Insecticidal and Repellant Activities of Sugar Apple Leave Extract against Stored Grain Pest, *Tribolium Castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae)

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**Abstract:** This study was conducted to test the insecticidal and repellent activities of sugar apple leave extract against stored grain pest *Tribolium castaneum* (Herbst) during December 2018 to March 2019. The objectives of the study were to test the repellency of sugar apple extract against red flour beetle 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 activity of 48.72 % when observed at the end of 24 h treatment, decreased to about 40.54% and 41.18 % respectively at the end of 48 h and 96 treatment. The extract of sugar apple leaves showed the highest level of repellant activity at the end of 72 h treatment. After 96 h of treatment, percentage of repellency was decreased about 41.18 %. Overall average of the repellency of sugar apple leave extract on *Tribolium castaneum* was 51.72 % and therefore Aqueous extract of sugar apple leaves belong to the repellency class III. The percentage of mortality was the highest (15%) after 96 h of treatment while the lowest after 24 h of treatment. The data pertaining in the experiment showed that higher the exposure time was higher the percent mortality. It was suggested that the extract showed effective repellency on stored grain pest red flour beetle and the results will be contributed to Integrated Pest Management programme and their structural elucidations are underway.

**Keywords:** Aqueous extract, sugar apple leaves, *Tribolium castaneum*, repellency class, mortality.
1. INTRODUCTION

Insect pests on stored grains are a problem throughout the world. Because they reduce not only the quantity and quality of grains but also their products are unfit for human consumption. Their damage to stored grains and grain products may amount to 5–10% in the temperate zone and 20–30% in the tropical zone (Nakakita, 1998). Such damage may range up to 40%, in countries where modern storage technologies have not been introduced (Shaaya et al., 1997).

The effective controlling method of grain protection from insect infestation is the use of chemical agents since it 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).

*Tribolium castaneum* is a major pest of stored grain products causing severe damage. This species is a long association with human stored food such as flour, cereals, pasta, biscuits, beans and nuts causing loss and damage (Pugazhvendan et al., 2009). Besides, this pest has developed resistance to many common pesticides, whose intensity differs in different regions.

In resolving these problems, biopesticides have gained huge importance in recent grain protection technology because of their insecticidal properties. Many plants have now been reported for their insecticidal properties. Among them, sugar apple leaves, seeds and barks are widely employ by small scale farmers to protect their crops and grains from infestations. They are traditionally and widely used against stored grain pests due to their easy accessibility and biodegradable nature (Anita et al., 2012). Moreover, there is a serious problem because most of people use unwisely chemical pesticides for stored grain pests and therefore these chemical residues may be persist in our food grains. Thus, there is an urgent need to develop safe alternatives to conventional insecticides and fumigants for the protection of grain products against insect infestations.

Therefore, in the present study, the repellency and insecticidal properties of sugar apple leave extract on the stored grain pests *Tribolium castaneum* was experimented under laboratory conditions. The results will provide data useful for the development of new repellants for stored-product pests. The aim of this study was to investigate the insecticidal activities of the leave extract of sugar apple against red flour beetle.

1.1 Statement of the problem

The statement of the problem is an alternative of chemical pesticide against the infestation of stored grain products.

1.2 Objectives

The objectives of the study were:

a. to test the repellency of sugar apple extract on red flour beetle and
b. to observe the mortality of beetle under the laboratory condition.

2. REVIEW OF LITERATURE


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.

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*...
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.

Pugazhvendan *et al.* (2012) stated the insecticidal and repellant activities of plants oil against stored grain pest, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). They recorded the five plants oil for their insecticidal and repellant activity against *Tribolium castaneum* (Herbst), a stored grain pest they were tested in the laboratory during the study period.

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.

Shahzad Saleem *et al.* (2014) also described the insecticidal activity of essential oils of four medicinal plants against different stored grain insect pests. To investigate the insecticidal activity of essential oils of four locally grown plants against three major insect pests, *Tribolium castaneum*, *Trogoderma granarium* and *Cryptolestes ferrugineus* responsible for economic loss to stored commodities of 5, 10, 15 and 20µL/L water laboratory conditions at 30±2°C and 65±5% relative humidity.

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 considerations, the present study is aimed to use the botanical pesticide from the local plants as a substitute of synthetic pesticide in order to fulfill the local needs and a part of integrated pest management.

### 3. Materials and Methods

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

#### 3.1 Test insects

In the present study, the adult specimens of species to be tested were reared with stored grains as they were collected, *Tribolium castaneum* in sesame 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. Working solution (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 *T. castaneum* 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 petri dish 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:

\[
\% \text{ mortality} = \left(\frac{x - y}{x}\right) \times 100
\]

Where, \(x\) is the % survivorship of the control group
\(y\) is the % survivorship in the experimental group.

