

POTENTIAL OF *Olax zeylanica* LEAVES IN THE SUPPRESSION OF *Sitophilus oryzae* L. INFESTATIONS

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ABSTRACT

In spite of the growth of paddy production, significant losses occur globally during post harvest storage of rice, due to the rice weevil *Sitophilus oryzae* attack, which is considered the major insect pest of stored rice. In order to develop green alternative to synthetic insecticides, the repellent and toxic properties of *Olax zeylanica* leaf powder, solvent extracts and essential oil were tested against *S. oryzae* under laboratory conditions. Laboratory reared one week old adults were used to determine the efficacy of plant products. Repellent effect was evaluated within an hour of exposure using a contact-repellency chamber by admixing leaf powder with 30g of rice grains at 4 doses (1, 3, 5 and 7 g). Repellency of leaf-solvent extracts (methanol, ethyl acetate, hexane and water) was investigated at 10%, 50% and 100% (v/v) while the repellency of essential oil was assessed at same concentrations using area-preference method and all the observations were recorded after 30 minutes. Observations on contact and fumigation toxicity of the essential oil were made 30 and 60 minutes after exposure to weevils. All tested plant products elicited significantly very high responses in *S. oryzae* when compared with the controls (0.00%). Leaf powder of *O. zeylanica* evoked 100% repellency at the highest dose (23.33% w/w) and the repellent rate increased with the increase in dose. In repellency assays using leaf extracts, methanol extract was found to be the most effective causing 96% repellency in the weevils, followed by ethyl acetate (95%) and hexane (89%) whereas the aqueous extract was least effective (70%) indicating the lowest level of repellency. Furthermore, three concentrations of the essential oil evoked extremely high repellent action in weevils (94%, 99%, and 100% respectively) within 30 minutes. Both contact and fumigant toxicity effects with the essential oil were dose dependent and 100% adult mortalities were observed at the highest concentration after one hour of exposure. The overall results of the present study indicate that, *O. zeylanica* leaves could be utilized as a very successful candidate in the development of bio-insecticides in controlling *S. oryzae* infestations.

Key words: *Olax zeylanica*, *Sitophilus oryzae*, essential oil, leaf-solvent extracts, repellency, toxicity

INTRODUCTION

Rice is the most important food crop for more than half of the world's population (Pratheep-Kumar *et al.*, 2004). *Sitophilus oryzae* L. is a very common insect pest infesting on rice and they are among the most economically important stored product insect pests (Pugazhvendon *et al.*, 2009).

Attempts have been made to get total suppression of these insect pests through fumigants and insecticide applications, but their extensive use has led to the escalation of some major problems including development of insect resistance to insecticides (Ribeiro *et al.*, 2003),

toxic residues in food, toxicity to consumers and increasing cost of application (Sighamony *et al.*, 1990; Mishra *et al.*, 2012). Moreover, unrestrained use of synthetic insecticides has caused many hazards to both environment and consumers due to their residual properties (White, 1995; Mishra *et al.*, 2012). Hence, there is a vital importance in developing safe and sound alternatives that are of low cost, convenient to use, environmentally friendly and less toxic to mammals (Jembere *et al.*, 1995; Pugazhvendan *et al.*, 2012).

In this context, highly evolved plants provide an important source of novel insecticides (Prakash and Rao, 1997). The use of sub-

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stances extracted from plants as bio-insecticides has many advantages when compared to synthetic preparations for insect pest control because the plant material is renewable and degrades rapidly so it does not persist in the environment (Knaak *et al.*, 2013). Numerous plant species and their extracts possess significant capability in affecting insects through repellency, contact toxicity or fumigation (Dev and Koul, 1997; Shaaya *et al.*, 1997; Hou and Taylor, 2006). The use of plant products as insecticides could be broadly classified under (a) powders or fresh application, (b) volatile oils, (c) non-volatile oils, and (d) extracts in application technology (Rajapakse, 2006).

Taking into account the enormous value of the plant derived green insecticides, a detailed investigation on repellent potential of powder, solvent-extracts, essential oil together with the toxic activities of the essential oil using the leaves of *Olax zeylanica* (Mella) was carried out against the rice weevil, *Sitophilus oryzae* L. to evaluate the potential of utilizing this plant in the post-harvest protection of stored rice. *O. zeylanica*, was selected for this study as it is a locally available plant known to possess a number of medicinal properties and is consumed by the people in Sri Lanka.

MATERIALS AND METHODS

Rearing of the test insects

White raw rice grains infested by the *S. oryzae* were collected from a stock of rice in the local market. Infested seeds were then set aside in a large jar and covered with muslin cloth till the emergence of adults. Healthy rice weevil adults emerged from the jar were shifted to another container and provided wholly, uninfested seeds for oviposition which was maintained at $29 \pm 2^{\circ}\text{C}$ and $84 \pm 2\%\text{RH}$. One week old adult rice weevils were used in all the experiments. The remaining insects were used to start new generations, so that the cultures with emerging adults are available continuously.

