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Ethnobotanicals for Storage Insect Pest Management: Effect of Powdered Leaves of *Olaxzeylanica* in Suppressing Infestations of Rice Weevil *Sitophilusoryzae* (L.) (Coleoptera: Curculionidae)

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Abstract

The rice weevil, *Sitophilus oryzae* is one of the major pests of stored rice in Sri Lanka. This study investigates the effectiveness of the botanical *Olax zeylanica* in controlling infestations of the rice weevil.In two separate bioassays, contact/feeding and fumigant toxicity of powdered leaves of *O. zeylanica* were tested against 1-7 days old adult *S. oryzae* under laboratory conditions. Contact/feeding toxicity was tested by directly exposing adult weevils to 1.0, 3.0, 5.0 and 7.5g of leaf powder mixed with 100g of rice grains while fumigant toxicity was evaluated by using the same doses where theadults were exposed to fumes emitted from the leaf powders. In both bioassays 100% mortality of the adult *S. oryzae* was observed within 18 hours of exposure to 3.0, 5.0 and 7.5g doses of leaf powder. Percentage adult weevil mortality in treated rice tested with three doses of *O. zeylanica* leaf powder at all the time intervals (except for 1.0g) was significantly higher ($p < 0.05$) than that of the corresponding control. No Contact/feeding toxicity was recorded when adult weevils were directly exposed to 1.0g leaf powder whereas only 14% adult weevil mortality was observed even after 24hours of exposure to fumes ofleafpowder. Results also revealed that mortality increased both with increasing dose and time of exposure. In both bioassays a 100% adult weevil mortality was obtained after18 hours of exposure to 3.0g leaf powder of *O. zeylanica* or to its fumes.Moreover, LD50 values of 2.55g and 2.08g for leaf powders obtained after 12 hours of exposure to adult *S. oryzae* in contact/feeding toxicity test and fumigation test respectively.The results indicatedthat leaf powder of *O. zeylanica* is more toxic to adults *S. oryzae* when they were in direct contact with it.Findings of this study bears out the exceptionally high efficacy of *O. zeylanica* leaves applied directly mixed with the rice grains or introduced as a fumigant to suppress weevil infestations in stored grains and strengthen the possibility of using powdered leaves of *O. zeylanica* as an alternative to synthetic chemicals in storage insect pest management.

Keywords: *Olax zeylanica, Sitophilus oryzae, feeding toxicity, direct fumigation toxicity*

1. Introduction

The utilization of plants with insecticidal properties to protect stored commodities against insect pest attack has a very long history (Belmain and Stevenson, 2001). Leaves, bark, roots, twigs and flowers locally available plantsmixed with various stored products have been used for major insect pests

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of stored products as protectants in different parts of the world for centuries (Hassanali and Lwande, 1989; Isman, 2006). The efficient control as well as removal of insect pests from stored food has long been the goal of many entomologists throughout the world. Scientific literature documenting bioactivity of plant materials to insect pests shows, that a great number of plant species from a wide range of families have been assessed for their toxic, antifeedant and repellant properties (Isman, 2006; Talukder, 2006; Dubey et al., 2008; Ogunleye et al., 2010). Many of the plant species that have been investigated are often those used as culinary spices or in traditional medicine by local communities (Lale, 1992). Some researchers surmise that these plant materials are therefore safe to use as insecticides. Many researchers are trying to validate the efficacy of ethnobotanicals which are readily available in the local environment for farmer use at village level (Ahmed and Koppel, 1985)

