treated with *F. gigantea* (Plot 2). The commercial molluscicide (Plot 3) only managed to kill 56.88% of snails. Meanwhile, 100% of snail was found alive in the untreated plot (Plot 1).

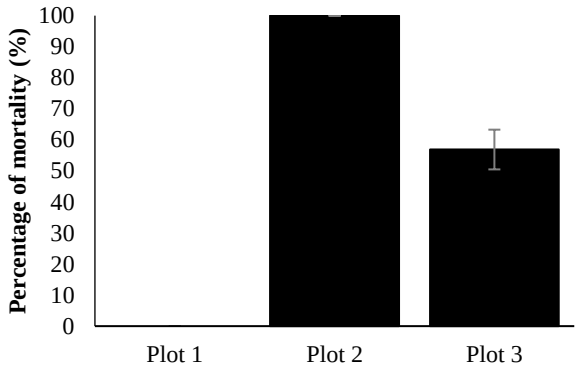

Figure-3. The mortality of GAS. Plot 1 is untreated plot, Plot 2 treated with *F. gigantea* cut leaves and Plot 3 treated with commercial molluscicide.

In all of our experiments (laboratory and field), we observed that most of the dead snails excreted their internal organs out of the shell and produced bad odour. Saponin kills snails by increasing the membrane permeability of the gills, thus leading to the losses of essential electrolytes and apoptosis effects which can cause uncontrolled cell death to the body system of the snail (De Geyter et al., 2007). This supported our findings. This compound can also cause hemolytic effects (Sidana et al., 2016) due to the effects on the blood circulation by destroying the blood cells of the snail. In the research by Yosef et al. (2011), the application of *F. Selloa* var marginata on *Biomphalaria alexandrina* snails reduced the levels of amino acid of the snail.

Measuring the effect of *F. gigantea* application on non-target organism

Catfish survival: All catfish survived in all replicates at all experimental plot 24 hours after the application. All catfish managed to swim away into the water canal after release.

Rice plant traits: All treatments showed no significant difference on chlorophyll content (F= 0.25, P=0.78) and shoot to root ratio (F= 0.83, P= 0.44). However, the rice plants that grew in the plot applied with *F. gigantea* cut leaves (Plot 2) had a higher plant height compared to the rice plants grown in the rice plot applied with commercial molluscicides (Plot 3) and untreated plot (Plot 1) (Figure 4). Similar to that, the plants grown in Plot 2 had higher plant dry weight compared to the plant grown in Plot 1 and Plot 3 (Figure 5). The results obtained showed that the application of *F. gigantea* cut leaves in the paddy field could enhance the plant growth by eliminating the damages caused by GAS.

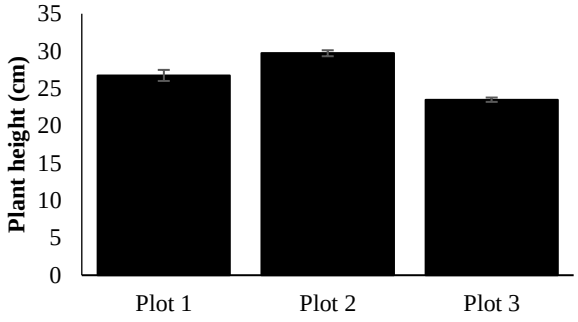

Figure-4. The height of paddy plant. Plot 1 is untreated plot, Plot 2 treated with *F. gigantea* cut leaves and Plot 3 treated with commercial molluscicide.

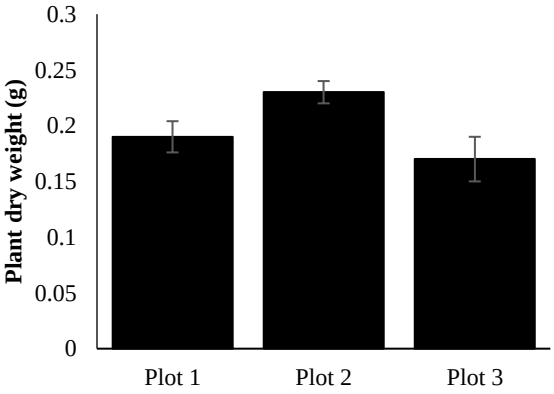

Figure-5. The dry weight of paddy plant. Plot 1 is untreated plot, Plot 2 treated with *F. gigantea* cut leaves and Plot 3 treated with commercial molluscicide.

The usage of plant-based biopesticide is not always rainbows and butterflies as always said. Some of the plant-based molluscicides were found to be not environmental friendly and they could harm the non-target organisms. For example, tea seed cake commonly used as a molluscicide is extremely toxic to fish and frog, and it can cause haemolysis in animals (Sin and Hamsein, 2017). It has also been found to reduce the growth of weed seedlings, early watergrass (*Panicum Crus-galli* L.), green foxtail (*Setaria viridis*

Beauv. L.) and white clover (*Trifolium repens* L.) (Kohata et al., 2004). The ethanolic and aqueous extracts of the leaves of *Agave cantala* and *Agave intermixta* were toxic to brine shrimps; and further investigations showed that the extract of *A. intermixta* could completely inhibit the cell division of *Allium cepa* after 24 hours of exposure (Sidana et al., 2016). The observations on the application of ethanol-water (7:3) extract of *Agave offoyana* on *Lactuca sativa*, *Lycopersicum esculentum*, *Lepidium sativum* and *Allium cepa* showed that it inhibited the root growth of those plants (Sidana et al., 2016).

In some conditions, the exposure to saponin might cause undesired side effects to aquatic organisms such as some species of fish (Jiang et al., 2018), and toxicity to cold-blooded animals (Cannon et al., 2004; Bhatia and Dahiya, 2015). However, a normal intake of the majority of saponins is not toxic to humans as evidenced by the fact that the saponin intake by vegetarians is in the range of 100 to 200 mg/day (Bhatia and Dahiya, 2015). In this research, we found that the application of *F. gigantea* cut leaves at the right amount in the paddy field is safe for the ecosystem, at least for the catfish and paddy plants.

Conclusion

F. gigantea is effective in killing GAS and safe for non-target organisms. When compared to the commercial synthetic molluscicide, *F. gigantea* managed to effectively kill 100% of GAS. Our field experiment showed that the application of 30 kilograms of *F. gigantea* in 0.5 ha of rice field did not alter the plant traits and was safe for catfish. However, further research should be done to investigate the side effects of the *F. gigantea* application on other non-target organisms, such as birds, beneficial insects, beneficial plants and frogs that use rice fields as their habitat. Detailed study should be done to evaluate the consequences of *F. gigantea* application before it can be applied commercially at a large scale. The results obtained from this study can be used to encourage researchers to formulate a plant-based, commercial molluscicides product that can be easily used by farmers.

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Contribution of Authors

Rejab MRM: Planned and managed the study, performed experiment, data collection, data analysis and wrote the manuscript

Manam NKA & Fauzi NS: Performed experiment, data collection and interpretation

Mohamed S: Interpreted data and wrote the manuscript

Ngah N: Conceived idea, designed research methodology, performed data analysis and wrote the manuscript