Collection and processing of plant samples

Fresh leaves of *Olax zeylanica* were collected from Piliyandala area. Plant material was rinsed, shade dried, powdered by using an electric grinder and then stored at 4°C in a refrigerator prior to use.

Preparation of leaf extracts

The leaf powders (60 g) were extracted with 99% methanol and distilled water in a soxhlet apparatus (Shanti Scientific Industries, India). After 18 hours of continuous extraction at 62°C , a fraction of methanol extract was further partitioned between 95% n-hexane and 99% ethyl acetate solvents. Methanol and distilled water leaf extracts together with the n-hexane and ethyl acetate fractions were then concentrated to 50 ml using a rotary evaporator at 65°C and stored in a refrigerator at 4°C . These final products were then re-dissolved in the respective solvent to obtain a series of dilutions of 10, 50 and 100% (v/v) of each plant extract for the experimental use.

Extraction of essential oil

The freshly ground leaf powder of *O. zeylanica* was subjected to hydro-distillation using a modified Clevenger-type apparatus continuously for 3 hours to yield the essential oil. Essential oils were stored in airtight glass vials in a refrigerator at 4°C . For the bioassays, three solutions at the concentrations of 10, 50 and 100% (v/v) were prepared by dissolving the essential oil in hexane.

Contact repellent effect of leaf powder

Leaf powder of *O. zeylanica* was tested for contact repellency adapting the method suggested by Mohan and Fields (2002) with some alterations. The freshly powdered plant leaves were admixed at 1, 3, 5, and 7g with 30g of uninfested rice grains in separate small plastic cups (height 8cm, diameter 7.5cm). One week old, 20 adult weevils were introduced into each cup. The top part of the small plastic cup was perforated through which the weevils are allowed to escape from the plastic cup if repelled by the plant powder. This cup was then placed

inside a large plastic bottle (height 15cm, diameter 7.5 cm) to trap the weevils escaping from the small cup. Before the onset of each experiment, these holes were covered with a sticky tape for several minutes to let the introduced rice weevils settle down inside the plastic cup. The bio-assay apparatus was then covered with muslin cloth held firmly with rubber bands to prevent the escape of the weevils and ensure adequate aeration. A similar bio apparatus setup without leaf powder served as the control. The number of repelled insects in the large plastic bottle was counted after an hour of their introduction to estimate rice weevil repellency. The experiment was replicated 5 times.

Repellent effect of leaf extracts

Repellent effect of leaf extracts against *S. oryzae* adults was tested using the area preference method described by McDonald (1970), Talukder and Howse (1993) and Mishra and Tripathi (2011) with slight modifications. Filtermann® (125mm) papers were cut into 2 equal halves. One half of filter paper was treated with 1ml of each prepared concentration as uniformly as possible. The other half (control) was treated only with 1ml of the solvent. Both the treated and control halves were then air dried to evaporate the solvent completely. These were then attached together lengthwise and edge-to-edge with adhesive paper and placed on the bottom of a glass petridish. About 10g of clean and un-infested rice grains was uniformly distributed over the petri dish. One week old, 10 adult rice weevils were released at the center of each filter paper disc and the covered petridish was kept in the dark. 10 replicates were made for each plant leaf extract and concentration. The number of insects present on both treated and untreated halves was recorded after 30 minutes of their introduction.

Repellent effect of essential oil

Area preference test as mentioned in the above section was carried out to evaluate the repellency of adult weevils to essential oils.

For the assaying, prepared concentrations of 10, 50, and 100% (v/v) as treatments and hexane as the control were used. One week old, 10 adult rice weevils were released on to the center of each filter paper disc of the petri dish. Each concentration and the control were replicated 5 times. The number of insects present on each strip was recorded 30 minutes after the introduction of the weevils.

Contact toxic effect of essential oil

Aliquots of 0.5ml of the prepared concentrations at 10, 50, and 100% (v/v) were applied evenly on the inner surface of glass vials (diameter 1.5cm, height 10cm, volume 10ml) and the screw caps. The n-hexane solvent was used as the control. After the complete evaporation of solvents within a few seconds, unsexed and one week old, 20 adult rice weevils were introduced into each vial containing the culture media and the cap was screwed tightly on to the vial. Mortality of insects was recorded 30 and 60 minutes after the introduction of weevils. Each treatment was replicated 5 times.

