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Potential Control of *Pomacea canaliculata* Using Botanical Extracts in Paddy Field

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Abstract

One of the major paddy pests is the invasive apple snail, *Pomacea canaliculata*. The common control method to reduce the invasion of apple snail is by using chemical pesticides. However, this chemical pesticide could cause detrimental effects on human health and the environment. Currently, botanical pesticide is another safer way of replacing chemical pesticides. In this study, *Carica papaya* and *Peltophorum pterocarpum* leaves were extracted and analysed for the phytochemical compound and tested for toxicity effects against the apple snail under field conditions. Results from the phytochemical screening test displayed high content of saponin and flavonoid in *C. papaya* and *P. pterocarpum* leaves. *P. pterocarpum* leaf extracts showed the highest percentage mortality of snails at 98.33% after 96 hours of treatment application. The pH water and water conductivity test also showed a positive relationship between plant extracts and water quality. Thus, *P. pterocarpum* extract has a high potency as an eco-friendly pesticide in controlling the invasive apple snails which can be used to replace chemical pesticides.

Disciplinary: Crop Protection, Agriculture.

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1 Introduction

Rice is one of the major staple foods in the world. About 60% of the world population consumes rice and Malaysia is among the Asian countries that are growing and producing its own paddy (Massaguni & Latip, 2015). Invasion of alien species snails has caused major paddy losses which in turn reduce rice production in Malaysia. The apple snails can feed a rice blade in less than 5 minutes with their rough tongue which is used to scrape on plant surfaces (Massaguni & Latip, 2015). The ability to reproduce rapidly in a short time is the main reason the apple snails have become a major pest to paddy fields (Arfan et al., 2015).

Several control methods have been adapted in the paddy field to reduce the infestation of the invasive apple snails such as cultural and biological but less preferred by farmers due to the management cost. Farmers frequently used synthetic pesticides because they can help to increase rice production and effectively control the apple snails. However, synthetic pesticides could also become detrimental to humans, the environment, and non-target organisms such as natural predators and beneficial plants (Kamatenesi-Mugisha et al., 2013). Molluscicide such as niclosamide can be harmful to the paddy ecosystem due to their toxic effects on aquatic animals such as fish. An excessive number of synthetic pesticides could lead to the development of residues in the soil.

The alternative use of botanical pesticides to reduce the invasive apple snails' damages is safer and less harmful in comparison to chemical pesticides. Botanical pesticides are formulated from natural active compounds of selected plants such as phenols, terpenes, flavonoids, alkaloids, and saponins. These plant chemicals are also known as secondary plant metabolites which can act as repellent, antifeedant, and insect growth regulators to control insect infestation. The presence of biologically active compounds reveals the ability of *Carica papaya* and *Peltophorum pterocarpum* to be used as botanical pesticides. The leaf of C. *papaya* is reported to contain alkaloids, saponin, and flavonoids which highly possess pesticide effects against pests and diseases for plant defense mechanisms. *P. pterocarpum* leaf also contains tannin, terpenoid, alkaloid, steroid, saponin, and flavonoid (Jiménez et al., 2014). However, most of the studies conducted were based on laboratory experiments and analysis. It is important to conduct a field study to further examine the laboratory result under field conditions. One of the aspects that need to be considered in field study is the effects of botanical pesticides on water quality. In this study, pH water and water conductivity are evaluated to understand the effects of selected plant extracts on paddy water. Most rice paddy can survive in soil pH between 4.5-6.0 (Sys, 1985). Water conductivity can act as an indicator of water quality problems. Therefore, this study is conducted to quantify the phytochemical contents of *C. papaya* and *P. pterocarpum* that are responsible for controlling the apple snails, and to evaluate the feeding deterrence activity of the plant extracts towards the apple snails under field conditions.

2 LITERATURE REVIEW

2.1 *Pomacea canaliculata* (Golden Apple Snail)

The scientific name for the golden apple snail is *Pomacea canaliculate*. The flexibility in feeding is believed to be the key feature for the invasive success and impact of apple snails as crop pests (Oosterom et al., 2016). The apple snail is a voracious feeder (Naylor, 1996) compared to other ampullariids and due to this feeding habit, quarantine implementation is clearly relevant (Cowie, 2005). It can consume 7 to 24 rice seedlings per day and mostly attack young stems and leaves of paddy which results in great damage to the paddy field (Salleh et al., 2012). In Malaysia, the apple snails caused a total estimated yield loss of RM 41,229,695 in 2009 during the main season. This value covered the main rice granary areas in Malaysia of about 197,279 ha of cultivated area, which included Muda Agricultural Development Authority (MADA), Kemudu Agricultural Development Authority (KADA), and Integrated Agricultural Development Authority (IADA).

