Mella (*Olax zeylanica)* Leaves as an Eco-friendly Repellent for Storage Insect Pest Management

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Abstract

Among the cereals, rice is the most important staple food supplying energy requirements for most of the worlds' population. However during storage a loss of about 10-20% rice grains occurs due to stored grain pests. Repellents are considered as the best source of protection against insect attack upon stored products as they have potential for the exclusion of stored product pests from grain, and thereby preventing insect feeding and oviposition on food materials. Various plant materials have been utilized effectively through time as safe and ecofriendly insect pest control measures due to their repellent activity. The aim of the present study was to investigate the potential of powdered leaves and leaf extracts of *Olax zeylanica* as repellents against the rice weevil, *Sitophilus oryzae*. All the experiments were carried out under laboratory conditions using 1-7 day old unsexed adults. Four different doses (1.0g, 3.0g, 5.0g and 7.0g).of powdered leaves were tested for fumigant repellency in a dual-choice bio-assay apparatus. Repellent action of leaf extracts was evaluated by means of an area preference test using methanol, ethanol and n- hexane as solvents. Repellent effect of powdered leaves against the adult rice weevils was found to be significantly high ($P< 0.05$) at all doses. The highest repellent effect was produced by 7.0g of leaf powder resulting in repellency of 97%, while the lowest dose (1.0g) also elicited more than 50% repellency in weevils indicating a very strong repellent action of the powdered leaves. In comparison, methanol extract of leaves produced the highest repellent effect (96%) on weevils whereas n-hexane extract elicited the lowest. Nevertheless, at higher concentrations all three extracts produced more or less significantly similar repellent effect on the weevils. The findings of the present study suggest that certain active materials of *Olax zeylanica* leaves have potential to act as a grain protectant and may be exploited for the control of *Sitophilus oryzae* in rice storage in an environment-friendly way.

Keywords: *Olax zeylanica, Sitophilus oryzae, Repellent effect, Stored rice*

1. Introduction

The practice of using plant derivatives or botanical insecticides in agriculture dates back at least two millennia in ancient China, Egypt, Greece and India (Isman, 2006). In many developing countries, utilization of locally available plant materials to protect stored products against pest damage is common practice in traditional farm storage systems (Akob & Ewete, 2007). As Martin & Gopalakrishnan (2005) state these plant materials were not only used as insecticides but also as insect repellents and insect antifeedants.

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Plants have developed effective morphological and chemical defense mechanisms that ensure survival under rough environmental conditions and in the presence of natural enemies. These chemical defenses either may produce mortality of the insect by acting as an insecticide or may affect common biochemical and physiological functions and act as repellents, antifeedants or oviposition inhibitors. Excessive use of conventional pesticides disrupts natural pest control mechanisms and enhances development of pesticide resistant insect strains. In addition, they may have lethal effects on non-target organisms and may pose toxicity to consumers (Dubey *et al*., 2008; White & Leesch, 1995).

Compared to conventional pesticides, crude or formulated botanical pesticides tend to have broad-spectrum activity, and are relatively specific in their mode of action, easy to process by the small scale farmers and would be safe for the higher organisms (Viglianco *et al*., 2008). According to Prakash *et al.* (2008) these pesticides do not contribute to resistance development or pest resurgence, nor do they cause negative effects on non-target organisms and also they do not affect food quality.

Deterioration of the quality and quantity of the food materials is a major problem in storage systems. Stored rice seeds and milled rice is prone to be infested by rice weevils, *Sitophilus oryzae* L. (Coleoptera: Curculionidae), causing heavy economic losses. Adult weevils feed on rice grains and lay eggs within the grain surface and the larvae develop inside the grain thus feeding preferentially on the germ of the grain. Because both larvae and adults feed on germ of the grain, the grain is completely damaged beyond any use. Prevention of food losses during postharvest storage without creating environmental problems is therefore, of paramount economic importance. Hence, the primary objective of this investigation was to evaluate the repellent activity of leaves of *Olax zeylanica* against adult *Sitophilus oryzae* as identification of effective as well as locally available botanicals will provide a sustainable alternative in controlling storage insect pests.

