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# Studies on the Insecticidal Activity of *LeucasAspera* against the Rice Weevil, *Sitophilus Oryzae*

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**Abstract:** ***The present study was aimed to analyze the insecticidal activity of extracts of Leucas aspera on (Rice weevil) Sitophilus oryzae. On analysis, a concentration of 250 mg/ml of the plant extract showed highest mortality. Certain secondary metabolites present in plant such as alkaloids, phenolic compounds and saponins were found to be responsible for the toxic effect, contributing inhibitory activity. A remarkable inhibitory effect was observed on the activity of Acetyl Choline esterase and Glutathione S transferase in the insect.***

**Keywords:** *Leucas aspera,* Acetyl Choline esterase, Glutathione S transferase, insecticidal activity

## 1. Introduction

Quantitative and qualitative loss of about 5 - 10% in temperate zone and 20-30% in tropical zone during post - harvest storage (*Haque et al., 2000*) is mainly due to the attack of more than 600 species of beetle pests, 70 species of moths and about 355 species of mites (*Sarker et al., 2006; Rajendran and Sriranjini, 2008*) on stored agricultural and animal origin products. Sitophilus oryzae commonly known as rice weevil, is known to be the most potent one which feed on rice and grow inside rice kernels (*Lucas and Riudavets, 2002*). Several chemicals have been in use such methyl bromide and phosphine to control the pests, however turned to be lethal for the stored products (*Negahban et al., 2006*). Alternative pest control methods such use of natural pesticides derived from environment friendly sources need to developed (*Negahban et al., 2006*). A wide range of medicinal plants such as Black pepper (Piper nigrum L.), physic nut (Jatropha curcas L.) was found to effective against many insects (*Salimon and Abdullah, 2008; Scott et al., 2008*).

*Leucas aspera* belonging tothe family Lamiaceae, found in all parts of India is known to possess antipyretic and insecticidal properties. It contains several bio active compounds which contribute to several physiological and pharmacological effects, thus making way for new therapeutic applications (*Kumar et.al, 2018*). Phytochemical screening can be used to identify the bioactive compounds which provide toxic effects to the plant extracts. Studies on enzyme inhibition provide a better understanding of enzyme specificity and the mechanisms by which enzymes and toxic agents work (*Lie et al, 2007*).

Majority of the studies reveal that the mode of inhibition of most of the plant-based pesticides is by inhibiting acetylcholinesterase enzyme which breaks down the neurotransmitter acetylcholine (*Lopez et.al, 2010*). On inhibition of acetylcholinesterase enzyme, the insects undergo disruption in nervous system leading to restlessness, hyperexcitability, tremors, convulsions and paralysis leading to death (*Rajashekaret.al, 2014*). Glutathion-S-transferases, a soluble dimeric enzyme has an important role in the interaction between the insects and host plants. This enzyme initiates the detoxification of several potential alkylating agents, bioactive compounds and electrophilic metabolites of carcinogens (*Iasonet al., 2001*). The present study investigates the role of *Leucas aspera* extracts as a potent intesticide against *Sitophilus oryzae* and its inhibitory effects on the activities of two enzymes (acetylcholinesterase and glutathion-s transferase). The investigation is aimed to bring light into the future application of plants extracts in pest management for controlling stored commodities.

## 2. Materials and Methods

### Sample Collection

The experimental organism, rice weevil was collected from infested grain samples (rice, wheat) from markets and houses at Ernakulam. The experimental plant, *Leucas aspera*, collected from different localities of Ernakulam was thoroughly washed and dried.

### Plant extract preparation

5gm of dried plants was crushed using motor and pestle, out of which 3gm was subjected to extraction using Soxhlet extractor apparatus with chloroform as solvent. The extract so obtained was diluted to required concentrations using the same solvent and stored for further assays.

### Insecticidal activity of plant extracts

The toxic activity of plant extracts against *Sitophilus oryzae* was analyzed by exposing five of them to 5gm of wheat powder with different concentrations of 50, 100, 200 and 250 mg/ml of plant extracts in each container respectively. The control was set with 1ml of chloroform and 5gm of wheat powder. At the end of observation time, the number of dead organisms was noted. The assessment of mortality was done by using the following formula,

$$\text{Percentage of mortality} = \frac{\text{Number of pest's dead} \times 100}{\text{Total no. of pests}}$$

Phytochemical screening

Chemical tests were done with aqueous plant extracts using standard procedures to identify the secondary metabolites. Preliminary phytochemical analysis was done for detection of alkaloids using Mayer's test, Dragendroff's test and Wagner's test, phenolic compounds using Ferric chloride test, flavonoids using Aqueous NaOH test and Concentrated $H_2SO_4$ test and saponins using Form test**.** Qualitative analysis of total alkaloids, phenolics and flavonoids was also performed with standard spectrophotometer.