2.2 *Carica papaya* (Papaya)

Carica papaya is a giant tropical herb that is widely grown in tropical areas for its fruit due to its high yield, nutritional value, functional properties, and year-round fruit production (Jiménez et al., 2014). *Carica papaya* is known for its medicinal value due to several parts of the plant that contains phytochemical compounds or also known as secondary metabolites (Vij & Prashar, 2015). The leaf has several secondary metabolites that enable the plant to protect itself from pests and diseases due to their pesticidal properties of alkaloids, flavonoids, saponins, steroids, and tannins (Akhila & Vijayalakshmi, 2015). These chemical compounds can act as repellents, antifeedants, and insect growth regulatory activities against pests, which are suitable to be used as a biopesticide. A study on *P. canaliculata* by Latip et al. (2016) showed that methanol extracts of *C. papaya* leaf can cause high mortality of the snails within 48 hours of treatment application, which suggested that the leaves of *C. papaya* possess the molluscicidal properties that can be used to control the molluscan pest.

2.3 *Peltophorum pterocarpum* (Yellow Flamboyant)

Peltophorum pterocarpum or yellow flamboyant can be classified as one of the medicinal plants due to the therapeutic values of their phytochemical compounds. The leaves of yellow flamboyant can produce several chemical compounds such as tannins, saponins, flavonoids, alkaloids, and phenols (Fernandes et al., 2015). Tannins and alkaloids have the properties of being toxic and repellent to insects while saponins can have the same action against insects and vertebrates. A study on the use of *P. pterocarpum* leaf extracts using ethanol and methanol showed a high mortality effect against apple snails within 96 hours of exposure on adult snails (Latip et al., 2015a). Another study also showed a similar result but it was tested on 2 weeks-old *Pomacea* hatchlings. The result showed that *P. pterocarpum* leaf powder is toxic to the apple snails with half the strength of commercial molluscicide (Ali, 2000).

3 Method

3.1 Apple Snails Sampling

The apple snails were collected from several paddy fields around Arau, Perlis, Malaysia based on the diagnostic standard by Cowie (2005) and laboratory trials. The apple snails were acclimated in a large tank at Malaysia Agricultural Research and Development Institute (MARDI), Seberang Perai, Malaysia, which were then labelled and measured by length (25-35mm). MR297 variety of paddy was transplanted in paddy field prior to the experiment after two weeks of sowing.

3.2 Plant Extraction

The leaves of *C. papaya* and *P. pterocarpum* were collected from their natural habitat in Arau, Perlis, Malaysia based on the recognition through Gilman and Watson (1994), and Jiménez et al. (2014), respectively. Soxhlet extraction was conducted following a protocol by Saad et al. (2014) using 95% methanol solvent. 10g of leaf powder was poured into a thimble and 100 ml of methanol solvent was added to a volumetric flask before the extraction. The Soxhlet extractor operated for 3 hours at 60°C to obtain the soluble extracts and was evaporated using a rotary evaporator for 60 minutes at 40°C to obtain the crude extracts. The crude extracts were kept in a freezer under dark conditions for further use. The plant extracts were diluted with distilled water to obtain a 50% concentration (50,000 ppm) before being applied in the field. Simple maceration was conducted by following the protocol of Khan et al. (2012). Fresh leaves of *C. papaya* and *P. pterocarpum* were shredded into small pieces before being soaked in a mixture of water and methanol according to a 1:1 ratio for three days (Wightman, 1999). The extracts were then filtered using a filter funnel and diluted with distilled water to obtain a 50% concentration before field testing.

3.3 Phytochemical Screening

The chemical compound in *C. papaya* was screened using standard methods of Sujatha et al. (2012). Flavonoids compound in selected indigenous plants was screened by using Alkaline Reagent Test. A few drops of sodium hydroxide solution were added to plant extracts. The formation of decolorised intense yellow colour into colorless after the addition of dilute acid indicated the presence of flavonoids compounds (Tiwari et al., 2011). Saponins compound in selected indigenous plants was determined through frothing tests. The formation of 1 cm of foam indicated the presence of saponins (Somkuwar & Kamble, 2013).

