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Effects of Several Plant Leaves on Rice Weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae) Productivity and Stored Rice Qualities

Nur Aida Hashim[1,*], Nurul Nadia Samsuddin[2], Khadijah Saad[3], Vu Thanh Tu Anh[4]

[1] School of Food Science and Technology,
Universiti Malaysia Terengganu,
21030 Kuala Nerus, Terengganu, Malaysia.

[2] School of Food Science and Technology,
Universiti Malaysia Terengganu,
21030 Kuala Nerus, Terengganu, Malaysia.

[3]School of Food Science and Technology,
Universiti Malaysia Terengganu,
21030 Kuala Nerus, Terengganu, Malaysia.

[4]Faculty of Resource Science and Technology,
Universiti Malaysia Sarawak,
94300 Kota Samarahan, Malaysia.

**Corresponding author's email: aida.hashim [AT] umt.edu.my*

ABSTRACT— ***Fresh, dried and powder forms from leaves of Murayya keonigii, Piper betel, Cymbopogon citratus, and Cosmos caudatus were tested for their repellent or attractant effects, developmental inhibition and infestation control towards Sitophilus oryzae in stored rice grain. A hundred of S. oryzae were released in the free choice bioassay chambers. The number of S. oryzae oriented within the chamber after three days exposure to different forms of the above leaves were counted and recorded. In no choice ovipositional test, five pairs of S. oryzae were introduced in separately treated rice grain (fresh and dried forms of leaves in 1g and powder leaves in 1% w/w) for five days. After four weeks, the number of newly emerged weevils were counted and compared. The repellent effect was showed in fresh form of M. koenigii with the less preferred by the weevils (10.00±2.08), dried (10.67±5.17) and powder form of C. citratus (17.00±3.00). Piper betel leaves showed an attractant effects toward the S. oryzae (20.33±9.82). The lowest number of new emergence was observed in rice grain treated with dried form of M. koenigii (0.33±0.33). In week 7, the powder form of M. koenigii still showed the lowest number of S. oryzae (32.00±19.50) while the highest showed in rice grain treated with powder form of C. citratus (127.33±32.75). The lowest weight of bore dust produce was observed in rice grain treated with powder form of M. koenigii (0.23g), followed by C. caudatus (0.40g), control (0.70g) and P. betle (1.17g). There is no significant difference in percentage of weight loss among the stored rice treated with different forms of the plant leaves were observed in this experiment (p >.05).***

Keywords— natural insecticide, herbs, rice pest

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1. INTRODUCTION

Rice (*Oryza sativa*) is an important food source for more than half of the world's population including Malaysia. It is also considered as a great economic importance [1]. Loss of cereal grains due to insect infestation during storage is a serious problem, particularly in the developing countries, where damage to stored grains and their products by insects may amount to 5 to 10% in the temperate countries and 20 to 30% in the tropical zones [2].

Sitophilus oryzae is one of the insect pests in stored rice and most widely distributed throughout the world [3]. This weevil is considered as primary stored-grain insect in warm climate areas. Previous study by [4] reported the infested rice grains in storage due to insect pests lead to losses of nutritional value and become unfit for marketing and for use as seed as the infestation starts in the rice kernel. This explains why consumers usually do not realize the infestation by the rice weevils because they infest the grains by laying eggs inside the kernel, where the eggs hatched in short period of time [5].

To control this pest, synthetic insecticides are used during storage of grains. However, synthetic insecticides can cause residual pollution to the environment, toxicity to consumers and residues on grains. In addition, it had been reported that *S. oryzae* developed resistance to synthetic insecticides [6]. In several countries, due to environmental concerns and human health hazards, several chemical insecticides have either been banned or restricted [7].

Therefore, there has been a pressing need for the development of safer ways which has led to the discovery of plant products as an alternative way of controlling insects [8, 9]. There are a number of plant substances that have been considered for use as insecticides, antifeedants or repellents which include terpenes, flavonoids, alkaloids, phenols, and other related compounds [10]. Besides, the production of repellents from plants is less expensive and easier compared to synthetic chemicals, and the plant products have minimum effect on the ecosystem and are safer for human beings [11].

