Repellency and Insecticidal Activities of Sugar Apple Leave Extract against Rice Weevil, *Sitophilus oryzae* (Linnaeus, 1763) (Coleoptera: Curculionidae)

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**Abstract:** This experiment was carried out to test the insecticidal and repellent activities of sugar apple leave extract against stored grain pest, rice weevil, *Sitophilus oryzae* (Linnaeus) during December 2018 to March 2019. The objectives of the study were to test the repellency of sugar apple leave extract against rice weevil and to observe the mortality of beetle under the laboratory condition. 50g of dried leaves powder of sugar apple was soaked in the one liter of pure water for 24h at room temperature to obtain 5 percent concentration. In this experiment, the results showed repellant activity of 5.00 % when observed at the end of 24 h treatment, followed by 10.00%, 15.79 % and 25.71% respectively at the end of 48 h, 72 h and 96 h treatment. The extract of sugar apple leaves revealed the highest level of repellent activity at the end of 96 h treatment. Overall average of the repellency of sugar apple leave extract on *Sitophilus oryzae* was 12.5 % and therefore aqueous extract of sugar apple leaves belong to the repellency class I. The percentage of mortality was the highest (25.71%) after 96 h of treatment while no mortality occurred after 24 h and 48h of treatment. The data pertaining in the experiment showed that higher the exposure time was higher the percent repellency and mortality. It was suggested that the extract showed effective repellency on stored grain pest rice weevil and the results will be contributed to Integrated Pest Management programme and their structural elucidations are underway.

**Keyword:** Aqueous extract, sugar apple leaves, *Sitophilus oryzae*, repellency class, mortality.
1. INTRODUCTION

Insect pests are a major problem in fields and warehouses. It is estimated that about 35% of crops all over the world are destroyed by them (Shani, 2000). They cause severe damage to stored grains and processed products by reducing their quality and nutritional quality making them unfit for human consumption and agricultural purposes (Frenmore and Prakash, 1992). Estimated loss of the world’s supply of stored grains from insect damage ranges from 5-10% of world production. The tropical countries alone suffer a loss of 20% due to favorable climatic and storage conditions (Rahman et al., 2009). Due to great economic losses caused by stored grain pests, control of infestation in warehouses, factories, ships and mills is of main interest to the food manufacturers and distributors (Frenmore and Prakash, 1992).

The effective controlling method of grain protection from insect infestation by chemical agents is the simplest and cost-effective means of concerning stored product pests (Hidalgo et al., 1998). However, chemical insecticides have serious problems such as pest resurgence and resistance, lethal effects on non-target organisms including human, the risk of user’s contamination, food residues and environmental pollution (Tapondjou et al., 2002).

Rice weevil, *Sitophilus oryzae* (L.), (Curculionidae: Coleoptera) is a major pest of cereals like rice, sorghum, wheat, barley and maize both in field before harvest and in storage. The white apodous grub and the reddish brown adults are internal feeders and cause serious quantitative and qualitative losses to cereal grains (Nalini et al., 2009).

As a result of the advantages of the botanical insecticides over the synthetic ones, for insect pest management on stored products these botanicals are extensively studied. Different types of plant preparations such as powders, solvent extracts, essential oils and whole plants are being explored for their insecticidal activity including their action as fumigants, repellants, anti-feedants, anti-ovipositors insect growth regulators (Isman, 2000; Weaver and Subramanyam, 2000; Erturk et al., 2004; Koul, 2004; Mordue, 2004; Negahban and Moharramipour, 2007) (cited by Nalini et al., 2009).

Therefore, in the present study, the repellency and insecticidal properties of sugar apple leave extract on the stored grain pests, *Sitophilus oryzae* was experimented under laboratory conditions. The aim of this study was to determine the insecticidal activities of sugar apple leave in suppressing the rice weevil *S. oryzae* damage in stored rice.

The statement of the problem is an alternative of synthetic pesticide against the infestation of rice in storage. The objectives of the study were:

a. to test the repellency of sugar apple leave extract on rice weevil and
b. to observe the mortality of rice weevil under the laboratory condition.

