Malaysian Applied Biology (2023) 52(4): 113-118
https://doi.org/10.55230/mabjournal.v52i4.d044

Research

Effects of Selected Plants Against Rice Weevil (*Sitophilus oryzae*)

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ABSTRACT

Rice weevils (*Sitophilus oryzae*) have caused significant damage and losses in rice storage. The use of chemical pesticides to control them has had negative environmental effects and limited efficacy. To address this issue, natural plant-based insect-control alternatives were investigated. This study aimed to assess the effectiveness of (*Pandanus amaryllifolius*), kaffir lime leaves (*Citrus hystrix*), and “asam gelugor” (*Garcinia atroviridis*) in repelling, inhibiting feeding, and reducing the offspring of rice weevils. Plant samples were extracted using the soxhlet extraction method, and the crude extracts were concentrated using a rotary evaporator. The extract solutions were then tested for their effects on rice weevils. The results showed that pandan leaves were the most effective, with a repellency percentage of 46.67% and a better anti-progeny effect of 2.175%. Kaffir lime leaves had no effect on rice weevils, while asam keping only showed an anti-progeny effect of 6.525%. The study revealed that low concentrations (0.002 - 0.006 g/mL) of insecticides from pandan extracts could provide a repellency effect against rice weevils. Therefore, plant extracts from Pandanus can be used as botanical insecticides to manage *S. oryzae* infestations.

Key words: Antifeedant, anti-progeny, plants, repellency, rice weevil

Article History
Accepted: 24 July 2023
First version online: 31 October 2023

Cite This Article:
Mohd Arafah, N.S., Jun, C.Y., Mohamed Shariff, S.S., Mohd Noor, N.Q.I., Zaharudin, N. 2023. Effects of selected plants against rice weevil (*Sitophilus oryzae*). 2023. Polycaprolactone/cellulose acetate loaded *Psidium guajava* essential oil electrospun nanofibrous mat dressing for healing wounds. Malaysian Applied Biology, 52(4): 113-118. https://doi.org/10.55230/mabjournal.v52i4.d044

INTRODUCTION

Rice is a widely consumed cereal grain and is the staple food for many people in Asian countries. According to (Devi *et al*., 2017)., it was the third-highest-produced agricultural crop globally in 2014 (741.5 million tonnes), after sugarcane (1.9 billion tonnes) and maize (1.0 billion tonnes). The quality of rice is closely monitored to ensure that it is maintained at an optimal level, given its importance as a primary food source. However, even with strict quality control measures, some rice weevils or their eggs may be present in rice packages after storage and processing, leading to their multiplication into adulthood and subsequent generations.

Rice weevil, which was also known as *Sitophilus oryzae* was one of the most serious stored grain pests worldwide. They were usually found in grain storage and processing plants. The insect is about 2 - 3 mm in length, stout in appearance, reddish-brown to black, and has four light yellow or reddish spots on the corners of its elytra, which are hard protective forewings (Koehler, 1999). The life cycle of the rice weevil consists of four stages: egg, larva, pupa, and adult, which can be completed in 28 days. When threatened, rice weevils may pretend to be dead by drawing their legs close to their bodies and remaining still.

Rice weevils cause damage to rice by chewing a hole into a seed or grain kernel to deposit eggs inside and then sealing up the opening to leave those eggs behind. When the eggs hatch inside the grain or seed, the larvae feed on the rice until they are fully grown, and the adult weevils eat their way out of the grain or seed, resulting in significant damage and loss of rice quantity and quality.

To control the infestation of rice weevils, several types of chemical pesticides and insecticides such as methyl bromide (PH_3) and phosphine (PH_3) have been widely used in the storage of grain and processing plants. However, the negative effects of methyl bromide and the limitations of phosphine have been discovered (Negahban *et al*., 2006).