This study was initiated to determine the effects of several plant leaves either as an effective repellent or oviposition deterrence towards the rice weevils, *S. oryzae* in stored rice grains. This study is important to discover an alternative way to eliminate or decrease the infestation of the rice weevils and consequently helping consumers to prolong the shelf life of storage rice.

2. MATERIALS AND METHODS

2.1 Insect rearing and identification

The adults of *S. oryzae* used in the experiments were obtained from stored rice grains in several households of Kuala Terengganu. They were reared in plastic containers for 25 days until the emergence of new adults. The adults of rice weevils were separated and sexed under stereos microscope following [12] methodology. The sexed rice weevils were kept in different containers before the start of the experiments.

2.2 Preparation of plant materials

Four types of plant leaves used in this experiment were curry leaf (*Murraya koenigii*), wild cosmos leaf (*Cosmos caudatus*), betel leaf (*Piper betle*) and lemongrass (*Cymbopogon citratus*). The leaves used were in the forms of fresh, dried and powder. For dried form of leaves, the leaves were dried in dryer at 40^{o}C for a week. For powder form, the dried leaves were ground separately in a mill blender into a fine powder.

2.3 Free choice chamber bioassay

The free choice chamber was a circular device of six transparent, 300ml plastic containers (Five containers for choice chambers and one was for releasing chamber) placed equidistantly to each other. The choice chambers were connected to releasing chamber placed in the center the device through plastic tube (1 cm in diameter and 4.5 cm long). The free choice chamber was fitted in a plastic basin having a diameter of 42 cm and 18 cm in height.

In each experiment, 50g of rice grains were dressed separately with four different plant leaves in three forms (fresh, dried and powder). The powder of leaves was dressed at the dose of 1% (w/w) and the fresh and dried leaves were used in amount of 1g for each treatment for both tests. The dressed rice grains were placed in four consecutive containers leaving one container, which was occupied by the control set of rice grains without any treatment. One hundred of adult weevils were introduced into the central container. The number of adults oriented in each treated and control set were counted in three days intervals for one month. All treatments were replicated thrice.

2.4 No choice ovipositional test

Fifty grams of rice grains treated separately with each plant material (fresh and dried leave forms in 1g and powdered leave form in 1% w/w) were kept separately in small plastic containers of 150 ml and covered. Five pairs of adult weevils were released in each container.

All of the adults were removed after 5 days of release and total number of adults emerged in each container with treated rice grains were counted after 30 days of their release. A control set was also be maintained without any treatment of plant leaves. The number of new emergence, percentage weight loss of treated and control rice grains and weight of bore dust were recorded. The experiment was repeated three times.

2.5 Parameters

2.5.1 Percentage weight loss of rice grains

Percentage of weight loss was calculated using the formula according to [13].

$$\%GWL = \frac{IWG - FWG}{IWG} \times 100$$

GWL = Grain Weight Loss

IWG = Initial Weight Grain

FWG = Final Weight Grain

2.5.2 Weight of bore dust

The grain dust or bore dust generated by the rice weevils after one month in the treated stored rice grains were sieved out of the grain, and the data were recorded as one of the rice grain damages. The amount of bore dust generated in treated stored rice grains was compared with the amount of dust in control stored rice grains.

2.5.3 Percentage of grain damages

Percentage of grain damages by the rice weevils was determined by calculating the percentage of damage (holed and infested) grain.

2.5.4 Data analysis

All the treatments were done in three replications. All the data were subjected to normality test. It was found that all data was not normally distributed even after transformation of the data was conducted. Therefore, non-parametric tests were used as statistical analysis. Chi Square Test goodness of fit was used to analyze the Free Choice Bioassays data and Chi Square Test for relatedness or independence for No Choice Ovipositional Test's data. Kruskal Wallis was used to analyse the weight of bore dust and percentage weight loss data.