Acetyl Choline Esterase inhibition assay

To 50 ml of test sample**,** 2.4 ml of 0.1 M phosphate buffer (pH 8.0) was added along with 0.1 ml of DTNB (0.4 mg/ml) and 0.2 ml of enzyme solution (acetyl choline esterase). The mixture was pre incubated at $25^0$ C for 5 minutes, after which enzyme reaction was initiated with 40 ml of acetylthiocholine (1mM) at $25^0$ C for 20 minutes. The control was set with 50 ml of solvent. The absorbance at 412 nm was measured spectrophotometrically (Ellman method).

Glutathione s- transferase inhibition assay

The reaction mixture comprises of 0.1 ml of Glutathione (1Mm), 0.1 ml of 1-chloro 2, 4- dinitrobenzene (CDNB) (1mM in ethanol) made up to 2.9 ml with phosphate buffer (0.1M, pH 6.5). The enzyme reaction was initiated with the addition of 0.1ml of plant extract. Absorbance was recorded at every 15 seconds at 340nm. The assay mixture without the extracts served as the control to monitor non-specific binding of the substrates.

## 3. Results and Discussion

Different concentrations of *Leucas aspera* extracts were evaluated against *Sitophilus oryzae* to determine its insecticidal property. The results showed 20% mortality in 50mg/ml, 40% in 100mg/ml, 60% in 200mg/ml and 100% in 250mg/ml concentrations of plant extracts (Table 1). The graphical representation of insecticidal activity at different concentration against percentage of mortality is shown in Figure 1. Qualitative analysis implied the presence of alkaloids, phenolic compounds and saponins in the plant extracts. The standard graphical representation obtained from quantitative analysis of alkaloids, phenols and flavonoids are shown in Figure 2, 3 and 4 respectively. The plant extracts have shown a significant inhibitory effect on acetyl choline esterase and Glutathione s- transferase upon measuring their enzyme activity. The results indicated about 74.5% of inhibition of acetyl choline esterase (Table 2) and 80.3 % Glutathione s- transferase (Table 3)with plant extracts against *Sitophilus oryzae.*

Since years plant based insecticides have been in use and have shown remarkable inhibitional activity (*Arannilewa et al. 2002, Kim et al. 2003 a, b, Akhtar et al. 2008*). A wide range of plant-based compounds have proved to be a potent insecticide such as pyrethrin extracted from flower of *Tanacetumcinerariaefolium* (*Singh 1993*), rhizomes of Acorus calamus t (*Abel 1987, Rees et al. 1993*), seeds of *Azadiraechtaindica* (*Schmutterer 1990*), however most of them where observed to cause harmful effects on the stored commodities. Plant extracts of *Leucas aspera* was conventionally known for its therapeutic properties. Several studies have supported the antipyretic, antimicrobial and insecticidal aspects of plant leaves and flowers (*Kumar et.al, 2018*). The present study also proves its inhibitory effect of with high mortality rate in sufficient concentrations, hence revealing its capability of usage as an efficient insecticide. Many plant secondary metabolites have shown insecticidal activity against stored grain insect pests [*Rajashekaret.al, 2012*]. On phytochemical screening and analysis, presence of alkaloids, phenolic compounds and saponins indicated that these compounds might be attributing to the toxic effect against pests. Most of the insecticides work by inhibiting the activity of some specific enzymes in the host pest. Acetyl choline esterase and Glutathione-S-transferases are two important enzymes which play a key role in host and pest interaction. The results of present investigation showed a significant inhibitory action of plant extract on these enzymes.

## 4. Conclusion

*Sitophilus oryzae,* destructive pest of stored grain products can be controlled by using the plant extracts of *Leucas aspera* (250mg/ml) instead of various harmful synthetic insecticides. Secondary metabolites in the plant extracts such as alkaloids, phenolic compounds and saponins contribute to the toxic effect of the plant extract. A significant inhibitory effect of plant extracts on the activity of Acetyl choline esterase and Glutathione-s-transferase in the insect body was also established. These results bring light into use of extracts of *Leucas aspera* as a potent insecticide, however further research need to be done on the active ingredients, pesticide preparations, application rates and environmental impact of botanical pesticides are a prerequisite for sustainable agriculture.

