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Potential of *Olax zeylanica* (Olacaeceae) as a botanical insecticide for the control of the Maize Weevil *Sitophilus zeamais* Motschulsky

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Abstract

The possible use of leaf powder of *Olax zeylanica* in managing the maize weevil, *Sitophilus zeamais* was investigated in the present study. Mortality of one week old adults was obtained for five different doses each for their insecticidal effect (1.0, 2.0, 3.0, 5.0 and 7.5g) and fumigation toxic activity (2.0, 3.0, 5.0, 7.5, 10.0g) separately. In each bioassay, the mortality was recorded after 3, 6, 9, 12, 18, and 24 hours after they were exposed to different doses of the plant powder. The results of both bioassays revealed that the leaf powder of this plant elicited a very high insecticidal activity as well as fumigation toxic activity in *S. zeamais* weevils. Mortality effect of the powders on the weevils was found to be dose and time dependent. The observations indicated that the adult mortality increased with the increase of the exposure time and the dose. No mortality was observed 3 hours after the exposure of weevils to leaf powder at all doses. A high mortality rate was observed after 9 hours of exposure with all doses except for the lowest and a100% mortality was recorded with 7.5g after 24 hours. Fumigation toxicity of plant powder against the weevils also gave similar results where no weevil deaths were observed at all doses of leaf powder after 3 hours of exposure. At the highest dose of 10.0g, 98% mortality was observed after 24 hours. The overall results imply the strong possibility of using leaves of *Olax zeylanica* as a potent bio-insecticide in protecting maize grains from *S. zeamais* infestations.

Key words: *Olax zeylanica*, *Sitophilus zeamais*, insecticidal effect, fumigation toxicity, adult mortality, leaf powder

Introduction

Maize (*Zea mays* L.) is one of the major cereal crops produced worldwide. It is a staple food in many countries throughout Asia, Africa and Latin America where it is a cheap source of dietary carbohydrate (Blackie and Jones, 1993). Post harvest storage of maize is greatly constrained by the pest, *Sitophilus zeamais* Motschulsky. It is associated primarily with maize although it is capable of developing on all cereal grain and cereal products (Bekele, 2002). Initial infestations of maize grain occur in the field just before harvest and the insects are carried into the store where the population builds up rapidly (Appert 1987). The huge post harvest losses and quality deterioration caused by this insect pest is a major obstacle to achieving food security. Reduction of insect damage in stored grains is a serious problem in developing countries in the tropics due to favorable climatic conditions and poor storage structures (Rouanet, 1992).

Insect pest control in stored food products relies heavily on the use of synthetic insecticides. However the use of these chemicals to protect maize grain against the attack of grain weevil in storage may cause serious health hazards (Talukder and Howse, 1994), problems of pest resistance and resurgence is quite expensive to smallholder farmer situation (Iloba and Ekrakene, 2006). These problems have necessitated research on the use of alternative eco-friendly cheaper insect pest control methods amongst which are the use of powdered plant parts and their extracts (Cobbinah and Appiah-Kwarteng, 1989).

A number of indigenous plants in Sri Lanka are known to possess some biological activity against insects (Bandara et al, 1987; Hewage et al, 1997). *Olax zeylanica* belonging to the family Olacaeceae is a native medicinal plant in Sri Lanka. Leaves of this plant are consumed as salads by local people. Also, Olax leaves are known to be used by rural farmers to repel storage insect pests. According to our knowledge, no scientific research has been carried out yet for the insecticidal activity or repellent action of this plant. The present investigation was therefore, undertaken to study the possible insecticidal properties of *Olax zeylanica* against the maize weevil, *S. zeamais* with the intention of isolating and chemically determining its bioactive components.

Materials and Methods

Adult maize weevils were obtained from infested maize seeds at the local market. They were introduced into 1 liter plastic containers containing clean, un-infested maize grains. The containers were then covered with muslin cloth held in place with rubber bands. Freshly emerged one week old adults were used for all the experiments. Weevil cultures were maintained under

laboratory conditions at ambient temperature of 30±1 °C and at a relative humidity of 84 - 86%. fresh mature leaves of *Olax zeylanica* were collected from Kalutara District, washed and air dried. These were ground using a domestic electric grinder (Multi national, 2101, India) into a fine powder and kept in an air tight jar before using in the experiments.