Botanical insecticides have long been touted as attractive alternatives to synthetic chemical insecticides for pest management because botanicals reputedly pose little threat to the environment or to human health (Isman, 2006). However, apart from localized use of traditional plant materials and isolation of a number of phytochemicals with insecticidal properties only a handful of botanicals such as pyrethrum, rotenone and neem are in widespread use (Dev and Koul, 1997; Isman, 1997). The increasing attempts to replace synthetic insecticides with less expensive and locally available pest control means have been undertaken especially in the tropics (Jermy, 1990). In the context of agricultural pest management, botanical insecticides can play a much greater role in the production and postharvest protection of food in developing countries (Isman, 2006) who have the best supplies of the natural resource and have the most to gain from the development and local use of simple plant extracts for crop protection (Koul and Dhaliwal, 2001). In many countries, plant tissues or crude products of the plants, such as aqueous or organic solvent extracts, are used directly as protectants of stored products. These practices are labour intensive, but are often economically and ecologically sound, and do not require sophisticated technology (Talukder, 2006). Considering these facts, the present investigation was aimed at evaluating the toxic properties of powdered leaves of a local medicinal plant *Olax zeylanica* (Malla) belonging to the family Olacaeceae against *Sitophilis oryzae*, one of the major storage insect pests of worldwide distribution.

2. Materials and Methods

2.1 Insect Cultures

Parent stock of *S. oryzae*was obtained from infested raw white rice bought from the local market. Laboratory cultures of *S. oryzae*were maintained on uninfected white raw (Sudukekulu) rice. 25 pairs of adult rice weevils were introduced into plastic jars containing 400g of rice. These plastic jars were then covered with a muslin cloth to prevent insects escaping and to allow ventilation. After two weeks the adults were removed and the rice medium was kept in ambient laboratory conditions (30 ± 1^0C and relative humidity of 85 ± 1 %) for the emergence of *S. oryzae* adults. For all the experiments 1-7 days old, unsexed adult weevils were selected from ongoing cultures.All the experiments were carried out under ambient laboratory conditions.

2.2 Plant Material

Fresh, mature leaves of *O. zeylanica* were used for all the experiments. These were washed, air-dried and ground to a fine powder using a domestic electric grinder (Multinational, 2101, India). Leaf powders obtained in this manner was used for all experiments.

2.3 Contact/Feeding Toxicity Test

Powdered leaves of *O. zeylanica*, weighing 1.0, 3.0, 5.0 and 7.0g, were mixed with 100g of white raw rice (Sudukekulu) in separate plastic containers (height 8cm, diameter 5cm) using a glass rod. Thirty adult *S. oryzae*weevils were then introduced into each container and the mouth of the container was covered with a muslin cloth. In the control, 30 adult insects were introduced into 100g of untreated rice. The number of dead adult weevils in each container was recorded after 6, 12, 18, 24 hours after their introduction. This experiment was replicated 5 times.

2.4 Fumigation Toxicity Test

The bio-apparatus for the direct fumigation toxicity test consisted of a plastic cup (height 8cm) inserted into a plastic container (height 12cm, diameter 8cm). Required doses (1.0, 3.0, 5.0 and 7.0g) of freshly ground plant leaves were placed on the bottom of this container. The bottom of the plastic cup was cut off and replaced with a muslin cloth so that fumes emitting from leaf powder would reach the insects above. After the plastic cup was inserted into the container, 30 adult weevils were introduced into it and the top was covered with a polythene film. A similar bioassay set up without any plant material was considered as the control. Observations on the adult weevil mortality were recorded after 6, 12, 18, 24 hours after the introduction of insects. Five replicates were made in this experiment.

2.5 Data Analysis

The means of mortality of each dose were compared using one-way analysis of variance (ANOVA) and Tukey's pairwise comparison test. LD50 and LD99 values were determined by Probit Analysis. For all statistical calculations, Miniatb 14 was used.

3. Results and Discussion

Contact/feeding toxicity of different doses of *O. zeylanica* leaf powder on *S. oryzae* at different time intervals are shown in Table1. According to the observations, all the doses of leaf powder except the lowest dose (1.0g) showed significantly very high insecticidal effect on *S. oryzae* adults. In fact, adult mortality varied with dose and exposure periods indicating the mortality effect of the leaf powder treatments were dose and time dependent. However, it was evident that 1.0g of leaf powder was not effective even after 24 hours of exposure exhibiting only 3% adult mortality.Moreover, 0% adultmortality was observed within 18 hours of exposure to both 1.0g of leaf powder and the control. All thethree other doses 3.0g, 5.0g and 7.0g of leaf powders exhibited 100% adult weevil mortality within 18 hours of exposure period to treatments.