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## Tables and Figures

**Table 1:** Percentage of mortality caused by plant extract against rice weevil

| Concentration Mg/ml | No: of pests living | No: of pests died | Total no: of pests treated | Percentage of mortality |
|---|---|---|---|---|
| control | 5 | 0 | 5 | 0 |
| 50 mg/ml | 4 | 1 | 5 | 20% |
| 100mg/ml | 3 | 2 | 5 | 40% |
| 150mg/ml | 3 | 2 | 5 | 40% |
| 200mg/ml | 2 | 3 | 5 | 60% |
| 250mg/ml | 0 | 5 | 5 | 100% |

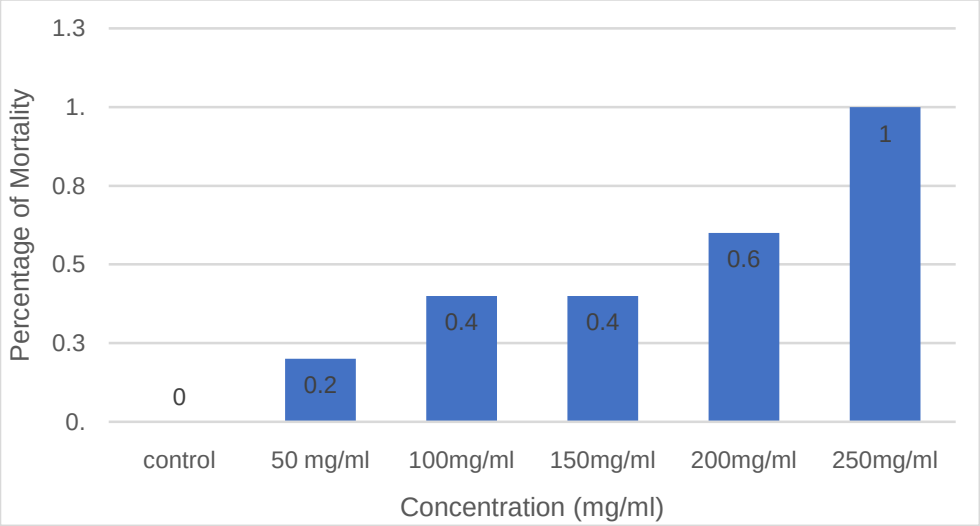


**Figure 1:** Percentage of mortality of rice weevil treated with plant extract

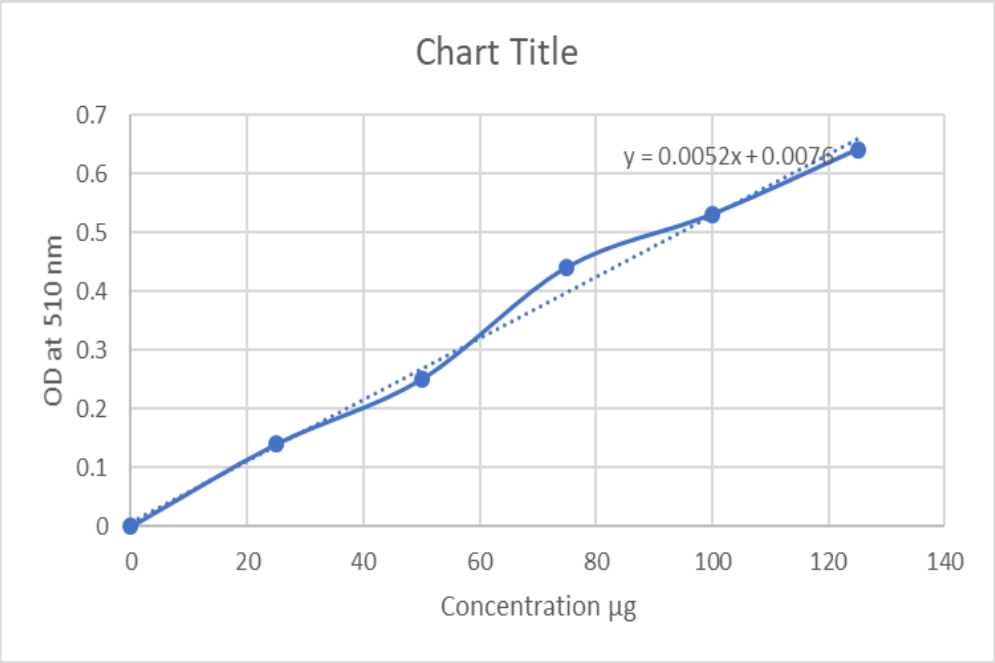