Therefore, the development of new natural insect-control alternatives with natural plants was necessary. Natural plants are a rich source of bioactive metabolites that exhibit toxicity, repellency, antifeedant, progeny, and fumigation effects in a wide range of insects and are believed to be safe for the environment and have medicinal properties for humans with low toxicity to mammals (Khani *et al*., 2011). This study was initiated to analyze the repellency, antifeedant, and anti-progeny effect of Malaysian plant extracts against rice weevils and to observe the most effective plant against rice weevils.

MATERIALS AND METHODS

Preparation of raw materials

Fresh pandan leaves (*Pandanus amaryllifolius*), kaffir lime leaves (*Citrus hystrix*), and "asam gelugor" (*Garcinia atroviridis*) were purchased from Tunas Manja Group (TMG) market in Kuantan, Pahang. All of the plant samples were removed from their package and washed under the flowing tap water. The water droplets that remained on the plant sample surfaces were shaken off. After washing, the plant samples were cut into small pieces (0.5 - 1.0 cm) by using a pair of scissors distributed uniformly as a thin layer on several trays and dried in an oven. The temperature of the oven was adjusted to 50 °C and the drying process of pandan leaves and kaffir lime leaves was carried out for 24 h while the drying process of asam keping was carried out for 48 h.

After the drying process, the plant samples were pulverized into powder form by using a large blender followed by a small blender. The plant's powder was sieved by using a sieve with a mesh size of 18 (1 mm) to remove large and unblended plant samples. The fine powder of pandan leaves, kaffir lime leaves, and asam keping were stored in 250 mL laboratory glass bottles and kept in the refrigerator at -4 °C.

Preparation of plant extract

A mass of 20 g of plant powder and 250 mL of acetone were used in a soxhlet extractor to extract volatile compounds from the plant samples. Soxhlet extracted was operated for 1 day at a temperature of 60 °C. After that, the plant extract was cooled down for 1 h and transferred into a rotary evaporator to obtain crude plant extract at operating conditions of 50 °C and 200 r.p.m. The crude extract obtained was then dissolved in acetone to form a plant extract solution with different concentrations.

Preparation of rice

The white rice was removed from the package and placed evenly on several trays. The trays were then put into the incubator and the temperature of the incubator was adjusted to 60 °C and the rice was sterilized for 24 h (Devi *et al*., 2014). After that, the sterilized rice was stored in 1000 mL laboratory glass bottles.

Rearing of rice weevil

The rice weevil was reared in a 1000 mL laboratory glass bottle that contained whole rice grain at 28 ± 2 °C in the incubator (Khani *et al*., 2011).

Testing on repellency effect

A piece of filter paper was divided into two equal parts, one act treated part while the other was the control part. The treated part with plant extract was air-dried in a fume hood to evaporate the excess solvent. Both filter papers were put inside the same petri dish with a diameter of 9 cm and 10 rice weevils were distributed in the middle of the petri dish. The number of rice weevils in the treated (NT) and control part (NC) was recorded after 3 h. The percentage of repellency (%R) was calculated by using Equation 1:

$$\%R = [(NC-NT)/(NC+NT] \times 100\%$$

Testing on antifeedant effect

Three small pieces of filter paper were prepared and applied with plant extract. The filter papers were then air-dried in a fume hood to evaporate the excess solvent. Both treated and control rice consisted of 150 g of rice and 20 rice weevils were placed in the petri dish and kept at room temperature (±28 °C). The difference was the treated part consisted of three filter papers with plant extract located at the bottom, middle, and upper part of the rice. The initial weight of rice (IW) was recorded. The rice weevils were allowed to feed on the rice for 4 weeks. The percentage of weight loss (%W) was calculated.

Testing on anti-progeny effect

Three small pieces of filter paper (diameter 4.7 cm) were prepared and applied with plant extract. The filter papers were then air-dried in a fume hood to evaporate the excess solvent. Both treated and control rice consisted of 150 g of rice and 20 rice weevils. The difference was the treated part consisted of three filter papers with plant extract located at the bottom, middle, and upper part of the rice. The rice weevils were allowed to survive on the rice for 4 weeks. The number of rice weevils on treated (NT) and control parts (NC) was recorded after 4 weeks. The percentage of Abbott-corrected mortality (%M) was calculated.