2. REVIEW OF LITERATURE

Leatemia (2003) reported the development of a botanical insecticide from Ambon and surrounding areas (Indonesia) for local use in which the extracts of *Annona squamosa* were the most active one among other botanicals such as *Annona squamosa*, *Annona muricata*, *Lansium* and *Sandoricum koetjape*.

According to Duke (2008), one of the *Annona squamosa* leaf contents, borneol, is the possibly effective as insecticides or repellent. Kesetyaningsih (2012) recorded that *Annona squamosa* leaves have not been much researched as insecticides and are still in discussion about the active compound of *Annona squamosa* by way of insecticide.

Nalini et al. (2009) examined the leaf extracts from eleven medicinal plants for their effect on repellency, mortality, progeny production and loss in grain weight caused by the rice weevil, *Sitophilus oryzae* (L.). They concluded that ethanolic extract of *Lippia*, *Piper* and *Gloriosa* possess toxic principles with significant insecticidal and repellent effect and could be a potential grain protectant against *S. oryzae*.

Anita et al. (2012) described the efficacy of pulverised leaves of *Annona squamosa* (L.), *Moringa oleifera* (Lam.) and *Eucalyptus globulus* (Labill.) against the stored grain pest, *Tribolium castaneum* (Herbst.) In the case of *M. olifera*, the minimum days required for 100% mortality with 2.0g concentration was 11, while with 0.05g it was 18 days.
Isman and Seffrin (2014) stated that the crude extracts from seeds, leaves, bark, twigs, and fruits obtained from the plant species of Annonaceae have been extensively tested in recent years for bioactivity to pest insects and related arthropods worldwide.

Thinzar Hlaing (2015) reported the efficacy of Annona squamosa L. 1753 leaf extract as pesticide for the control of Spodoptera litura, Spodoptera exigua and Phenococcus solenopsis were tested with spraying method of Annona squamosa leaves extract concentrations 0% (control), 60%, 75% and 90% respectively.

For the above respects, the present study is aimed to use the plant based pesticide from the local plants instead of synthetic pesticide in order to fulfill the local needs and contribute Integrated Pest Management.

3. Materials and Methods

The experiment was carried out at the laboratory of Department of Zoology, University of Magway during December 2018 to March 2019.

3.1 Test insects

In the present study, the adult specimens of rice weevil to be tested were reared with stored rice as they were collected in the container.

3.2 Preparation of aqueous extract of Annona squamosa leaf

Leaves were collected from the surrounding area of Magway University, dried in the shade and finely chopped. Then 50g of dried leaves powder was soaked in the one liter of pure water for 24h at room temperature to obtain 5 percent concentration (Cruz et al., 2004 cited by Paul et al., 2016).

3.3 Area preference and mortality test

Area preference tests were performed using the method of Tapondjou et al. (2005) with modifications. One ml of the aqueous extract of sugar apple leaves was uniformly applied to half a filter paper disk to single dose concentration of five percent. The same volume of the solute without extract (pure water) was applied to the other half to serve as a control. Paper disks were placed in 90-mm petri dishes and the solvent allowed drying. Half an hour after the application, ten adults of S.oryzae were placed in the center of each paper disk. The dishes were added with 50 g rice food supplied for insects and covered by plastic and maintained at room temperature. Insects were counted in treated and control areas at 24 hour (h), 48h, 72 h and 96 h after insect release. With ten insects per dish and four replicate dishes, a total of 40 insects were used. The experimental design was completely randomized, with four replicates. Insect mortality was evaluated after every 24 hours of exposure to impregnated filter paper (Moreira et al., 2007).

Percentage of mortality was calculated using Abbott’s formula by counting number of dead insects in each petridish 24 h, 48 h, 72 h, and 96 h after treatments. Adult insects were considered dead when no response was observed after probing them with fine brush. At the end of each assessment, dead insects were removed. The experiment was arranged in completely randomized design (CRD).