**Figure 2:** Standard graph of Alkanoids

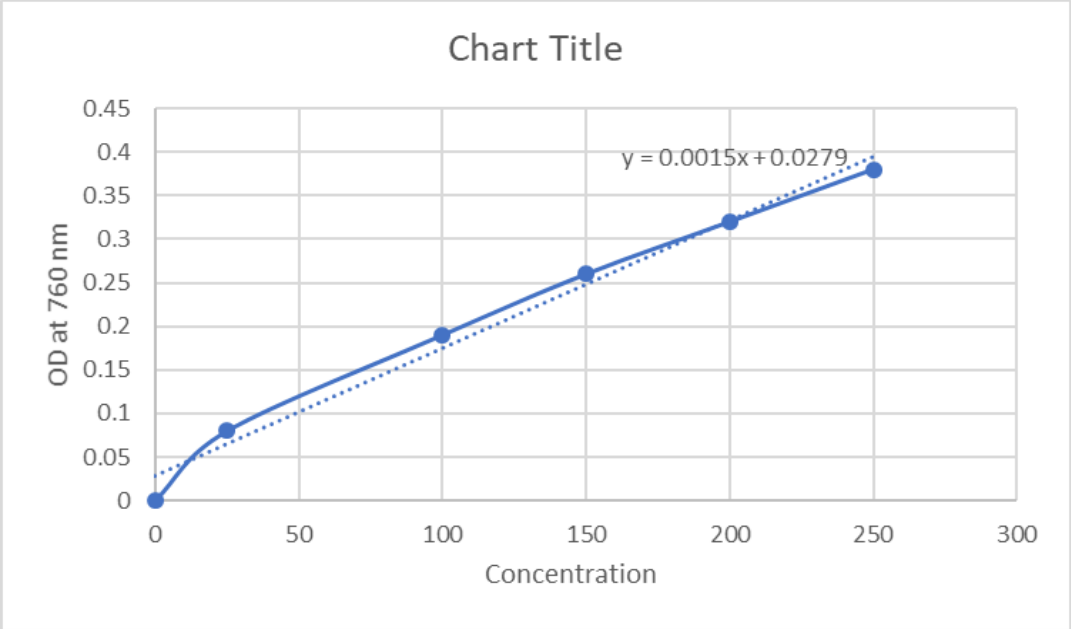


**Figure 3:** Standard graph of Phenol

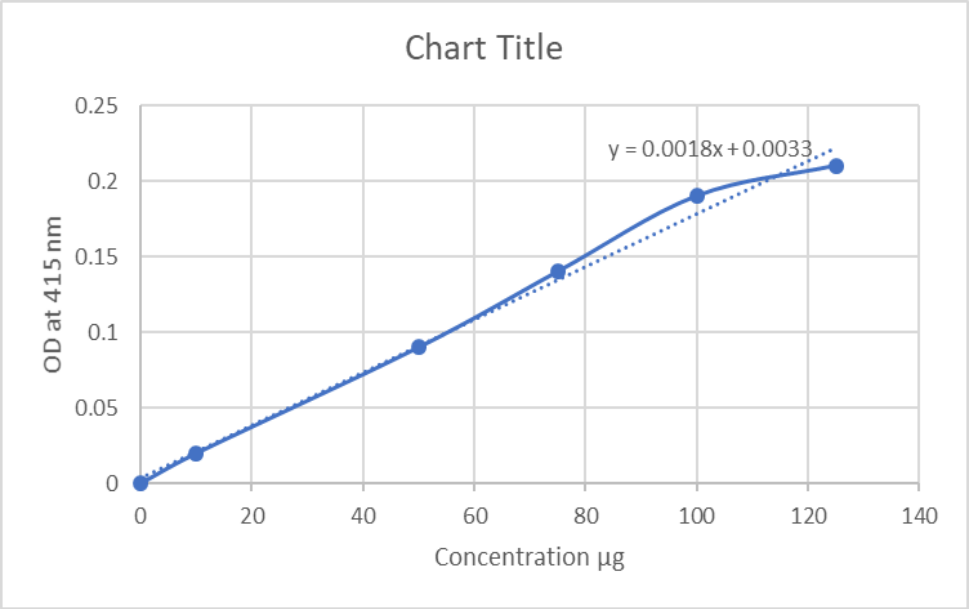


**Figure 4:** Standard graph of Flavonoids

**Table 2:** Absorbance for Acteyl choline esterase activity

| | **Optical Density:1** | **Optical Density:2** |
|---|---|---|
| **Blank** | 0.00 | 0.00 |
| **Test** | 0.31 | 0.31 |
| **Control** | 1.22 | 1.22 |

**Table 3:** Absorbance for glutathione S transferase activity

| | **Optical Density 1** | **Optical Density 2** |
|---|---|---|
| Blank | 0.00 | 0.00 |
| Test | 0.11 | 0.11 |
| Control | 0.60 | 0.60 |