Insecticidal Effect

Powdered plant leaves at the rate of 1.0g, 2.0g, 3.0g, 5.0g and 7.5g were admixed with fifty grams (50g) of clean and un-infested maize grains in separate plastic cups (8cm diameter x 6.5cm height). A control experiment was set up without mixing any leaf powder with the grains. Batches of thirty, unsexed adult insects were then introduced into each cup. Muslin cloth was used to secure the mouths of the plastic cups to ensure aeration and also to prevent insects escaping from containers.

The number of dead insects in each plastic cup was recorded after 3, 6, 9, 12 and 24 hours and percentage adult weevil mortality was calculated for each treatment. Adults that showed no visible movement after 30 seconds were considered as dead. Each treatment was replicated five times. Data on percentage adult weevil mortality were corrected using Abbott's (1925) formula.

$P_T = (P_0 - P_c) / (100 - P_c)$

Where P_T = corrected mortality (%)

P_0 = observed mortality (%)

P_c = control mortality (%)

Fumigant Toxicity Effect

The fumigation chamber used in this experiment is shown in Figure 1. Batches of thirty, unsexed adult insects were introduced into the small plastic cup (8cm× 6.5cm??) which contained 50g of clean and un-infested maize grains. The plastic cup was fitted into a large plastic container (15cm × 7cm). Fresh, powdered plant leaves at the rate of 2.0, 3.0, 5.0, 7.5 and 10g were placed in the bottom of the large container separately. The hole on the bottom of the small plastic cup was covered with a thin muslin cloth so that plant vapour would pass through it and saturate the atmosphere of the plastic cup containing the maize weevils. The top of the fumigation chamber was capped with an airtight lid. The control consisted of a similar setup but without any plant material. This experiment was replicated five times. Adult mortality counts were made 3,6,9,12,18 and 24 hours after treatment. Correction of natural mortality in the control was made by using Abbott's (1925) formula. (Figure 1)

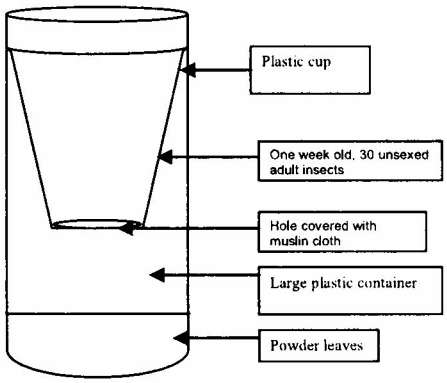

Figure 1. Schematic diagram of the Bioassay chamber for testing fumigation toxicity effect of *Olax zeylanica* on *S. zeamais*

Data analysis

Data were subjected to one-way analysis of variance (ANOVA) and means of different treatments were separated using Tukey's test in 'Minitab 14' at a 0.05 level of significance.

Results and Discussion

Insecticidal effect

The insecticidal effect of various doses of *O. zeylanica* leaf powder on the adults of *S. zeamais* is presented in Figure 2 and Table 1. Mortality effect of the powders on *S. zeamais* adults was found to be dose and time dependent. The data in fact indicated that, the adult mortality increased with the increase of the exposure time and as well as the dose. Also, there was no mortality of *S. zeamais* in the untreated grains. (Figure 2)

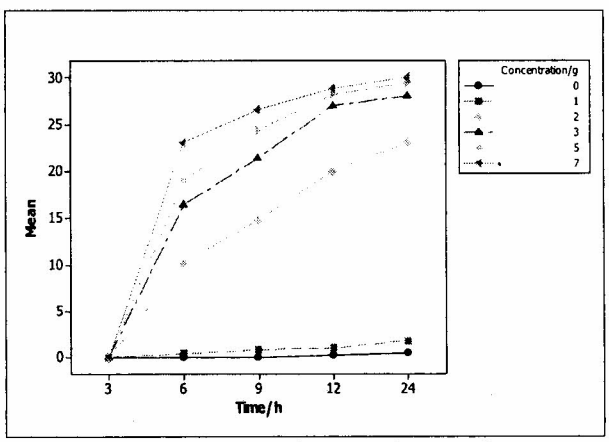

Figure 2. Insecticidal effect of *Olax zeylanica* leaves on adult *Sitophilus zeamais* after different times of exposure