3.4 Data analysis

Average percentage repellency from four replicates was calculated for each time period (24 h, 48 h, 72 h and 96 h) in the experiment. Positive (+) values indicated repellency and negative (-) values showed the attractancy. The overall average values with respect to the exposed period were calculated and assigned a repellency class using the following scale described by Mc Govern et al., (1977). The percent repellency was calculated by following Liu et al., 2013;

\[
PR\% = \frac{N_c - N_t}{N_c + N_t} \times 100
\]

Where, \(N_c\) = no. of individuals in the control group
\(N_t\) = no. of individuals in the treatment group
According to Mc Govern et al., 1977

<table>
<thead>
<tr>
<th>Class</th>
<th>Repellancy Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>I</td>
<td>0.1-20</td>
</tr>
<tr>
<td>II</td>
<td>20.1-40</td>
</tr>
<tr>
<td>III</td>
<td>40.1-60</td>
</tr>
<tr>
<td>IV</td>
<td>60.1-80</td>
</tr>
<tr>
<td>V</td>
<td>80.1-100</td>
</tr>
</tbody>
</table>

Mortality percent was conducted by using calculation of Abbott’s formula (1925). Analysis of data was carried out with hand calculation method.

Abbott’s formula:

\[
\% \text{ mortality} = \frac{x-y}{x} \times 100
\]

Where, \(x\) is the % survivorship of the control group

\(y\) is the % survivorship in the experimental group.

4. RESULTS

4.1 Repellent activities of \textit{A. squamosa} leave extract on the adults of \textit{Sitophilus oryzae}

In adult bioassay, the repellency of aqueous extract of sugar apple leaves on rice weevil was tested for single dose of five percent concentration under laboratory condition. In the experiment, the calculated repellency are presented in Table 1. The result shows a relation in the percentage repellency as the time of treatment was increased (Fig. 1). In this case, the lowest repellency of 5.00 % was observed at the end of 24 h treatment and then increased to about 10.00% and 15.79 % respectively at the end of 48 h, 72 h and 96 h treatment. The extract of sugar apple leaves showed the highest level of repellent activity at the end of 96 h treatment.

Based on the overall average percentage of repellency at 24 h, 48 h, 72 h and 96 h plants were categorized into different repellency classes (MC Govern et al., 1977), as mentioned in the methods section. The repellency classes are; class I, 0.1 to 20%; class II, 20.1 to 40 %; class III, 40.1 to 60%; class IV, 60.1 to 80%; and class V, 80.1 to 100% of repellency. Overall average of the repellency of sugar apple leave extract on \textit{Sitophilus oryzae} was 12.00 % and therefore aqueous extract of sugar apple leaves belong to the repellency class I.

4.2 Percent mortality of \textit{Sitophilus oryzae} after treatment

Another observation of this experiment was that after 24 h and 48 h exposure, no dead insects were found. However, about 5.00% insects were found dead after 72 h whereas the mortality percent increased to the highest mortality 12.5% after 96 h of exposure (Table 2). No mortality was found in the control half of filter paper disk. The data pertaining in the experiment showed that higher the exposure time was higher the percent mortality (Fig. 2).

<table>
<thead>
<tr>
<th>Exposed period</th>
<th>Repellency (%)</th>
<th>Overall average percentage of % repellency</th>
<th>Repellency class</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hours</td>
<td>5.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48 hours</td>
<td>10.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72 hours</td>
<td>15.79</td>
<td>12.0</td>
<td>I</td>
</tr>
<tr>
<td>96 hours</td>
<td>25.71</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Data presented in the Table are the percentage of repellency presented as means of 4 replicates

![Repellency Graph](image1)

Fig. 1 Percent repellency of the aqueous extract of sugar apple leaves on *Sitophilus oryzae* during exposure

<table>
<thead>
<tr>
<th>Exposed period</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hours</td>
<td>0.00</td>
</tr>
<tr>
<td>48 hours</td>
<td>0.00</td>
</tr>
<tr>
<td>72 hours</td>
<td>5.00</td>
</tr>
<tr>
<td>96 hours</td>
<td>12.50</td>
</tr>
</tbody>
</table>

