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Ecotoxicity of *Furcraea gigantae* leaves on non-target organism

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The use of raw toxic plant in controlling Golden Apple Snail (GAS) at paddy field has become an eco-friendly alternative to synthetic pesticide. This pest management technique could reduce the undesired environmental impacts and side-effects on human health. Recently, paddy farmers in Malaysia were encouraged to apply the *Furcraea gigantae* chopped leaves in controlling GAS infestation at paddy field. The result was satisfactory, but the effects of this toxic plant application on the non-target organisms have been little studied. This work intends to contribute with novel information on the effects of short-term exposures of *F. gigantae* chopped leaves application on paddy seeds germination, paddy plant traits and catfish mortality. The percentage of paddy seeds germination was reduced when exposed to the *F. gigantae* soaked water. The amount of chlorophyll content was increased, but the shoot:root, and height of paddy plant were reduced when grown in the tray contained *F. gigantae* chopped leaves. More than 50% of catfish population was dying when exposed to 30 g of *F. gigantae* chopped leaves in 1200 ml of water for 24 hours. Overall, the application of *F. gigantae* chopped leaves reduce the seeds germination, altered the plant traits and harmful for catfish if exposed to the high amount of *F. gigantae* leaves. These results highlight the need for a more detailed risk assessment on non-target organism during the development of any pest management techniques.

Keywords: *Furcraea gigantae*, Golden Apple Snail, non-target organism, pest management technique

INTRODUCTION

Golden Apple Snail (*Pomacea canaliculata)* is listed among 100 worst invasive species in the world (Lowe et al. 2000), which cause major problems at the paddy cultivation area in most Asian countries (Ranamukhaarachchi and Wickramasinghe, 2006). Since 2002 until 2008, Golden Apple Snail (GAS) have infested almost 20,000 ha of paddy growing areas in Malaysia, thus threatened the livelihoods of paddy farmers (Yahaya et al. 2017). Golden Apple Snail is difficult to control because of its good adaptability to the harsh environmental conditions, their high reproductivity rate and their ability to move through the drainage and irrigation system (Mokhtar et al. 2019).

In Malaysia, most of the paddy farmers applied the Integrated Pest Management (IPM) methods that cover cultural techniques, biological control and the usage of molluscicides to control the GAS infestation. The cultural techniques includes the usage of plant attractant such as pineapple, papaya and cassava leaf to easy hand pick the snail, control the water level to avoid the dispersion of the GAS, and the usage of duck and fish to eat the young snail (Su Sin, 2006; 2001). One of the new techniques in controlling GAS at paddy field is the application of toxic plant. This technique was encouraged by the agriculture authority of Malaysia as toxic plant has greater biodegradability, cheaper compared to the synthetic molluscicides, does not pose any hazard to farmers and for the environment (Taguiling, 2015). Toxic plants such as *Sandorium vidalii* (Ngaloy and Andrada, 2005), *Sandoricum Koetjape* (Taguiling and Buyuccan, 2009) and *Furcraea selloa* (Ramli et al. 2017) were commonly used to control GAS at paddy field. These plants contains saponins compound that toxic to the GAS.

Furcraea plant which belongs to family Agavacea is known to have rich steroidal saponins such as furostanol saponin, furcreafurostatin and spirostanol saponins (Ramli et al. 2019); that are capable in killing GAS (Calle et al. 2016; El-Sayed et al. 2006). Saponins are naturally occurring plant glycosides which form a soapy lather with water; it is consist of a sugar moiety and an aglycone unit. Plants that contains saponins is organic and does not pose any hazard to farmers and the environment (Taguiling, 2015). However, saponins is also reported to have diverse biological properties such as haemolytic properties and high toxicity to most cold- blooded animals (Sparg et al. 2004). Therefore, instead of its benefit to control pest, saponins also might cause the undesired side-effects to the aquatic organisms (Jiang et al. 2018).