Fumigation toxic effect of essential oil

The fumigant toxic activity of the *O. zeylanica* essential oil was investigated according to the method adapted after Liu and Ho (1999) and Fang *et al* (2010) with some modifications. A Filtermann® (125mm) paper strip (diameter 2cm) was impregnated with 0.5ml of the prepared concentrations of essential oil, and then placed on the underside of the screw cap of a glass vial (diameter 1.5cm, height 10cm, volume 10ml). The solvent was allowed to evaporate for a few minutes before the cap was placed tightly on the glass vial. Subsequently one week old, 20 adult rice weevils were introduced into each vial containing rice grains to form a sealed chamber. The solvent n-hexane was used for the control. Five replicates were made for all treatments and controls. Fumigant mortality of insects was recorded 30 and 60 minutes after the introduction of weevils.

Statistical Analysis

The data relevant to the observations were subjected to one-way analysis of variance (ANOVA) using the “Minitab”, version 14.0 software package. The mean values of the experiments were separated using Tukey’s multiple comparison test where significant differences existed ($p < 0.05$).

RESULTS AND DISCUSSION

The repellency effect of leaf powders of *O. zeylanica* on rice weevils after one hour of exposure in the contact repellency bioassay is shown in Table 1.

Table 1: Repellency effect of leaf powders of *Olax zeylanica* on *Sitophilus oryzae* after 1 hour in the contact repellency bioassay

Dose / g	*Mean % Repellency \pm SD
Control	0.00 \pm 0.00 ^a
1	70.00 \pm 3.54 ^b
3	88.00 \pm 2.74 ^c
5	97.00 \pm 2.74 ^d
7	100.00 \pm 0.00 ^d

*Means followed by the same letters in the columns are not significantly different according to the Tukey’s test at $P < 0.05$

*Mean Percentage Repellency \pm SD for five replicates ($n = 100$)

Leaf powders of *O. zeylanica* elicited the highest repellency of 100% at the highest dosage. Repellent rate of the leaf powders gradually increased with the increase in dose. Accordingly, leaf powders of *O. zeylanica* exhibited significantly similar, but relatively high influence on *S. oryzae*. It is quite noteworthy that the leaf powders showed as high as 70 % repellent action even at the lowest dosage of 1 g, thus demonstrating the extraordinary ability of *O. zeylanica* leaves have in grain protection.

Normally, the simplest way to apply plant leaves to a stock of grains is collecting leaves and mixing them with seeds. Many plants have been tested in the laboratory as powders to estimate their possible anti-insect effects. The Modes of action of these powders vary, but with low to moderate dosages, the effect is always repellent or toxic, never mechanical (Rajapakse, 2006).

The results of repellent effect of different leaf extracts applied using the area preference method are presented in Table 2. Among the leaf-solvent extracts of *O. zeylanica* (Hexane, Ethyl Acetate, Methanol and Aqueous), Methanol and Ethyl Acetate extracts revealed very strong repellent activities accounting for 96% and 95% respectively, whereas that aqueous extract exhibited comparatively

Table 2: Percentage repellency for *Olax zeylanica* leaf extracts on *Sitophilus oryzae* after 30 minutes of exposure in area preference bioassay

Concentration (%v/v)	*Mean % Repellency \pm SD			
	Hexane	Ethyl Acetate	Methanol	Water
10	69.00 \pm 7.38 ^a	71.00 \pm 6.38 ^a	79.00 \pm 7.38 ^a	59.00 \pm 8.76 ^a
50	83.00 \pm 8.23 ^b	88.00 \pm 6.89 ^b	93.00 \pm 6.75 ^b	63.00 \pm 6.75 ^{ab}
100	89.00 \pm 7.38 ^b	95.00 \pm 4.27 ^b	96.00 \pm 3.16 ^{ab}	70.00 \pm 8.17 ^b

*Means followed by the same letters in the columns are not significantly different according to the Tukey’s test at $P < 0.05$

*Mean Percentage Repellency \pm SD for ten replicates ($n = 100$)

lower repellent activity (70%) against rice weevils. Nevertheless, all the leaf extracts produced over 50% repellent action even at the lowest concentration. Since larvae of *S. oryzae* stay inside the grain for a long period before emerging as an adult, it is very difficult to control them with insecticides while the leaf extract repellents can protect the grains and diminish the initial infestation (Highland & Cline, 1986).

Essential oil of *O. zeylanica* elicited the highest repellency (100%) effect on weevils at the highest concentration after 30 minutes of exposure in area preference bioassay (Table 3). although there was no significant difference between 50 and 100% (v/v) treatments, indicating its strong potential in controlling rice weevils even within short time durations. The repellent effect of essential oil was verified by the fact that the number of adult weevils in treated rice grains decreased as the concentration of essential oil increased.