![Mortality Graph](image2)

Fig. 2 Percent mortality of *Sitophilus oryzae* against *Annona squamosa* leaves with respect to exposure time

5. DISCUSSION

The problems given by synthetic insecticides have caused to the environment as well as to human health, there has been a surge of research on plant extracts and plant natural products for insect control (Castillo-Sánchez *et al*., 2010). The practice of using plant extracts in agriculture for pest control is not new. They have been used for at least two millennia, when botanical insecticides
were considered important products for pest management in ancient China (Long et al., 2006), Egypt, Greece, and India (Isman, 2006). Even in the United States and some European countries, botanical insecticides were widely used before the discovery of organochlorine and organophosphate insecticides in the late 1930s and early 1940s (Isman, 1997).

To protect the stored products from insect infestation, there is an increasing need for the use of locally available plants as repellent that are more effective, more persistent and more economical than existing synthetics available. The present study shows that the aqueous extracts of the sugar apple leaves tested were effective in producing repellent activity and mortality of Sitophilus oryzae.

In the present experiment, the exposure period given was up to 96 h. Results presented in the experiment indicates that highest level of repellency occurred at the 96 h of exposure of the insects. The minimum repellency was at 24 h of the treatment. However, all the samples tested except 96 h treatment exhibited less than 20% repellency. Rahman et al. (2007) tested the ethanol extract of melgota, Macaranga postulata at different concentrations for their repellency activity and reported that repellent effect was proportional to the concentration and higher concentration had stronger effect. The results of present experiment showed in agree with the findings of Rahman et al.

According to the data pertaining from the experiment, the repellency was likely to be concerned to the exposure time. Furthermore, the repellency class of the extract on Sitophilus oryzae showed Class I. Use of insect repellents offer a hope for protection of stored grains from insect attack because they are relatively specific and may have low mammalian toxicity.

Sahayaraj and Paulraj (2001) reported that there is no relationship between toxicity and repellency. Present study is in conformity with the above findings. It was suggested that it acted as a very effective insecticide for a long period of time and high concentration of extract.

Soujanya et al. (2016) examined efficacy of plant extracts against rice weevil Sitophilus oryzae (L.) in stored maize in which they revealed mortality increased in dose dependent manner. The results of present experiment is similar to that of Soujanya et al. However, substances that are more toxic to the insects were in great abundance in this extract.

In 2012, Anita et al. reported that A. squamosa was found to be the most effective compared with other botanicals and produced 100% mortality within a short time with more than 50% mortality exhibited within first two days of exposure. In the present study, the percentage of mortality was maximum at 96 hours of exposure but the mortality in the present study showed lower than the results reported by Anita et al. No mortality occurred in 24h and 48h treatment. It is likely to be the reason of lower concentration of extract. The time required for 100% mortality may be decreased if the concentrations of leaf extract was increasing.

From the present study, the effectiveness of A. squamosa to control post-harvest food grain losses during storage is highly recommendable. This is of practical importance to the farmers who could improve their traditional methods of seed protection with the use of aqueous leaf extract as they are easily available and potentially less expensive.

6. CONCLUSION

From this study, it was found that A.squamosa leave extracts were the high efficiency for controlling the adults of rice weevil, Sitophilus oryzae. Moreover, it gives baseline information to researchers and local farmers as botanical pesticides alternative to chemical pesticide. It was suggested that the extract showed effective repellency on stored grain pest, rice weevil and the results will be contributed to Integrated Pest Management programme.

7. RECOMMENDATION

a. The aqueous extract of A.squamosa (Sugar apple) leaves should be used to control the stored grain pest rice weevil, Sitophilus oryzae.
b. Farmers should increase the concentrates of aqueous extract of sugar apple leaves to control effectively the rice weevil in stored rice.
c. Farmers should be recognized to use the botanical insecticide instead of chemical pesticides to contribute integrated pest management (IPM).
8. ACKNOWLEDGMENT

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9. REFERENCES