The effectiveness of Furcraea plant is undeniable. Research shows that the application of Furcraea powder at paddy field is effective to reduce the infestation of GAS (Ramli et al. 2017, 2019). Research by Mokhtar et al. (2019) stated that the survival rate, growth rate and egg laying capacity of GAS was decreased when the time of exposure to Furcreae saponin increased. Even though it is impressing to find out that Furcraea plant can be used as part of pest management program in controlling GAS, there is no research was done to investigate the consequence effect of the application of this plant material to the non-target pest. Thus, this study was designed to investigate the ecotoxicity of *F. gigantae* leaves on the non-target pests; which are the paddy plant and catfish in the control environment. The objective of this study is to investigate the toxicity effect *of F. gigantae* leaves on paddy seeds germination, paddy plant traits and catfish mortality.

MATERIALS AND METHODS

Study system

Matured and full-grown leaves of *F. gigantea* were collected from the garden area at the Universiti Sultan Zainal Abidin (UniSZA) Gong Badak Campus, Terengganu Malaysia (5°23'50.3"N 103°04'45.7"E). Leaves were brought to the Toxicology Laboratory, UniSZA Besut Campus on the day it is collected and were chopped into small pieces. The leaves preparation was done a day before the experiment started to guarantee the freshness of the leaves.

The certified paddy seeds variety MR220CL1 were purchased from the agriculture store at Besut Terengganu Malaysia. Seeds germination test was done before experiment and the result obtained showed that the germination rate of the paddy seeds is 98%. Only fertile seeds were used for the experiment.

The catfish (2-inch size) were bought from the catfish breeder at Besut Terengganu Malaysia a week before the experiment started. Catfish were kept in the aquarium at the Toxicology Laboratory, supplied with oxygen and fed with fish food pellet (Star Feed, Malaysia) as required until the day of experiment. The condition of catfish were checked daily and before the experiment started; the weak catfish were discarded and only healthy catfish were used for the experiment.

Experiments were done in the Toxicology Laboratory, UniSZA at the ambient temperature (25-30°C) and humidity (80-90%). The experimental organisms were exposed with 12 hours dark and 12 hours light.

Assessment of ecotoxicity effect of *F. gigantae* on paddy seed germination

Approximately 10 g, 20 g and 30 g of *F. gigantea* chopped leaves were soaked in 1 litre of dechlorinated tap water in respective containers for 24 hours. After that, 20 ml of *F. gigantae* soaked water from those containers and 20 paddy seeds were placed into the respective petri dishes (size 100 mm × 15 mm). The petri dish containing 20 ml dechlorinated tap water and 20 paddy seeds were made as control. All treatments were replicates 10 times. The germinated paddy seeds were counted every 24 hours until all seeds emerge which took 6 days.

Assessment of ecotoxicity effect of *F. gigantae* on paddy plant traits

Plastic trays (50 cm x 25 cm x 10 cm) contained 1 kg of paddy mud soil collected from Kampung Oh Besut Terengganu (5°39'54.1"N 102°31'58.3"E), 1200 ml of dechlorinated tap water and 20 g, 40 g, 60 g, 80 g and 100 g of *F.*

gigantae chopped leaves were prepared at the laboratory. Two hundred of paddy seeds were sowed into each trays. Control treatment was the paddy plant grown in the tray without *F. gigantae* chopped leaves. The emerging plants were checked daily and supplied with dechlorinated tap water as required. Plants were let to grow in the trays for 21 days after seed sowing process. Each treatment was repeated for ten times.

After 21 days, the chlorophyll content of paddy plant leaves was measured by using the handheld chlorophyll content meter (SPAD 502 Plus Chlorophyll Meter, Japan)(Mendoza-Tafolla et al., 2019). After that, five plants were harvested randomly from each replicates, and washed through the running tap water to remove all the soil and dirt. The height of the plant was measured from the base of the plant to its highest point using calliper (Vernier Caliper N15R, Japan). Plants then were dried in the drying oven (Memmert U Universal Heating Oven, German) until it reaches the constant weight. The dry weight of shoot and root of the plant was measured separately by using scientific balance (RADWAG AS 220 R2, Poland). The shoot root ratio was counted by dividing the dry weight of shoot per the dry weight of root.