2. Materials and methods

2.1 Host material

Whole and un-infested white raw rice was used for bioassays and as rearing media to prevent contamination of laboratory cultures. Unsexed, 1-7 days old adult rice weevils were used for bioassays. All bioassays were carried out under ambient laboratory conditions of 29±2^{0}C and 84 ± 2% RH.

2.2 Preparation of plant powder

Freshly collected leaves of *Olax zeylanica* were washed thoroughly under running tap water to remove any contaminants. These were then shade dried until the water was evaporated. The dried leaves were ground to fine powder using a domestic electric grinder (Multinational®, 2102, India).

2.3 Preparation of plant extracts

Hundred grams of freshly ground leaf powder was mixed with 350ml of solvent and kept for 48 hours while stirring from time to time. Three solvents, methanol (99.85%), ethanol (96%) and n- hexane (95%) were used for the bioassays. After 48 hours each of the resulting crude extract was filtered using Filtermann® (125mm) filter papers. The filtered extract was then concentrated using a vacuum rotary evaporator (Microsil, India) at 65^{0}C until the extract was reduced approximately to 60ml and was considered as the stock solution. A series (1ml, 3ml, 5ml, 7ml, and 10ml) of this stock solution was dissolved in 10ml of the appropriate solvent and five dilutions (T1, T2, T3 T4 and T5) each were made.

2.4 Fumigation repellency assay for plant powders

The bioassay apparatus for fumigation repellency test consisted of two plastic containers separated by a perforated lid (Figure1). Powdered leaves were introduced into the bottom container (height10cm,

diameter 6cm) and the upper container consisted of 50g of rice. The upper part of this container was perforated with a thick needle to allow the insects escape from the container if they were repelled by the fumes emanating from the powdered leaves.

The bioassay apparatus was placed inside a larger container (height 25cm, diameter 10cm) to trap the weevils that escaped through the holes in the test container. The mouth of the larger container was covered with a piece of polythene which has tiny holes in it to allow ventilation.

Test insects were then introduced into the container consisting rice, and the holes on the container was covered with a sticky tape for 10 minutes to let the introduced insects settle down inside. A similar bio-apparatus without leaf powder was used as the control. Number of escaped insects was recorded after half an hour and one hour of introduction of weevils. Four doses (1.0g, 3.0g, 5.0g and 7.0g) of powdered leaves and the control were assayed against the weevils. This test was replicated five times.