3. RESULTS

3.1 The effects of different forms of plant leaves towards Sitophilus oryzae

The results of bioassay studies indicate significantly different effect of different forms of plant towards the rice weevil, *S. oryzae* (Figure 1). The weevils preferred fresh leaves of *C. citratus* and *C. caudatus* but powder form of *P. betle* and dried form of *M. koenigii*. Among the leaves, the weevils oriented toward the leaves of *P. betle* the most and of *C. citratus* the least, regardless of the leaf form, [χ^2 (8, N = 222) = 20.209, $p < .05$].

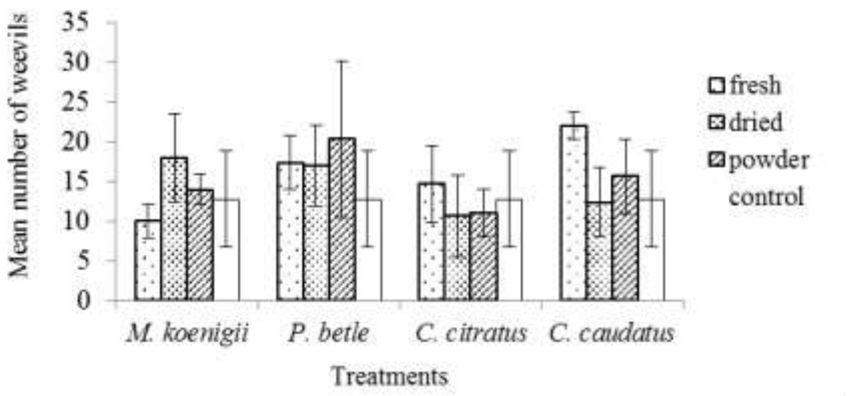

Figure 1: Mean number of *S. oryzae* oriented in free choice chamber bioassay.

3.2 The effects of different plant leaves treated rice grains towards emergence of Sitophilus oryzae.

3.2.1 Fresh form

Figure 2 shows the number of weevils emerged from the grains treated with fresh form of different types of leaves for seven weeks. In the first week, control grains showed the lowest mean number of weevils emerged (1.00±0.58) while the rice grains treated with *C. citratus* have the highest number of weevils emerged (9.33±1.76). At the end of the experiment, the lowest mean number of *S. oryzae* emerged was found in control treatment (55.67±13.38), while the highest mean number of weevils was found in the rice grains treated with *C. citratus* leaves (128.00±5.86). There were three treatments showed over 100 adult weevils emerged from the treatments at the end of the experiments: *M. koenigii* (110.00±59.27), *C. citratus* (128.00±5.86) and *C. caudatus* (124.00±67.04). Chi square test showed a significant differences between the numbers of weevils emerged from rice grain treated with different fresh form of leaves, and the number of weevils was lowest in the control treatment [χ^2 (4, N = 516) = 32.45, $p < .05$].

3.2.2 Dried form

The effect of dried form of several plants leaves towards the emergence of *S. oryzae* is shown in Figure 3. After one week, the minimum mean number of *S. oryzae* emerged was 0.33±0.33 in rice treated with dried form of *M. koenigii* while the highest number was 4.00±1.00 in rice grains treated with *C. citratus*. On week 7, *M. koenigii* treated rice grains maintained as the lowest mean number of *S. oryzae* emerged with the total emergence was 23.33±8.33 while *C. caudatus* treated rice grains were heavily infested by the *S. oryzae* with the mean number of emergence was 126.33±27.61. The chi square test on week 7's data showed a significant difference in the number of weevils emerged from rice grains treated with dried form of different leaves. The results show that the number of weevils is lowest in the rice grains treated with *M. koenigii* dried leaf [χ^2 (4, N = 358) = 85.212, p <05].