Assessment of ecotoxicity effect of *F. gigantae* on the catfish survival

Approximately 10 g, 20 g and 30 g of *F. gigantae* chopped leaves were added into the respective containers containing 1200 ml dechlorinated tap water with 10 catfish. Each treatments were repeated for ten times. After 24 hours, the number of dead and alive catfish was counted and recorded.

Statistical analysis

Data obtained were analysed using R statistical software version 3.4.0 (R Core Team, 2017) . The plant traits data were transformed when necessary to meet the assumptions of normality and homoscedasticity. Significance of differences between means values were determined by using LSmeans and separation by post-hoc Tukey tests using treatments as explanatory variables. The data of catfish mortality was transformed to percentage. The exact amount of *F. gigantae* that can kill 50%, 90% and 95% of catfish was calculated by using GLM function with MASS package (family binomial).

RESULTS

Assessment of ecotoxicity effect of *F. gigantae* on paddy seed germination

The percentage of paddy seeds germination in all treatment are significantly differed ($F= 186.889$, $P < 0.001$). The time taken by the seeds to emerge also differed between treatments ($F= 500.762$, $P < 0.001$). Our important finding is the percentage of paddy seeds germination were reduced when exposed to the soaked water from the containers contained *F. gigantea* chopped leaves (Figure 1). The higher the amount of *F. gigantae* chopped leaves soaked in the water, the lower the paddy seeds germination percentage. In the normal condition (0 gram of *F. gigantae* chopped leaves), paddy seeds only took 2 days to achieve 60% of germination. However, the seeds exposed to 10 g and 20 g of *F. gigantae* chopped leaves soaked water took at least 4 days to achieve at least 50% of germination. On the other hand, at least 6 days are needed for the seeds treated with 30 g of *F. gigantae* chopped leaves soaked water to germinate at least 50%.

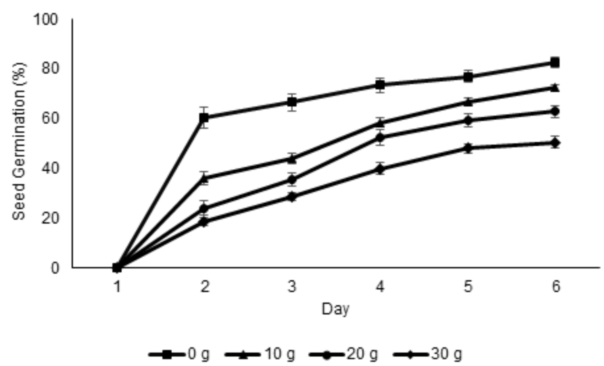

Figure 1: The percentage of seeds germination in the *F. gigantae* soaked water

Assessment of ecotoxicity effect of *F. gigantae* on paddy plant traits

Result obtained shows that the amount of chlorophyll content ($F= 14.14$, $P < 0.001$), dry weight ($F= 3.65$, $P < 0.001$), shoot:root ($F= 8.51$, $P < 0.001$) and plant height ($F= 9.59$, $P< 0.01$) are differed between treatments. Overall, the application of *F. gigantae* chopped leaves enhanced the amount of chlorophyll content, but reducing the plant height and shoot:root of paddy seedlings. However, this result is not so obvious on the plant dry weight (Figure 2).