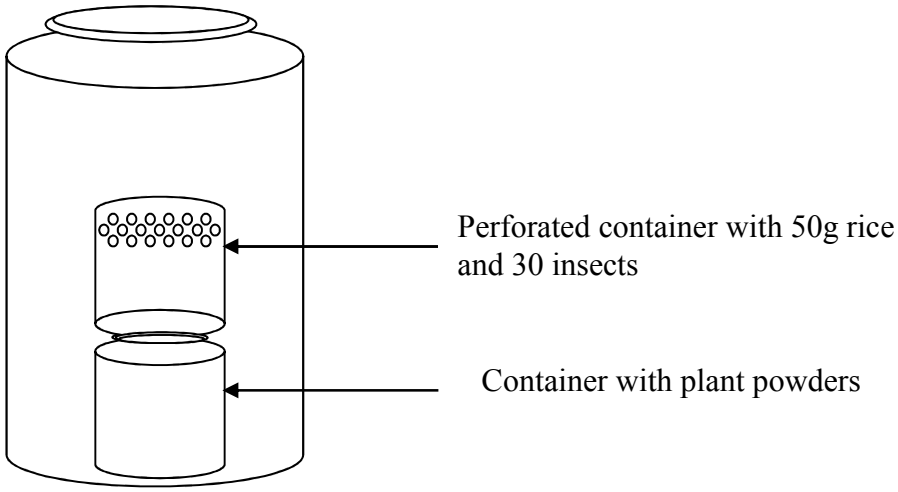

Figure1: Bioassay apparatus for the fumigation repellency test

2.5 Repellency assay for leaf extracts

Repellency of adult weevils for leaf extracts was tested using an area preference test. The test area consisted of a Filtermann® (125mm) paper disc that was cut into two parts. One part of the filter paper was treated with 1ml of the prepared extract as uniformly as possible and the other part was treated with 1ml of the solvent which was considered as the control. Both the treated half and the control half of the filter paper were then air dried to evaporate the solvent completely. A full disc was carefully re-made by attaching the treated part to the control part with adhesive tape and then placing it in a petri-dish. Before the onset of the experiment, glycerin was applied on to the side walls of the petri-dish to prevent the weevils moving to the top. 10g of rice seeds were distributed uniformly on the remade full disc as an attractant for the weevils to stay on the paper. Ten unsexed adult weevils were then released on to the centre of each filter paper disc and a lid was placed over the Petri dish. Thirty minutes after the introduction, the adult weevils present on each half of the filter paper was counted. Ten replicates were made for five concentrations of three solvent-plant extracts of *O. zeylanica.*

2.6 Analysis of Data

Statistical package "Minitab 14" was used for all the statistical analyses. Data obtained were subjected to one way analysis of variance (ANOVA). Tukey's multiple comparison test ($p<0.05$) was used to separate mean values of the experiments.

3. Results and Discussion

According to Figure 2, it is clear that the volatiles of leaf powders of *O. zeylanica* played a significant role on rice weevils by repelling them away from the treated chamber to the outer container. One hour after introduction of weevils, the highest repellency (88.67%) was observed for 7g of leaf powder.

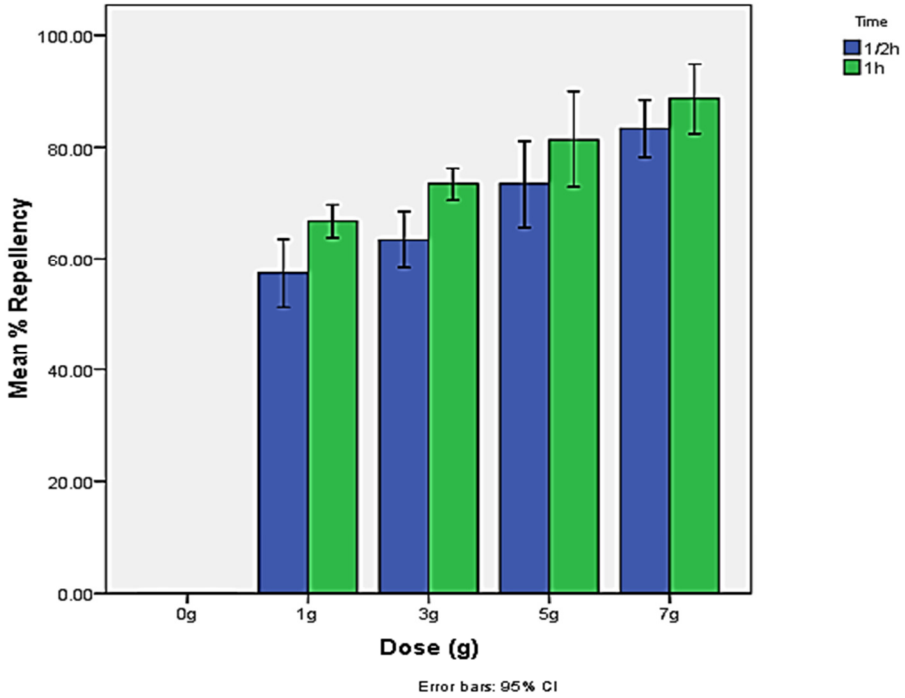

Figure 2: Mean percentage repellency of *S. oryzae* to different doses of plant powders
*Mean Repellency ± SD for ten replicates (n= 100)

The repellency rate of methanol, ethanol and n- hexane extracts at different doses on *S. oryzae* is presented in Table 1. The findings of this experiment revealed that the rate of repellency increased with the increase of dose level. For this bioassay, solvents with different polarity were selected in order to extract differently polarized compounds from *O. zeylanica* leaves into the solvents

Table1: Repellency of *S. oryzae* to different concentrations of three plant solvent extracts in an area preference test.