Statistical analysis

Data were expressed as mean ± SD. The GraphPad Prism 5.0 software for Windows (GraphPad Software, California, USA) was used to perform one-way fixed effects ANOVA and Tukey's tests (significance at $p<0.05$) for the assays of antifeedant activity.

RESULTS AND DISCUSSION

Repellency effect

The purpose of this experiment was to evaluate the repellent properties of Malaysian plants, namely pandan leaves, kaffir lime leaves, and "asam gelugor," against rice weevils (*Sitophilus oryzae*). The results of the experiment, presented in Figure 1, demonstrate the percentage of repellency at different concentrations of the plant extracts.

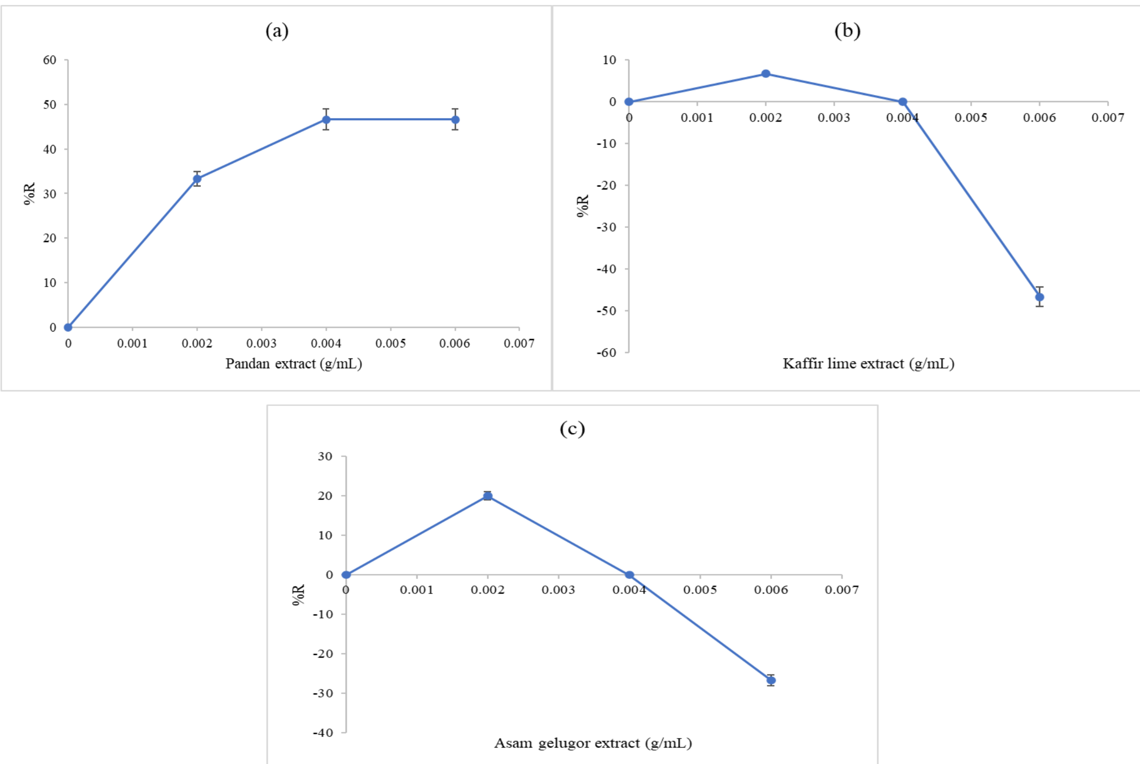

Fig. 1. Percentage of repellency (%R) of (a) pandan extracts, (b) kaffir lime extracts, and (c) “asam gelugor” extracts at different concentrations (0.002, 0.004 & 0.006 g/mL).