The chemical compound in *P. pterocarpum* was screened out using Ultraviolet-Visible (UV-VIS) spectrophotometer by following the protocol of Telange et al. (2014). The stock solutions of standard (rutin, kaempferol, quercetin, catechin, salicylic acid, and lupeol) were prepared by diluting them in methanol. The working solution of each standard was then prepared using the serial dilution technique and vortexed for a minute to mix the solution. The maximum absorbance (λmax) was measured at absorption maxima of 271.4 nm (rutin), 395 nm (kaempferol), 370 nm (quercetin), 290 nm (catechin), 320 nm (salicylic acid), and 285 nm (lupeol). The regression equation for the standard compound was measured. The extract was measured three times. The amount of each standard in the extract was calculated using the regression of each standard.

3.4 Field Testing

The field testing was conducted in a paddy field experimental plot at MARDI Seberang Perai. An experimental cage (25cm x 25cm x 65cm) was used during the experiment for data collection, which was labeled and embedded in the soil prior to transplanting the paddy. Four hills of paddy were transplanted in each cage of its own bed (area of 1m^2). Ten apple snails were added to each cage (10 snails/m^2) before the extracts were sprayed on the paddy. Bioassay's procedure by

San Martín et al. (2008) was followed accordingly using three replicates for each treatment (two types of plant extracts using two different methods) and controls (diluted methanol as negative control and fentin acetate as positive control). The mortality of snails was monitored and recorded every 24, 48, 72, and 96 hours after treatment application as well as the weight of the snails. The death of the snails was determined through their responses toward the needle probe (Kijprayoon et al., 2014). Water pH values and water conductivity values were measured to assess the quality of paddy water after treatment application. The samples of water were taken prior to the application of plant extracts and after 96 hours of treatment application. The water samples were tested by using pH metre and conductivity probe within 24 hours after sampling to reduce any reading error.

3.5 Data Analysis

All data were subjected to t-test and ANOVA tests and Tukey's Honestly Significant Difference (HSD). Probit analysis was also conducted to find the Lethal Time (LT_{50}) of the plant extracts. All the mortality data were corrected using Abbot's formula (Abbott, 1925) as follows:

$$\text{Corrected \%} = [1 - (\text{n in T after treatment} / \text{n in C after treatment})] \times 100 \qquad (1),$$

where n is the number of snails, T is the treated samples, and C is the control samples.

4 Result and Discussion

Phytochemical analysis in Table 1 and Table 2 shows methanolic extracts of *C. papaya* and *P. Ptrerocarpum* leaves contained saponin, phenolic acid, and flavonoid compounds. From the obtained results, it showed that saponin had the highest compound quantified in both plants compared to phenolic acid and flavonoids. By referring to Table 1, saponin was the highest (1.27%) compound quantified in methanolic extracts of *C. papaya* leaf, followed by phenolic acid (1.08%) and flavonoids (1.08%). Table 2 indicates that the saponin compound was also the highest (9.91%) compound quantified in *P. pterocarpum* leaf, whereas 9.58% of the flavonoid compound was quantified by 2.22% of phenolic acid. A study by Devi et al. (2017) stated that various chemical compounds can be found in methanolic extract of *P. pterocarpum* such as terpenoids, sterols, alkaloids, flavonoids, tannins, phenols, and glycoside.

Table 1: Phytochemical screening analysis of *Carica papaya*

Quantity of compound 100,000ppm of *Carica papaya*			
The yield of compound (%)	Saponin	Phenolic Acid	Flavonoid
	1.27%	1.08%	1.08%

Table 2: Phytochemical screening analysis of *Peltophorum pterocarpum*

Quantity of compound 100,000ppm of *Peltophorum pterocarpum*			
The yield of compound (%)	Saponin	Phenolic Acid	Flavonoid
	9.91%	2.22%	9.58%