As shown in Table 1, all the doses tested for their insecticidal efficacy had an effect on the percentage weevil mortality and was found to be directly proportional to the amount of powder used. However, no mortality was observed 3 hours after the exposure of weevils to leaf powder at all doses. Similarly, a very low amount of mortality resulted at all times when the weevils were exposed to the lowest dose (1g) of the leaf powder. The powder of *O. zeylanica* affected the survival of the adult weevils in treatments (2.0, 3.0, 5.0 and 7.5g) with a mortality rate ranging from 76.66 - 100 percent after 24 hours of their introduction in to maize. Moreover, the results revealed that at all doses except1.0 and 2.0g, more than 50% weevils were dead immediately after 6 hours of exposure to the leaf powder (Table1).

Table1. Percentage mortality of adult *Sitophilus zeamais* exposed to different doses of *Olax zeylanica* leaf powders

Leaf concentration (g)	***Time/hour***				
	3	**6**	**9**	**12**	**24**
1.0	0.00±0.00[a]	1.33±0.54[a]	2.66±0.44[a]	3.33±0.00[a]	6.00± 0.83[a]
2.0	0.00±0.00[a]	34.00±1.30[b]	49.33±2.49[b]	66.66±3.24[b]	76.66±2.12[b]
3.0	0.00±0.00[a]	54.66±4.09[c]	71.33±5.07[c]	90.00±2.82[c]	93.33±2.34[c]
5.0	0.00±0.00[a]	63.33±8.30[c]	81.33±4.03[c]	94.00±0.83[c]	98.00±0.89[c]
7.0	0.00±0.00[a]	76.66±2.44[c]	88.66±3.28[c]	96.00±0.44[c]	100.00±0.00[c]
F value	NS	30.04	69.80	278.64	492.02

- Percentage Means followed by the same letter are not significantly different from each other (p>0.05 Tukey's test)
- Mean value for five replicates (Mean±SD)
- NS- Not significant at p= 0.05 level

Fumigation Toxicity Effect

Figure 3 illustrates the mean number of adult morality at different doses of plant leaf powder preparations and that of the control. Mortality of the exposed *S. zeamais* adults increased with the increase of the exposure time and dose. Adult mortality at the dose of 2.0g of leaf powder was more or less similar to that of the control. (Figure 3)

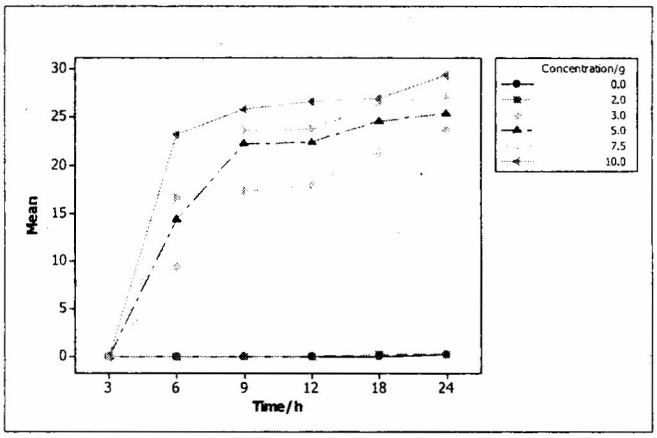

Figure 3. Fumigation toxicity effect of *Olax zeylanica* on adult *S. zeamais* at different times of exposure

The fumigant effect of leaf powder of *O. zeylanica* on the cumulative mortality of adult *S. zeamais* is depicted in Table 2. Similar to the insecticidal effect, the fumigant toxicity effect of the powders on *S. zeamais* adults was also found to be dose and time dependent. There was no mortality of *S. zeamais* in the untreated grains. Also, no mortality was recorded for all the doses after 3 hours of exposure to the treatment. Mortality of rice weevil was almost nil at the dose of 2g of leaf powder. In fact the mortality increased from 0.0 % to only 0.66% even after 24 hours of exposure. However, the highest dosage (10.0g) of plant leaf powder tested induced an extremely high level of mortality (98%) in the weevils, 24 hours after treatment. This was significantly different ($p>0.05$) to the mortality of all the other treatments. In addition 10.0g of the leaf powder elicited 77% mortality just after 6 hours of post treatment.