Table 3: Mean percentage repellency of essential oil isolated from of *Olax zeylanica* on *Sitophilus oryzae* after 30 minutes in area preference bioassay

Concentration (%v/v)	*Mean % Repellency \pm SD
10	94.00 \pm 1.18 ^a
50	99.00 \pm 0.24 ^b
100	100.00 \pm 0.00 ^b

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

*Mean Percentage Repellency \pm SD for five replicates (n = 100)

Insecticidal effect of essential oil extracted from *O. zeylanica* on rice weevil in contact toxicity bioassay is presented in Table 4.

Table 4: Mean percentage mortality of essential oil isolated from *Olax zeylanica* on *Sitophilus oryzae* in the contact toxicity bioassay

Concentration (%v/v)	*Mean % of Contact Mortality \pm SD	
	½ HAT	1 HAT
Control	0.00 \pm 0.00 ^a	0.00 \pm 0.00 ^a
10	85.00 \pm 5.00 ^b	97.00 \pm 2.74 ^b
50	89.00 \pm 4.18 ^{bc}	100.00 \pm 0.00 ^c
100	92.00 \pm 2.74 ^c	100.00 \pm 0.00 ^c

*Means followed by the same letters in each column are not significantly different according to the Tukey's test at $P < 0.05$

*Mean Percentage of Contact Mortality \pm SD for five replicates (n = 100)

*HAT – Hours After Treatment

All treatments displayed extremely high insecticidal actions against rice weevils after 60 minutes rather than after 30 minutes. Highest concentrations (50 and 100% v/v) were extremely effective in inducing 100% and 92% weevil mortalities within 60 minutes and 30 minutes respectively when compared to those of the control which gave no mortality. In addition, the mean percentage mortality at the lowest concentration was also higher than 75%, thus indicating the strong insecticidal action of essential oil extracted from the leaves of *O. zeylanica*.

According to the data presented in Table 5, essential oil extracted from leaves of *O. zeylanica* produced the highest percentage in fumigation mortality of 100% at highest concentration after an hour of weevil exposure, whilst no mortality was observed in the control. Moreover, 95% and 79% of induced *S. oryzae* mortalities were recorded within 60 and 30 minutes respectively at the lowest concentration, indicating the very high potential of using leaf essential oil of *O. zeylanica* as a fumigant.

Table 5: Mean percentage mortality of essential oil isolated from *Olox zeylanica* on *Sitophilus oryzae* in the contact toxicity bio-assay

Concentration (%v/v)	*Mean % of Fumigation Mortality \pm SD	
	$\frac{1}{2}$ HAT	1 HAT
	Control	0.00 \pm 0.00 ^a
10	79.00 \pm 4.18 ^b	95.00 \pm 2.54 ^b
50	86.00 \pm 4.18 ^c	99.00 \pm 0.24 ^c
100	90.00 \pm 3.54 ^c	100.00 \pm 0.00 ^c

*Means followed by the same letters in each column are not significantly different according to the Tukey's test at $P < 0.05$

*Mean Percentage of Fumigation Mortality \pm SD for five replicates (n = 100)

*HAT – Hours After Treatment

Plant essential oils and their constituents in relation to contact and fumigant insecticidal actions against stored product pests have been well documented. Especially their main compounds, the monoterpenoids, offer most promising alternatives to classical fumigants (Papachristos and Stamopoulos, 2003) and also have some effects on biological parameters such as growth rate, life span and reproduction (Pascual-Billalobos, 1996; Mahfuz and Khalequzzaman, 2007). Treatments with essential oils may cause symptoms that indicate neurotoxic activity in insects including hyperactivity, seizures and tremors followed by paralysis (knock down effect), which are very similar to those produced by the insecticides pyrethroids (Kostyukovsky et al., 2002; Leyva et al., 2012).

Earlier literature indicate the importance of plants in the protection of grains by the way of direct mixing of dried leaves, plant powders, solvent extracts, essential oils on grains during post harvest storage (Rajapakse, 1996; Shifa-vanmathi *et al.*, 2010). Law-Ogbomo

(2007) reported that plant treatments of grains have no effect on seed viability. He further stated that, such plant products could reduce the infestation of stored grain pests without causing any deleterious effect on grain quality.

CONCLUSIONS

Even though the scientific literature documenting the efficacy of plant products to different insect pests continues to expand, so far only a handful of botanicals are being successfully used. On the basis of the results of the present study, it is highly encouraging to note that the use of leaf powder, extracts and essential oils of *O. zeylanica* with its excellent repellent, insecticidal and fumigant activity would be economically feasible to alleviate the rice weevil problem. This is due to the fact that these products are highly promising, relatively available, cheap, and easy to prepare and apply on rice grains when compared to synthetic insecticides.

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