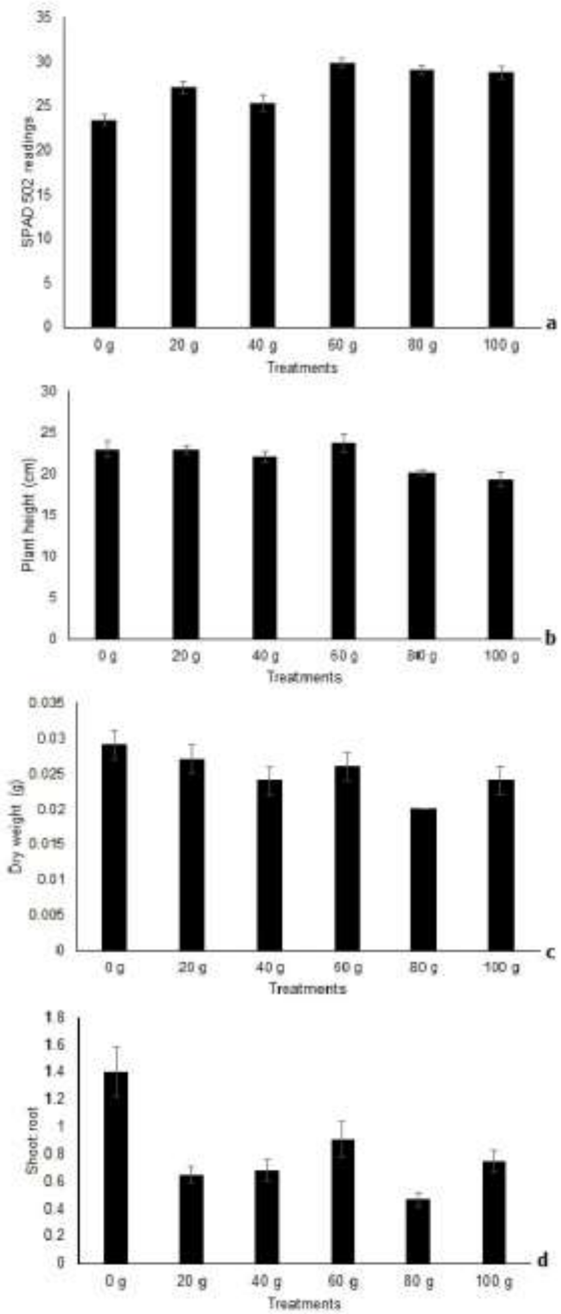

Figure 2: Plant traits of paddy; a: SPAD 502 readings represented the amount of chlorophyll content in plant leaves, b: plant height (cm), c: plant dry weight (g), d: shoot:root of the paddy plants.

Assessment of ecotoxicity effect of *F. gigantae* on the catfish survival

The catfish mortality are significantly differed among treatments (F= 6.52, P < 0.005). There is no significant difference was observed on the catfish mortality in containers without *F. gigantae* chopped leaves and the containers contained 10 grams of *F. gigantae* chopped leaves. Result shows that the *F. gigantae* chopped leaves is harmful to catfish when the amount of leaves is more than 10 grams. The higher the amount of *F. gigantae* chopped leaves, the higher the mortality percentage of catfish.

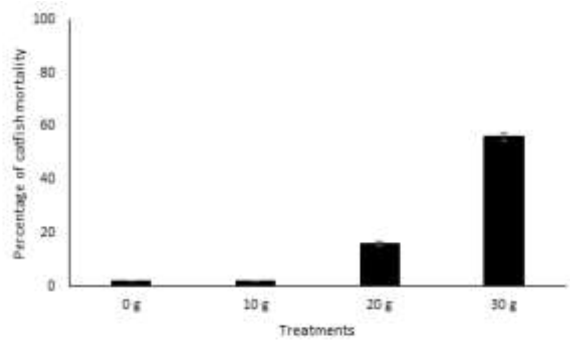

Figure 3: Percentage of catfish mortality

Our findings shows that the application of 28.69 g of *F. gigantae* chopped leaves in 1200 ml of water can kill at least 50% of catfish population in the control environment 24 hours after exposure. On the other hand, the mortality of catfish was achieved 90% after expose to 48.55 g of *F. gigantae* chopped leaves and achieved 95% at 58.06 g of *F. gigantae* chopped leaves (Table 3).

Table 1: The amount of *F. gigantae* needed to kill 50%, 90% and 95% of catfish population

	Amount (g)	LCL (g)	UCL (g)
LD50	28.69 ± 1.06	25.50	32.28
LD90	48.55± 1.16	36.22	65.07
LD95	58.06± 1.20	40.38	83.48

*LD50, LD90 and LD95 represent the amount of *F. gigantae* chopped leaves that can kill 50%, 90% and 95% of catfish population respectively.