3.2.3 Powder form

Figure 4 indicates the effect of plant leaves in form of powder towards the emergence of *S. oryzae* in rice grain. The lowest mean number of *S. oryzae* emerged in the first week was 0.00±0.00 in control treatment followed by rice treated with *M. koenigii* and *P. betle* (0.33±0.33). The highest number of weevils was observed with the rice grains treated with in *C. citratus* and *C.caudatus* (1.00±0.58). On week 7, *M. koenigii* treated rice grains had the lowest number of emergence of *S. oryzae* which was 32.00±19.50. *C. citratus* treatment showed the highest number of weevil's emergence which was 127.33±32.75. The chi square test showed a significant difference in the number of weevils emerged from the rice grains treated with different plant leaves in the powder form. The number of weevils was lowest in powder form of *M. koenigii* treated rice grains [χ^2 (4, N = 445) = 56.978, $p <.05$].

3.3 The effects of different forms of individual plant leaves on emergence of Sitophilus oryzae.

The comparison of the mean number of weevils emerged from the rice grains treated with different form of plant leaves for seven weeks is shown in Figure 5. The chi square values are significant for *M. koenigii, C. citratus* and *C. caudatus* leaves ($p <.05$), which indicates significant differences in the number of weevils in different forms of *M. koenigii, C. citratus* and *C. caudatus* leaves. However, there was no significant difference in the number of weevils towards different form of betel leaves treated rice grains.

3.3.1 Murayya koenigiii

The number of weevils increased over seven weeks of experiment but slightly decreased after five weeks for dried and powder forms of *M. koenigii* (Figure 5) due to some mortality of the weevils. The number of rice weevil was lowest in dried form of *M. koenigii* treatment (23.33±8.33) compared to fresh (110.00±59.27) and powder form (32.00±19.50) treatments. There was a significant difference in the number of weevils emerged in different form of *M. koenigii* leaves [χ^2 (2, N = 170) = 77.024, $P<.05$].

3.3.2 Piper betle

Figure 6 shows the number of weevil emerged from rice treated with different form of *P. betle*. The number of weevil emerged from the rice treated with different form of *P. betle* was not significantly different [χ^2 (2, N = 304) = 4.605, $p > .05$].

3.3.3 Cymbopogon citratus

The number of weevils was lowest in dried form of *C. citratus* treatment (71.67±7.33) compared to fresh (126±5.86) and powder form (127.33±32.75) treatments (Figure 7). Chi square test showed that there was significant different in the number of weevils emerged in different form of *C. citratus* treatments [χ^2 (2, N = 318) = 16.83, $P < .05$].

3.3.4 Cosmos caudatus

The number of weevils increased over seven weeks of experiment except for powder form of *C. caudatus* after week 5 as it showed a slightly decreasing pattern in number of weevils as there were some mortality occurred. The number of weevil was lowest in powder form of *C. caudatus* treatment (62.67±17.03) compared to fresh (124.00±67.04) and dried form (126.33±27.61) Figure 8. Chi square test showed that there was significant different in the number of weevils emerged in different form of *C. caudatus* treatments [χ^2 (2, N = 319) = 19.680, $p < .05$].

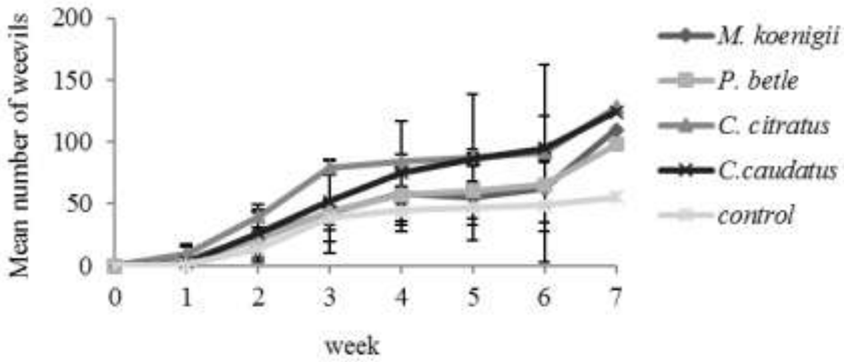

Figure 2: Comparison of mean number of rice weevil, *S. oryzae* emerged from rice treated with fresh form of different plant leaves.