The results indicate that pandan extract exhibited a repellency effect on rice weevils, as shown by the positive values of the percentage of repellency in Figure 1. This could be attributed to the presence of repellant compounds in pandan extract that deter the rice weevils from approaching the treated part, leading to fewer rice weevils compared to the control. This finding is consistent with the research by Fernando & Karunaratne (2013), which showed that higher doses of insecticides from pandan extract result in greater repellency. At low concentrations (0.002 - 0.006 g/mL), pandan extract provided a repellency effect against *S. oryzae*. Conversely, kaffir lime and “asam gelugor” showed an attractive effect rather than a repellency effect on rice weevils, as indicated by the negative values of the percentage of repellency in Figure 1. It can be observed that pandan extract exhibited the highest percentage of repellency (46.67 ± 0.44%) at a concentration of 0.006 g/mL, compared to kaffir lime (-46.67 ± 0.26%) and “asam gelugor” (-26.67 ± 18%).

It could be concluded that pandan extract possesses repellent compounds that can repel rice weevils, whereas kaffir lime and “asam gelugor” might contain attractant compounds that can attract rice weevils. Therefore, pandan extract could be developed into a natural rice weevil repellent, while kaffir lime and “asam gelugor” could be developed into a natural rice weevil attractant to lure rice weevils away from rice.

Antifeedant effect

Table 1 shows the average percentage of weight loss of rice treated with different plant extracts. From the table, it can be seen that the average percentage of weight loss for pandan extract-treated rice was 0.108 ± 0.081%, for kaffir lime extract-treated rice was 0.102 ± 0.062%, for “asam gelugor” extract-treated rice was 0.1 ± 0.009% while for control rice was 0.180 ± 0.01%. The average percentage of rice weight loss treated by plant extracts was lower than the average percentage of rice weight loss of control.

Table 1. Average percentage of weight loss (%W) of rice with different plant extracts

Rice with plant extracts	Average %W
Pandan	0.108±0.081[a]
Kaffir lime	0.102±0.062 [a]
Asam gelugor	0.100±0.009 [a]

[#] Values in each row having the same superscript letter (a) were not significantly different (*p*>0.05).

The results of this study indicate that the use of plant extracts can reduce the risk of weight loss in rice caused by *S. oryzae*. This finding is consistent with the research of Yazdgerdian *et al*. (2015), which demonstrated that *S. oryzae* can cause up to 30 - 40% weight loss. The percentage of weight loss in rice may vary depending on the concentration and chemical composition of the plant extracts, as well as the ratio of the constituents present in the mixture, which can prevent the rice weevils from feeding on the rice. It is worth noting that the concentration and chemical composition of plant extracts can differ even among closely related species (Yazdgerdian *et al*., 2015). Pandan leaves contain terpenoid compounds, while kaffir leaves contain citronellal compounds, both of which have been shown to exhibit potent toxicity against *S. oryzae* (Lee *et al*., 2001; Balamurugan *et al*., 2019; Ikawati *et al*., 2017). This suggests that these plant extracts possess antifeedant properties that can be utilized to control rice weevils from feeding on rice.

Anti-progeny effect

Figure 2 shows the graph of the percentage of Abbot corrected mortality against the concentration of plant extracts treated rice.

As depicted in Figure 2, the rice treated with pandan and "asam gelugor" extracts displayed a favorable percentage of Abbott-corrected mortality, indicating a lower number of rice weevils compared to the control group. This could be attributed to the presence of anti-feedant compounds in these extracts, which could slow down the growth or hinder the reproduction of rice weevils. On the other hand, kaffir lime exhibited a negative percentage of Abbott-corrected mortality, which could be due to the concentration range being insufficient to display its anti-feedant activity against rice weevils. Therefore, further concentration increments of kaffir lime extract are necessary to ascertain its effect on rice weevils.