Treatment	% Repellency± SD		
	Methanol	Ethanol	n- Hexane
T1	75.0 ± 0.85 ^{ab}	68.0 ± 0.79 ^{b}	57.0 ± 1.16 ^{b}
T2	78.0 ± 1.03 ^{ab}	85.0 ± 1.43 ^{a}	67.0 ± 0.95 ^{b}
T3	93.0 ± 0.95 ^{a}	86.0 ± 0.84 ^{a}	68.0 ± 0.79 ^{b}
T4	94.0 ± 0.84 ^{a}	86.0 ± 0.96 ^{a}	80.0 ± 1.33 ^{ab}
T5	96.0 ± 0.52 ^{a}	94.0 ± 0.69 ^{a}	83.0 ± 1.06 ^{a}
Probability	$p< 0.05$	$p < 0.05$	$p < 0.05$

Means followed by the same letters are not significantly different according to Tukey's test at p<0.05

Among all the concentrations and solvents tested, methanol extract was found to be the one with the strongest repellent effect on *S. oryzae* with percentage repellency of 96% at the highest concentration followed by ethanol extract (94%). Hexane extracts elicited significantly lower repellency rate when compared with Ethanol and Methanol extracts Nevertheless at higher concentrations repellent effect of all three extracts were not significantly different ($P< 0.05$) from each other in spite of their different polarity levels. This suggests that the repellent activity of the leaf extracts may be due to a combination of different types of allelochemicals. Regardless of the solvent used, all three extracts at all concentrations produced considerably high repellent activity in weevils signifying that even lower amounts of the leaf material could be used effectively as a repellent against the rice weevil.

The present study reveals that the repellent effect of *O. zeylanica* on *S. oryzae* was directly attributable to the volatiles emitted from the leaves as the weevils were not in direct contact with the leaf powder or extracts. Moreover, previous studies conducted on fumigant toxic effect of *O. zeylanica* leaf volatiles on *S. oryzae* have shown 100% mortality in weevils even at small doses (Fernando & Karunaratne, 2012). These observations verify the fact that some volatile constituents of *O. zeylanica* leaves elicit both repellent and insecticidal effects. In view of these results it is quite apparent that *O. zeylanica* leaves can be used as an effective fumigation agent in controlling rice weevil infestations.

In recent years, many research workers have given greater attention to the control of stored grain pests especially *S. oryzae* using leaf and seed powders, extracts and essential oils of various plant species. Mishra et al (2012) demonstrated that essential oils of *Eucalyptus globulus* and *Ocimum basilicum* leaves were effective in repelling rice weevils. Khani *et al* (2011) evaluated the toxicity and repellency of crude extracts of *Piper nigrum* and *Jatropha curcas* on *S. oryzae* adults and stated that both species of plant extracts could be applied against rice weevil effectively. Leaf powder of *Mentha viridis* has been observed to be highly effective as a repellent to *S. oryzae* (Gunaratne & Karunaratne (2009).

The findings of the present study indicate that active compounds present in the powders and extracts of *O. zeylanica* leaves may play a role in the biological activity against adult *S. oryzae.* These compounds may independently or jointly contribute to cause repellent action against *S. oryzae*. These results also suggest that leaves of this plant have potential to provide rice grain protection and may be exploited for rice weevil control in storage in an environmental friendly way. However, further studies are needed to isolate and identify the active chemical compounds responsible for this activity and to examine the effect of powdered leaves and leaf extracts of *O. zeylanica* against a wider-range of storage insect pests.

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