Figure 1 shows the result of the bioassay test on apple snails for different treatments according to the different times of exposure. The highest mortality was caused by treatment PS (*P. pterocarpum* leaf extracted using Soxhlet extraction) at 98.33% ± 0.05, followed by treatment CS (*C.*

papaya leaf extracted using Soxhlet extraction) at 57.78% ± 0.23 after 96 hours of treatment application, whereas the lowest mortality was caused by treatment CM (*C. papaya* leaf extracted using simple maceration) at 8.33% ± 0.05 after 96 hours of treatment application. Mortalities of the snails can be seen to gradually increase across time for every treatment's application. This result suggested that treatment PS and treatment CS could produce a higher mean percentage mortality of apple snails over time. Treatment PS was comparable to treatment C+ (fentin acetate) at 100% mortality after 96 hours of treatment application, while treatment CS only demonstrated half of the percentage mortality as compared to treatment C+. This result indicated that the leaf extracts of *P. pterocarpum* can be used as botanical pesticides which can be as good as the commercialized pesticide. The R^2 values for all treatments showed the best fit as the R^2 value was closer to 1. This result indicated that the data had a high relation of regression model which showed that the data were precise with less error.

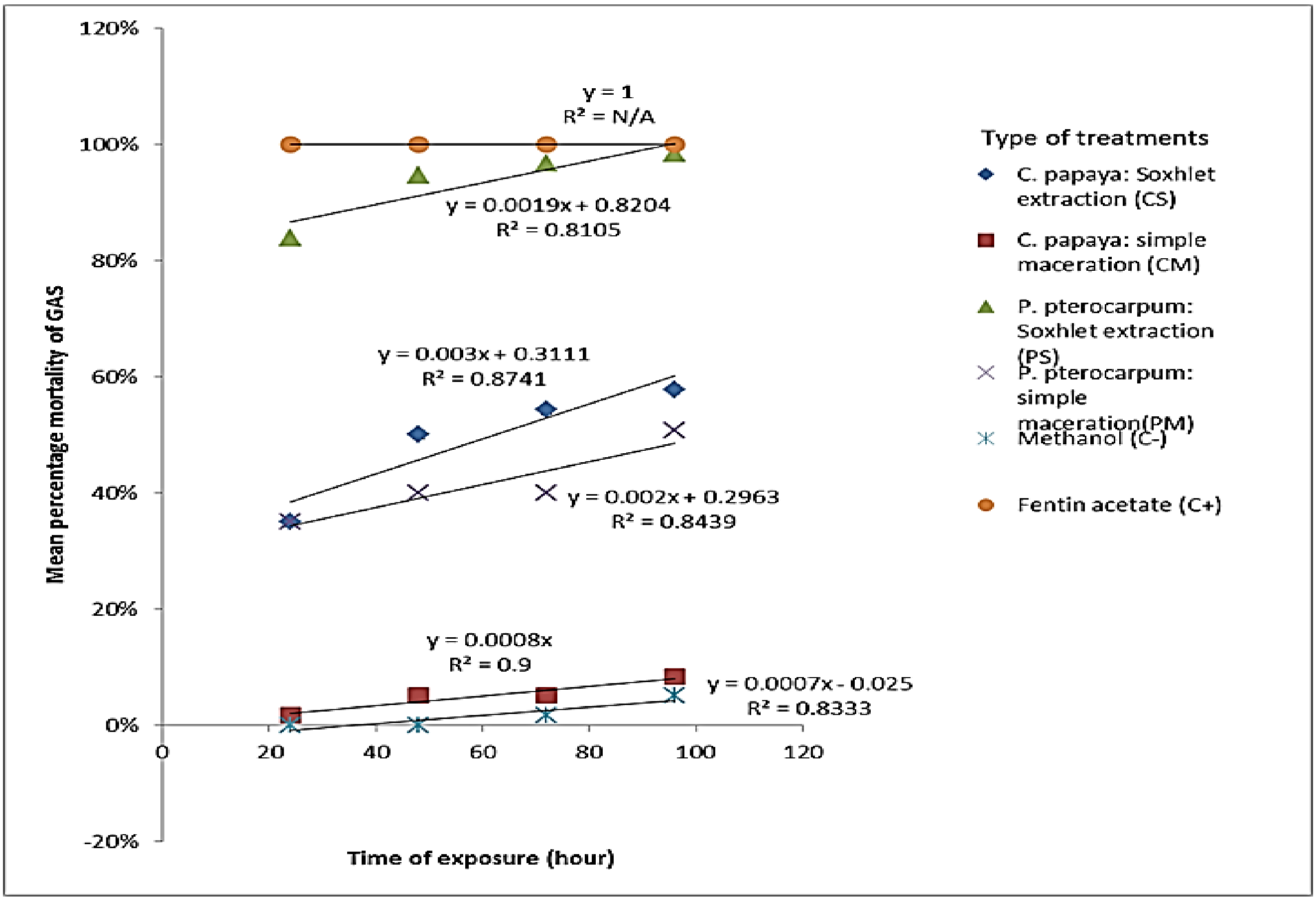

Figure 1: Relationship between mean percentage mortality of snails (%) and time of exposure (hour) for different types of treatment