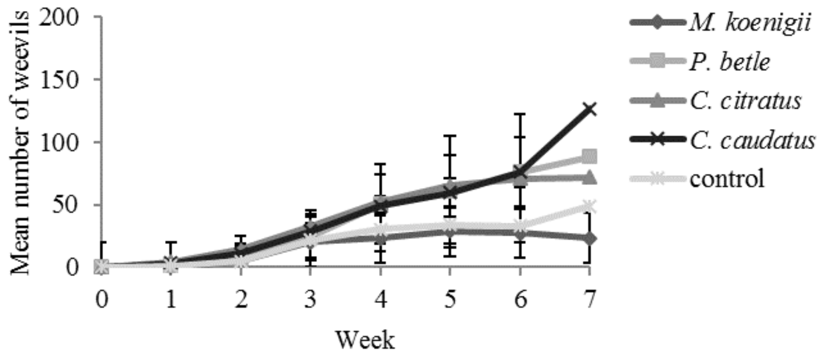

Figure 3: Comparison of mean number of rice weevil, *S. oryzae* emerged from rice treated with dried form of different plant leaves.

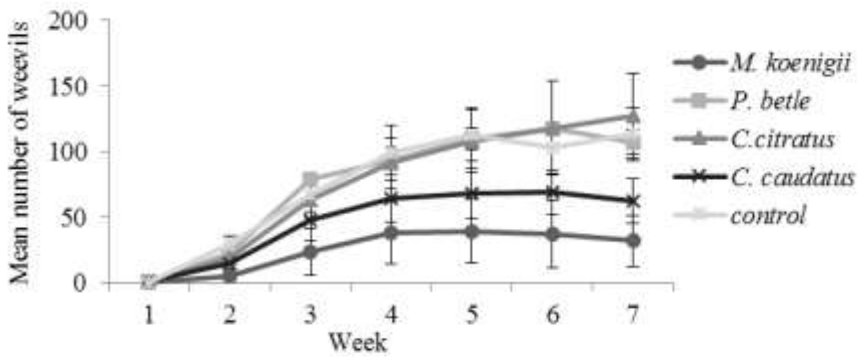

Figure 4: Comparison of mean number of rice weevil, *S. oryzae* emerged from rice treated with powder form of different plant leaves.

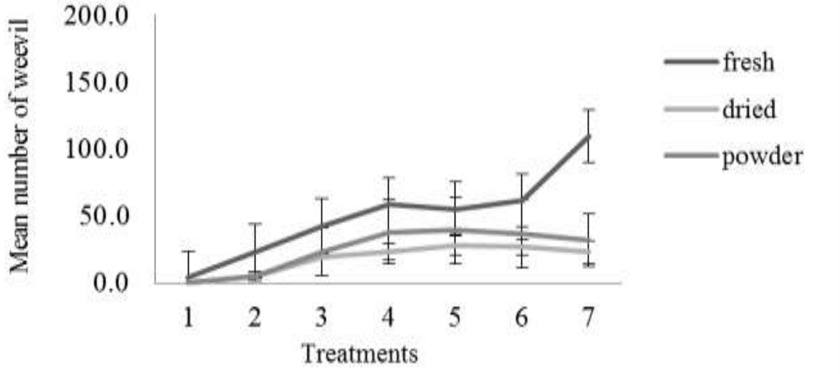

Figure 5: Mean number of *S. oryzae* emerged in rice grain treated with *M. koenigii*