Table2. Percentage fumigation mortality of adult *Sitophilus zeamais* at different doses of powdered leaves of *Olax zeylanica*

Leaf Concentration (g)	Time/hour					
	3	6	9	12	18	24
2.0	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [a]	0.66±0.44 [a]	0.66±0.44 [a]
3.0	0.00±0.00 [a]	31.33±5.03 [b]	57.33±0.83 [b]	60.00±0.00 [b]	71.33±2.51 [b]	78.66±2.07 [b]
5.0	0.00±0.00 [a]	48.00±1.51 [c]	74.00±3.03 [c]	74.66±3.28 [c]	82.00±3.13 [c]	84.66±2.40 [b]
7.5	0.00±0.00 [a]	55.33±0.54 [c]	78.66±2.70 [c]	79.33±2.95 [c]	88.00±3.28 [c]	90.66±2.58 [c]
10.0	0.00±0.00 [a]	77.33±3.11 [d]	86.00±3.83 [c]	88.66±3.13 [d]	90.00±2.82 [c]	98.00±1.34 [d]
F-value	NS	69.47	131.10	148.60	142.86	294.86

- Percentage Means followed by the same letter are not significantly different from each other ($p>0.05$ Tukey's test)
- Mean value for five replicates (Mean±SD)
- NS – Not significant at $p= 0.05$ level

The overall results of the experiments clearly indicated that the leaf powder of *O. zeylanica* at relatively high doses is extremely toxic to the adult weevils. Considerable attention has been focused on many plant derivatives, potentially useful as commercial insecticides. World wide reports on insecticidal effects of different plant materials including plant leaf powders have shown that many of them are toxic to stored-product insects. (Araya and Emana, 2009; Epidi and Odili, 2009; Law -Ogbomo and Enobakhare, 2007; Shukla *et al.*, 2007). Danjumma *et. al* (2009) reported that powders of *Nicotiana tabacum, Allium sativum* and *Zingiber officinale* are highly toxic to *Sitophilus zeamais*. Similarly, Asawalam *et al.* (2007) discussed the potential of powders of *Piper guineense* and *Capsicum frutescens* as attractive candidates in upgrading traditional post-harvest protection practices. Mulungu *et al.* (2007) observed that leaf powder of *Carica papaya*, *Azadiracta indica* and *Eucalyptus macrorhyncha* can be used as good alternatives to synthetic pesticides against *S. zeamais*. It is of great interest to note that the present study carried out using powdered leaves of *O. zeylanica* too produced extremely high mortality of the adult weevils.

Insecticidal property of plant powders may depend on the presence of bioactive chemicals which need to be identified, isolated and synthesized. These compounds may act as fumigants, stomach poisons, contact poisons and physical barriers blocking the spiracles (Law-Ogbomo and Enobakhare,

2007; Mulungu et al., 2007). The presence of these bioactive compounds may have independently or jointly contributed to cause toxic action against *S. zeamais*. Furthermore the highly significant fumigant toxicity to the weevils in this study strongly indicates the presence of volatile compound/s in the plant. In fact, leaves of this plant emitted a very strong, pungent odour when ground to a powder. The volatile compounds may have exerted fumigant toxic effect on the weevils by disrupting their normal respiratory activity, thereby resulting in subsequent death (Adedire and Ajayi, 1996). The results of the current study strongly demonstrate the possibility of using the leaves of this plant as a toxicant against *S. zeamais*. Furthermore, it is of importance to state that no previously reported scientific information was found on the effects of *Olax zeylanica* on any insect pest including *S. zeamays*.

The present study clearly indicated that leaves of *O. zeylanica* possess insecticidal and fumigant properties that can be used in the control of *S. zeamais* in stored grain at the same level as synthetic insecticides. This finding therefore, is of great value for further research to identify the active compound(s) responsible for its insecticidal activity and also to examine the effect of the leaves of this plant against a wider range of insects. Also, the availability of this plant in rural areas makes it an attractive candidate as a grain protectant. Moreover, due to the reasons that it is used as a medicinal plant and the leaves are consumed as salads by the local people, any adverse toxic effect/s of this plant on non-target organisms would be minimal. Finally, the significantly very high mortality (attaining 100%) induced by *O. zeylanica* suggests an excellent protectant most suitable for incorporation into an integrated management system of storage insect pests.

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