Table1: Direct contact/feeding toxicity effect of different doses of *O. Zeylanica* leaf powder on *S. oryzae*

Dose (g)	Percentage Mortality			
	6 HAT	12 HAT	18 HAT	24 HAT
Control	0.00 ± 0.00 ^a^	0.00 ± 0.00 ^a^	0.00 ± 0.00 ^a^	0.00 ± 0.00 ^a^
1.0	0.00 ± 0.00 ^a^	0.00 ± 0.00 ^a^	0.00 ± 0.00 ^a^	3.33 ± 3.33 ^a^
3.0	22.00 ± 3.80 ^b^	69.33 ± 4.34 ^b^	100.00 ± 0.00 ^b^	100.00 ± 0.00 ^b^
5.0	71.33 ± 7.30 ^c^	96.67 ± 4.08 ^c^	100.00 ± 0.00 ^b^	100.00 ± 0.00 ^b^
7.0	87.33 ± 6.41 ^d^	99.33 ± 1.49 ^c^	100.00 ± 0.00 ^b^	100.00 ± 0.00 ^b^
Probability	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$

Means followed by the same letters in each column are not significantly different according to Tukey's test at p > 0.005 significance level.
Mean Percentage Mortality± SD for five replicates (n = 150)
HAT – Hours AfterTreatment

The analyzed results of the present study indicated a similar dose and time dependent pattern of mortality when adult *S. oryzae* weevils were directly exposed to fumes of *O. zeylanica* leaf powder (Table2). Furthermore, results of this bioassay showed that fumigation toxic effect produced by the powdered leaves was higher than that of the contact or/and feeding toxicity effect it elicited. Moreover, even 3.0g of leaf powder produced weevil mortality as high as 91% when they were exposed to the leaf fumes for 12 hours. On the other hand, only 69% mortality was noted for the same dose and exposure period, when the leaf powder was mixed with the rice medium (Table1). However, with the higher doses (5.0 and 7.0) 100% adult weevil mortality was observed after 18 hours in both types of bio-assays. The extremely high weevil mortality seen in the fumigation bio-assay suggests that some volatile substance/s emanating from the powdered leaves may have a lethal effect on *S. oryzae* weevils. Furthermore, the strong repulsive odour detected from *O. ceylanica* leaves when ground to powder indicates the release of some volatile substance/s.

Table 2: Direct fumigation toxicity effect of different doses of *O. zeylanica* leaf powder on *S. oryzae*

Dose (g)	Percentage Mortality				
	6 HAT	9 HAT	12HAT	18 HAT	24 HAT
Control	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	0.00 ±0.00 [a]	0.00 ±0.00 [a]
1.0	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	1.67 ± 2.79 [a]	12.23 ± 7.79 [a]
3.0	0.00 ± 0.00 [a]	28.33 ± 6.91 [b]	91.67 ±3.50 [b]	100.00 ± 0.00 [b]	100.00 ± 0.00 [b]
5.0	24.43±14.56 [b]	88.90 ± 8.07 [c]	97.78 ±2.72 [c]	100.00 ± 0.00 [b]	100.00 ± 0.00 [b]
7.0	73.33 ± 5.96 [c]	97.23 ± 2.51 [d]	100.00 ± 0.00 [c]	100.00 ± 0.00 [b]	100.00 ± 0.00 [b]
Probability	P < 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05

Means followed by the same letters in each columns are not significantly different according to Tukey's test at p>0.005
** * Mean Percentage Mortality± SD for five replicates (n= 150)*
** HAT – Hours AfterTreatment*

The probit mortality lines for leaf powder for both contact/feeding and fumigation toxicity tests were analyzed at 6 and 12 hours after the treatment. Values of LD50 and LD99 and their respective 95% fiducial confidence limits for both contact/feeding toxicity as well as fumigation toxicity are presented in Table 3.