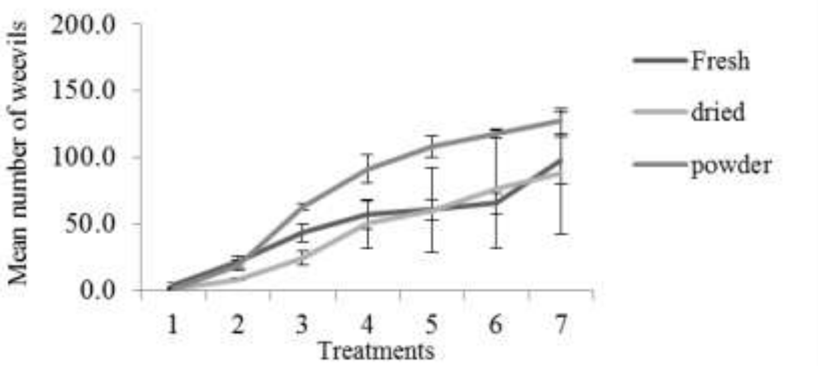

Figure 6: Mean number of *S. oryzae* emerged in rice grain treated with *P. betle*.

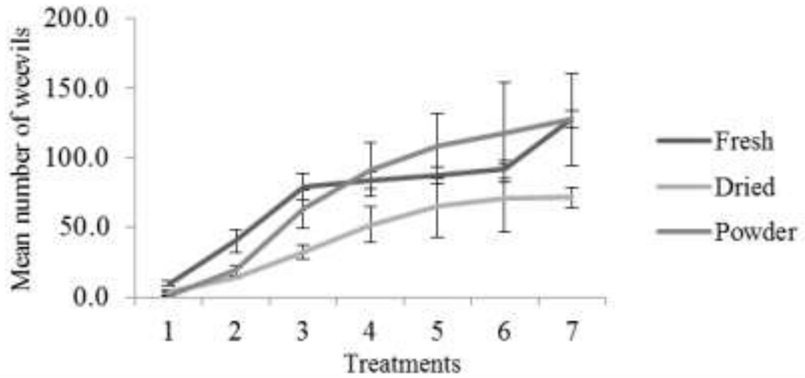

Figure 7: Mean number of *S. oryzae* emerged in rice grain treated with *C. citratus*.

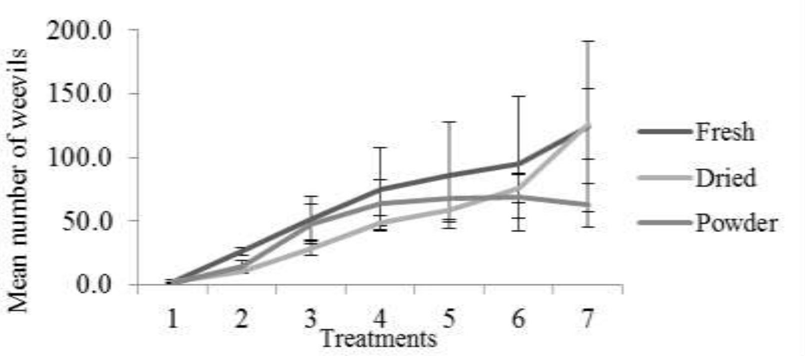

Figure 8: Mean number of *S. oryzae* emerged in rice grain treated with *C. caudatus*.

3.4 Grain damage assessment based on weight of bore dust and percentage weight loss.

Figure 9 shows the weight of bore dust produced by *S. oryzae* on the week 7 of observation. Between the three forms of the plant leaves, the weight of bore dust produced by *S. oryzae* in rice treated with fresh and dried form of plant leaves did not differ significantly across the five treatments [χ^2 (4, *N* = 15) = 4.466, P > .05] and [χ^2 (4, *N* = 15) = 9.46, p > .05]. The weight of bore dust in the rice grains treated with powder form of leaves was significantly difference across the five treatments [χ^2 (4, *N* = 15) = 10.523, p < .05]. Among the powder form treatments, *S. oryzae* in the rice grains treated with powder of *C. citratus* produced the highest amount of bore dust (1.5g) followed by *P. betle* (1.17g), control (0.70g), *C. caudatus* (0.40g) and *M. koenigii* (0.23g).