4. RESULTS

4.1 Repellent activities of A. squamosa leave extract on the adults of Tribolium castaneum

In adult bioassay, the repellency of aqueous extract of sugar apple leaves on Tribolium castaneum was tested for single dose of five percent concentration under laboratory condition. In the experiment, the calculated repellency are presented in Table 1. There was no relation in the percentage repellency as the time of treatment was increased (Fig. 1). In this case, the activity of 48.72 % when observed at the end of 24 h treatment, decreased to about 40.54% and 41.18 % respectively at the end of 48 h and 96 treatment. The extract of sugar apple leaves showed the highest level of repellent activity at the end of 72 h treatment. After 96 h of treatment, percentage of repellency was decreased about 41.18 %.

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 Tribolium castaneum was 51.72 % and therefore Aqueous extract of sugar apple leaves belong to the repellency class III.
4.2 Percent mortality of *Tribolium castaneum* after treatment

Another observation of this experiment was that after 24 h exposure, a remarkable percentage of insects were found dead. The mortality percentage increased with increase in treatment period. After 24 h, about 3.00% insects were found dead whereas the mortality percent increased to 8% after 48 h and 10.0% mortality after 72 h and the highest mortality 15 % after 96 h of exposure respectively (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>Overage average of % repellency</th>
<th>Repellency class</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hours</td>
<td>48.72</td>
<td>(51.72%)</td>
<td>III</td>
</tr>
<tr>
<td>48 hours</td>
<td>40.54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72 hours</td>
<td>77.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>96 hours</td>
<td>41.18</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data presented in the Table are the percentage of repellency presented as means of 4 replicates.

Table 1 Repellant effect of *Annona squamosa* leave extract on *Tribolium castaneum*

![Graph of repellency](image)

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

Table 2 Mortality percent of *Tribolium castaneum* against *Annona squamosa* leave extract (five percent) under laboratory condition

<table>
<thead>
<tr>
<th>Exposed period</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hours</td>
<td>3.00</td>
</tr>
<tr>
<td>48 hours</td>
<td>8.00</td>
</tr>
<tr>
<td>72 hours</td>
<td>10.00</td>
</tr>
<tr>
<td>96 hours</td>
<td>15.00</td>
</tr>
</tbody>
</table>
5. DISCUSSION

Since 1980, Golob and Webley had reported that repellents might be used more widely for temporary control in restricted areas. Insects might be directed to other areas for control by insecticides or chemosterilants. Botanical insecticides are broadly used in pest control and many are safe, unique in action and can be easily managed and used. For protection of 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 T. castaneum. The time required for 100% mortality may be decreased if the concentrations of leaf extract was increasing.

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 72 of exposure of the insects. In the present study, the maximum repellency was recorded at 72 h of exposure while minimum repellency was at 48 h and 96 h of the treatment. However, all the samples tested exhibited more than 40% repellency. High repellency observed may be due to the occurrence of highly volatile components present in the extract. Further treatment for longer period (up to 96 h) gave similar pattern of response. Similar effects reported by Ahmed and Eapen (1986) (cited by Bajpai and Chandel, 2010) in the case of repellency of essential oils of eucalyptus and cineole against M. domestica was due to volatilization of the oil. According to their study, these extracts were highly effective during the 72 h of exposure when maximum volatilization of extract took place. Thereafter, the volatilization and also the repellency may decline suddenly.

Sahayaraj and Paulraj (2001) reported that there is no relationship between toxicity and repellency. Present study is in conformity with the above findings. Even though, A.squamosa leave extract did not act as a persistent repellent against Tribolium castaneum adult, it was suggested that it acted as a very effective insecticide for a long period of time and high concentration of extract.

Karmmal (2004) also recorded that the presence of highly volatile chemical components in the extracts may result in the immediate repellent effect against the tested insect and loss of this components may lead to the decline of repellent effect. In other words, the substances that cause persistent repellency may be absent in this extract. However, substances that are more toxic to the insects were in great abundance in this extract.

![Fig. 2 Percent mortality of Tribolium castaneum against Annona squamosa leaves with respect to exposure time](image-url)
In 2012, Anita et al. reported that \( A. \text{ squamosa} \) 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. Similar effect was found 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. It is likely to be the reason of lower concentration of extract.

According to the data pertaining from the experiment, the repellency was not likely to be concerned to the exposure time. Furthermore, the repellency class of the extract on \( T. \text{ castaneum} \) showed Class III.

From the present study, the effectiveness of \( A. \text{ squamosa} \) 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

Repellent activity of the aqueous extract of sugar apple leaves was tested against \( T. \text{ castaneum} \). From this study, it was found that \( A. \text{ squamosa} \) leave extracts were the high efficiency for controlling the adults of stored grain pest \( T. \text{ castaneum} \). 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 red flour beetle and the results will be contributed to Integrated Pest Management programme and their structural elucidations are underway.

7. RECOMMENDATION

a. The aqueous extract of \( A. \text{ squamosa} \) (Sugar apple) leaves should be used to control the stored grain pest \( T. \text{ castaneum} \).

b. Farmers should increase the concentrates of aqueous extract of sugar apple leaves to control the stored grain beetle.

c. Farmers should be acknowledged to use the botanical insecticide instead of chemical pesticides to contribute integrated pest management (IPM).

8. ACKNOWLEDGMENT

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9. REFERENCES