A phytochemical screening test showed a high content of saponin and moderate content of flavonoid in the leaf of *C. papaya* (Latip et al., 2017), while a similar test on *P. pterocarpum* showed a high content of flavonoid and alkaloid (Satapathy & Swamy, 2012). A study from Latip et al. (2017b) proved that *C. papaya* leaf extracted using Soxhlet extraction with methanol solvent gave mean mortality of 5.64 after 96 hours of application at 50 g/L. Meanwhile, *P. pterocarpum* leaf extracted using Soxhlet extraction with methanol solvent gave 100% mortality of snails after 96 hours of application at 100% concentration (Latip et al., 2015a). Nevertheless, a high percentage of mortality was still achieved by *P. pterocarpum* leaf extracted using Soxhlet extraction which was comparable to the regular pesticide (fentin acetate). This result suggested that *P. pterocarpum* leaf has potent toxicity effects on the invasive apple snails as compared to *C. papaya* leaf.

Probit analysis results and the LT_{50} values for all the treatments applications are presented in Table 1. LT_{50} shows the time taken to kill 50% of the population tested. Based on the result, the lowest LT50 was achieved by treatment PS (20.43 hours) whereas the highest LT_{50} was from treatment CM (145.46 hours) which means that the apple snails were more sensitive toward *P. pterocarpum* leaf extracts compared to *C. papaya* leaf extracts. This result suggested that *P. pterocarpum* leaf possesses high toxicity effects on the apple snails as it can kill the snails within a short amount of time. A study by Latip et al. (2015b) demonstrated that the leaf of *P. pterocarpum* extracted using methanol solvent showed the lowest LC_{50} at 4.68% compared to the same leaf extracted using ethanol solvent. The LT_{50} and LC_{50} decrease with an increase of mean exposure concentration and times, respectively (Mohammad et al., 2011).

Table 3: Probit analysis for toxicities of *C. papaya* and *P.pterocarpum* leaves extracts toward the apple snails

Treatments	LT_{50} (Hours) 95% CI	Slope ± SE
C. papaya leaf using Soxhlet extraction (CS)	67.09 (42.38 – 126.85)	0.016 ± 0.006
C. papaya leaf using simple maceration (CM)	145.46 (97.65 – 193.28)	0.023 ± 0.018
P. pterocarpum leaf using Soxhlet extraction (PS)	20.43 (9.71 – 30.70)	0.068 ± 0.019
P. pterocarpum leaf using simple maceration (PM)	84.56 (54.60 – 414.14)	0.012 ± 0.006

Note: LT – Lethal time; CI – Confidence interval.

Figure 2 shows the comparison of water conductivity values for different treatments. There was an increase in the value of water conductivity after 96 hours of treatment application for all treatments. The highest value of water conductivity was indicated by treatment PS at 2301µS/cm ± 185 after 96 hours of treatment application while the lowest value was from control treatment C- at 1386 µS/cm ± 44. The change in water conductivity value is a good indicator of water quality monitoring (Pal et al., 2015). In this study, the increase in the value of water conductivity might be due to the temperature change during water sampling. Higher temperatures will result in higher water conductivity levels (Langland & Cronin, 2003). Rice can grow in an optimum state of water conductivity level below 2000 µS/cm (Sys, 1985).

The comparison of water pH values for different treatments is presented in Figure 2. The result indicated that the pH value of water increased after 96 hours of exposure time for all the treatments. The highest pH value of water after the treatment's application was displayed by control treatment C+ (7.24 ± 0.04) while the lowest value was shown by treatment PM (6.98 ± 0.09). Even though there was a significant difference between the pH values of water before and after the treatment's application (p-value = 0.0011), the values still fluctuated within the same range (6.0 –

7.5). These pH values are suitable for the survival of other aquatic insects as pH below 6.0 or above 8.0 is unfavorable for the reproduction of most aquatic insects (McCaffrey, 2013). The absorption of dissolved carbon dioxide during photosynthesis of aquatic plants might have changed the pH values of water (Ito, 1987). However, a suitable soil pH range for optimum growth of the paddy plant is between 5.5-7.5, which can be largely influenced by soil properties (Sys, 1985).