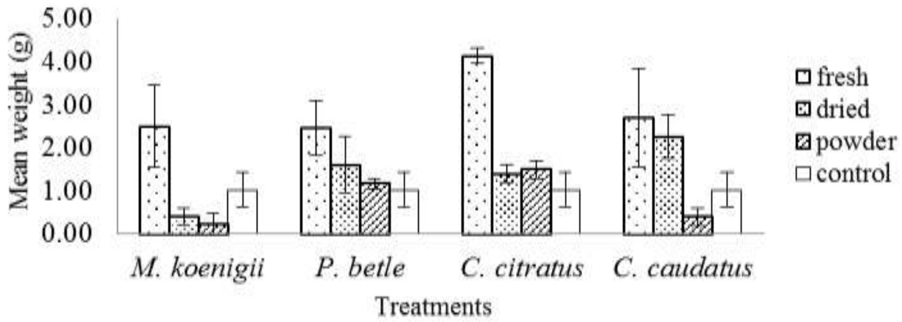

Figure 9: Weight of bore dust produced by *S. oryzae* in rice grain treated with different type of leaves and forms on week seven.

Figure 10 shows the percentage weight loss of rice grains treated with different form of *M. koenigii, P. betle, C. citratus, C. caudatus* and control treatment in no choice ovipositional test. At the end of the experiment, the lowest percentage weight loss was observed in rice treated with *P. betle* (3.73±2.48) while the highest was *C. citratus* (12.33±1.20). In dried form, *M. koenigii* shows the lowest percentage of weight loss (1.80±0.80) while the highest was *C. caudatus* (9.80±0.83). Powder form of M. *koenigii* showed the lowest percentage weight loss (3.33±2.25) followed by *C. caudatus* (6.27±1.67), control (7.20±1.59), *and P. betle* (9.93±0.53) and *C. citratus* (10.33±1.97). Kruskal wallis test showed that weight loss do not significantly differ across the fresh [χ^2 (4, *N* = 15) = 4.100, p > .05], dried [χ^2 (4, *N* = 15) = 8.486, p > .05] and powder form [χ^2 (4, *N* = 15) = 7.569, p > .05].

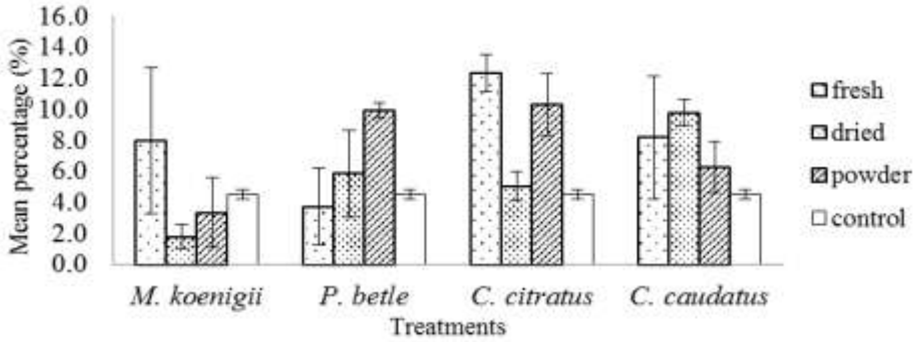

Figure 10: Percentage weight loss of rice grain treated with different forms of leaves.

4. DISCUSSION

Free choice chamber bioassays was used in this experiment to determine whether there are any effects from different forms of plant leaves toward *S. oryzae* in the rice grain. The results showed that fresh form of *M. koenigii* and dried and powder form of *C. citratus* have less preference by *S. oryzae* compared with others. Chemical constituents in *M. koenigii* leaf showed there is small percentage of α-pinene and β-myrcene that has potential to be used as active ingredients for natural-based insect repellents [14, 15, 16]. Some monoterpenes such as α-pinene, cineole, eugenol, limonene, terpinolene, citronellol, citronellal, camphor and thymol are common constituents in essential oil that representing the repellent activity [17].