Table 3: Probit analysis for contact and fumigation toxicities of *O. zeylanica* leaf powder on adult *S. oryzae* after 6 and 12hrs of exposure

Time	*LD $_{50}$ value (g)		**LD $_{99}$ value (g)	
	Fumigant	Contact	Fumigant	Contact
6 HAT	5.95 (5.68-6.23)	4.16 (3.84-4.48)	10.29 (9.17-12.36)	12.21 (10.24-15.78)
12 HAT	2.08 (1.84-2.29)	2.55 (2.28-2.78)	4.31 (3.83-5.07)	6.04 (5.29-7.33)

**LD 50 – lethal dosage that kills 50 % of the weevil population*
***LD 99 – lethal dosage that kills 99 % of the weevil population*
95% lower and upper fiducial limits are shown in parenthesis
HAT – Hours After Treatment

Based on this analysis it can be clearly stated that LD 50 value of fumigation toxicity exceeds the corresponding value of contact toxicity only at 6 hrs. after treatment but in all the other instances, the dose required to attain 50% weevil mortality was lower in the fumigation test than it was in the contact toxicity test. Also, the lowest LD50 values of 2.55g and 2.08g for leaf powders were obtained after 12 hours of exposure to insects in contact/feeding toxicity test and fumigation test respectively.

When these results are taken into consideration, it is quite apparent that *O. zeylanica* leaves could be to some extent more effective when used as a fumigant for the control of rice weevil. However, the contact toxicity of *O. zeylanica* leaves on *S. oryzaeweevils* is also nearly as high as fumigation toxicity. Therefore, the ability of this plant material to produce lethal effects against *S. oryzae* within a time period as short as 12-18hrs after treatment can be attributed to both fumigant and contact or/and feeding toxic properties of the leaf powder on *S. oryzae*.

Powders of various plant species with insecticidal activity have been used previously by several researchers in laboratory trials for the control of stored product pests including *S. oryzae* (Niber, 1994; Haq et al., 2005; Akob and Ewete, 2007; Law-Ogbomo and Enobakhare, 2007). Large variation in the sensitivity of stored grain pests to fumigation toxicity of volatiles of many plants have also been reported by several workers (Lee et al.,2001; Schmidt et al.,1991; Shaaya et al.,1997; Tripathi et al., 2002).

Plants belonging to the genus *Olax* reportedly have antimicrobial and anti-inflammatory properties (Ayandele and Adebiyi, 2007). In traditional Sri Lankan medicine, leaves of *O. zeylanica*are used to treat many ailments such as Hypercholesterolemia (Ediriweera et al., 2010) and snake bites. As *Olax zeylanica* is also a plant material consumed by Sri Lankans this would be ideal to use it as a grain protectant against rice weevil infestations.

The insecticidal activity of botanicals may possibly be dependent on different factors such as the presence of bioactive chemicals with diverse activities. The powdered leaves of *O. zeylanica* tested may act as a fumigant and a stomach poison. Also the powder may act as a physical barrier blocking the spiracles of the insects thus impairing respiration leading to their death (Law-Ogbomo and Enobakhare, 2007; Mulungu et al., 2007).

4. Conclusion

The extremely high fumigation activity observed in the present study shows that insecticidal properties of leaves of *O. zeylanica* could be a source of some biologically active volatile compound/s that is potentially an efficient insecticide. Consequently, the possibility of utilizing this natural fumigant to control stored product insectpests is worthy of further investigations. Moreover, Sri Lankan farmers who are aware of the importance ofnon- chemical based eco-friendly control methods would certainlyprefer to use such ethno-botanicals as stored product protectants.

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