DISCUSSION

In this study, the ecotoxicity effect of *F. gigantea* chopped leaves application on paddy seeds germination, paddy plant trait and catfish mortality were determined. Overall, the *F. gigantae* chopped leaves soaked water was found gave negative effect on the paddy seed germination, altering paddy plant traits and cause mortality to the catfish. This ecotoxicity effect might be influenced by the saponin content in the *F. gigantae* chopped leaves soaked water. *Furcraea gigantae* contains bisdesmosidic furostanol saponin and spirostanol saponin (Da Silva and Parente, 2006). When soaked in the water, the saponin in the chopped leaves will be extracted and dissolve (Rao et al. 2016; Mustapha and Harun, 2015).

Saponin has the allelopathic effect that can significantly reduce seed germination rate and alters the plant traits (Ghimire et al. 2019). As an example, the extraction of *Leucaena leucocephala* leaf contributed by mimosine effect reduce the seed germination, root growth, hypocotyl growth and fresh weight of *Ageratum conyzoides, Tridax procumbens* and *Emilia sonchifolia* (Ishak et al. 2016). The seed germination of wheat was reduced when exposed to *Astragalus mongholicus* root extract (Mao et al. 2006). The *Furcraea hexapetala* leaves extract give a detrimental effect on the growth of *Lactuca sativa* root (Calle et al. 2016). The allelopathic effect of these plants were influence by its saponin compound.

Our result shows that the strength of allelopathic effects of *F. gigantae* leaves is depended on the amount of chopped leaves soaked in the water or put in the experimental containers. The higher the amount of *F. gigantae* chopped leaves used in the treatment, the higher saponin concentration dissolve in water; thus cause the greater effect on the seeds germination and plant traits.

The application of *F. gigantae* chopped leaves is harmful to the catfish if used in the high amount. Again, this negative effect on catfish survival might cause by the amount of saponin release by the plant in the soaked water. Previous research found that the irreversible toxic effect of saponin on fish occurs at its 2.0 mg/L concentration (Grib et al. 2006). Unfortunately, in this experiment, we did not quantify the amount of saponin released by the plant in the soaked water; as our intention was to evaluate the effect of current pest management technique applied by the paddy farmers. As note, the amount of *F. selloa* applied by farmers is approximately 60kg/ hectare (Amzah, 2013). However, we found that the immediate toxic reaction exhibited by the catfishes when exposed to the container contained 30 grams of *F. gigantae* chopped leaves includes gulping for air, erratic swimming and loss of reflex. After 24 hours, some fish shows slow opercular movement and some others are settling at the bottom motionless and having discolouration.

Long ago, the usage of saponin is common in controlling the predacious fishes in shrimp pond (Tang, 1961). The soft skin organisms such as worms, snails and fish embryos are sensitive to saponins (Jiang et al. 2018). Saponins also found to be significantly functioning as anti-feedants, and affect the growth and reproduction in lower animals (Sami and Shakoori, 2014).

CONCLUSION

These findings explain the important of the usage of toxic plant at appropriate amount in controlling GAS. Even though the application of *F. gigantae* chopped leaves is effectives in controlling GAS, it also important to make sure that pest management technique is safe and not harming the non-target organism. The understanding on the concept of Good Agriculture Practices will help farmers to control GAS infestation effectively, save the cost of production and keep the environment safe. Results of this study may benefit the Department of Agriculture Malaysia to draw rules and regulation for *F. gigantae* application as molluscicides. Further research should be done to investigate the side effect of the *F. gigantae* application on the other non-target organism such as birds and frog that make paddy field as their habitat.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

MRMR, NKAM and NSF conducted the experiments. NN conceived and designed research. NN, MRMR and SM analysed the data. MRMR wrote the first draft of the manuscript. The manuscript was revisited and edited by NN and SM. All the authors read and approved the manuscript.

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