It is reported that *Cymbopogon* essential oils and their major constituents function as allelochemicals. Allelochemicals affect insect biology and behavior and hence are used in biocontrol [18]. The constituents of *C. citratus* such as citral, geraniol, citronellol, citronellal and piperitone also have been known to possess insect repellent for a long time [18]. One of the constituent which is citronella has showed repellent activity towards beetles in muesli and wheat by using the extract of citronella [19].

These studies also showed that *P. betle* have high preference by the weevils for all the three forms of *P.betle*. There are studies illustrated that *P. betle* have early insecticidal activities, but some insect species are known to be attracted to methyl eugenol, the major component in this leaves for unknown reasons, while others may be attracted and stimulated to undergo pharmacophagous feeding [20].

Based on the effect of different plant leaves treated rice grain towards emergence of *S. oryzae*, the study showed that control have the lowest mean number of weevils emerged on week 1 and week 7 in fresh form of different plant leaves. This finding shows that the fresh form of plant leaves selected showed poor effects towards oviposition, emergence and development of *S. oryzae* in the stored rice grain. This probably happened due to moisture content of fresh plant leaves provides humid condition in the rice grain containers. High environmental temperatures and moisture along with dockage and broken kernels provide conditions that accelerate mould and insect development within the grains, thus increasing grain losses [21].

The result in rice grain treated with dried and powder form of plant leaves showed that *M. koenigii* treated rice grains had the lowest mean number of weevils emerged in week 7. *Murayya koenigii* showed strong activity against the oviposition, emergence and development of the *S. oryzae* in the treated rice grain [22]. It also had been reported that *M. koenigii* alters the behaviour and physiology of the insects affecting the egg laying and F1 emergence due to semiochemical nature of the plant [22]. Among the four plant leaves, *P. betle* did not show any deterrence towards the oviposition, emergence and development of *S. oryzae* but showed high preference towards the *P. betle* treated rice grain.

Researcher found that the major component of *P. betel* is eugenol, which is also present in several other plant species. Due to the high volatility and short duration of activity as well as its non-persistent nature, the use of eugenol on agricultural and stored commodities is not recommended [23].

Bore dust was produced by the larvae and adult of *S. oryzae* during feeding activity of the weevils inside the rice grain kernel and on the surface of the grain. It was also produced during the oviposition which is after the female making the hole to lay the eggs [24]. For grain damage assessment, weight of bore dust was the lowest in powder form of *M. koenigii* leaf treated rice grain on week 7. In previous study, *M. koenigii* powder was reported to have antifeedant activity towards *C. chinensis* [25]. The effect was probably attributed to its sesquiterpenoids content as this compound is one of the most associated with deterrence against insects [26]. This probably gives the same effect to *S. oryzae* as an antifeedant, thus decreases the amount of bore dust produced.

5. CONCLUSION AND RECOMMENDATION

In conclusion, the plant leaves selected have shown several effects towards *S. oryzae* in rice grain such as repellent from fresh form of *M. koenigii*, dried and powder form of *C. citratus* while the attractant from *P. betle*. Dried and powder form of *M. koenigii* reduced the infestation of *S. oryzae* and the grain damages. This was probably due to an antifeedant activity towards the weevils in the stored rice grain.

Fresh form of *M. koenigii* and dried and powder form of *C. citratus* have potential to be used as repellent on *S. oryzae* in stored rice grain in higher amount or concentration. Besides, dried and powder form of *M. koenigii* can be used as an antifeedant on *S. oryzae* in stored grain. For bulk storage, the plant leaves in other form such as plant extract essential oil or applied as fumigant can be tested. For future research, it is recommended that plant leaves that do not gives any effects toward *S. oryzae* such as *C. caudatus* can be tested on other storage pest such as *Tribolium castaneum* or *Tribolium confusum*.

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