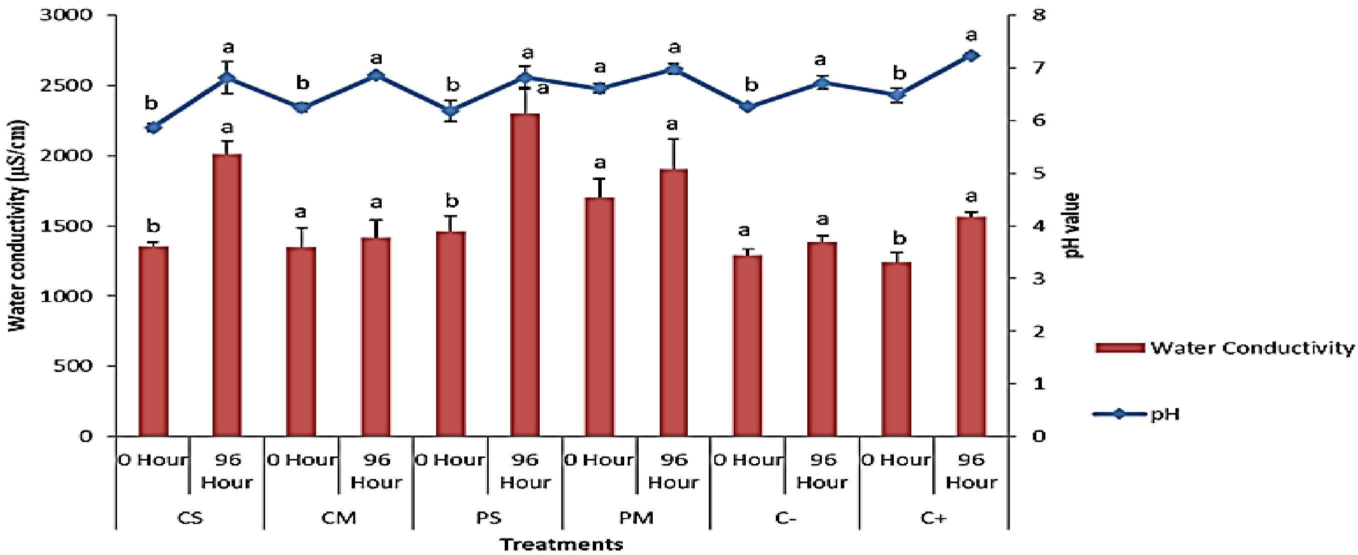

Figure 2: Comparison of water conductivity and pH values of water before (0 hours) and after (96 hours) the application of different treatments

Note: The different letters for each treatment between 0 hours and 96 hours are significantly different by Tukey's HSD tests at $p < 0.05$. Data are means of three replicates. CS: *C. papaya* leaves using Soxhlet extraction. CM: *C. papaya* leaves using simple maceration, PS: *P. pterocarpum* leaves using Soxhlet extraction, PM: *P. pterocarpum* leaves using simple maceration, C-: Methanol, C+: Fentin acetate.

5 Conclusion

The leaf of *P. pterocaprum* has the potent effects to be used as botanical pesticides against the invasive apple snails utilizing toxicity compared to *C.papaya* leaf. *Peltophorum pterocarpum* leaf can be best used as molluscicide as it can cause high mortality effects (98.33%) within a short amount of time. The phytochemical analysis also showed that high saponin (9.91%) and flavonoid (9.58%) contents in *P. pterocarpum* leaf extracts are the chemical compounds responsible for this mode of action. On the other hand, *C. papaya* leaf extracts might possess the feeding deterrence ability as suggested by other literature. The test on water quality also showed a promising result, where there is only a slight change in pH of water between 7.0 to 7.5, and 1400 to 2300 µS/cm for water conductivity. Nevertheless, the values are still within the safer range for the paddy environment. The result suggested that botanical pesticides can be another alternative use of chemical pesticides in reducing their harmful effects on humans and the environment. Therefore, further tests on water quality and other aquatic organisms are recommended for it to be feasible to be used by farmers. To attain the forecasted rice output by 2020, government bodies should provide the necessary support to acknowledge this alternative method towards sustainable rice production using ecologically safe methods.

6 Availability of Data And Material

Data can be made available by contacting the corresponding author.

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