The mortality rate of *S. oryzae* in this study was observed to begin after 12 h at low concentrations (0.002 - 0.006 g/mL) of plant extracts, as demonstrated in previous studies. The concentration of plant extracts is likely to have an impact on the mortality rate, and the way insecticides work is affected by differences in the concentration of toxicity that causes mortality (Fauzi & Prastowo, 2021). Adeleye *et al*. (2022) found that a higher concentration of plant crude extracts mixed with rice resulted in a faster mortality rate. In the case of pandan and “asam gelugor” extracts in this study, increasing their concentration may enhance the percentage of mortality as they contain anti-progeny compounds that slow down the growth or prevent the reproduction of rice weevils. Ravi *et al*. (1996) suggest that this effect may be due to the insecticidal properties of volatile organosulfur constituents in insecticides, which penetrate the cuticle and disrupt gas exchange in the respiration of rice weevils, leading to death.

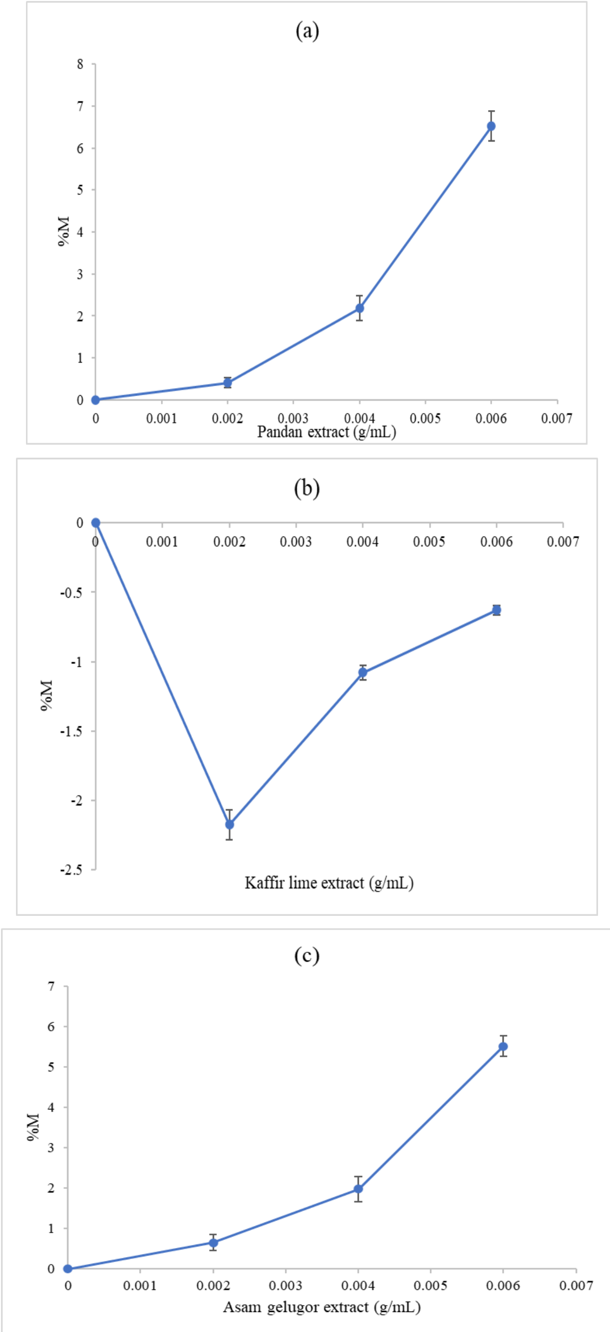

Fig. 2. Percentage of Abbott corrected mortality (%M) of (a) pandan extracts, (b) kaffir lime extracts and (c) “asam gelugor” extracts at different concentrations (0.002, 0.004 & 0.006 g/mL).

CONCLUSION

To summarize, the study found that pandan leaves were effective in repelling rice weevils and inhibiting their reproduction, while kaffir lime leaves had no effect, and "asam gelugor" only inhibited reproduction. Pandan leaves proved to be the most potent plant against rice weevils compared to the other two plants. It is possible that the presence of volatile compounds in the plants contributed to their insecticidal effects, and further research is needed to identify these compounds. These plants could serve as natural insecticides against rice weevils, replacing chemical pesticides that can harm the environment.

ACKNOWLEDGEMENTS

This research was supported by Universiti Malaysia Pahang (Project ID: PGRS210351). None of the authors declare any conflicts of interest.

ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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