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Laboratory evaluation of some Sri Lankan plants as post-harvest grain protectants for the control of rice weevil *Sitophilus oryzae*

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Abstract

Powdered leaves of thirty seven plant species were screened under laboratory conditions with the temperature of 29±2^0 C and 84±2 % relative humidity for their repellent properties against rice weevil, *Sitophilus oryzae* (L). The screening was carried out with the view of producing more eco-friendly agents suitable for post-harvest rice protection and as sustainable alternatives to synthetic pesticides in the control of *S. oryzae.*

Reared weevils were exposed to 15.0 g of leaf powders admixed with rice grains in laboratory screening test using a modified cup-bioassay. The results revealed that twenty treatments caused significant repellency of adult *S.oryzae* than the control. Out of these, *Ocimum gratissimum* (Lamiaceae), *Cinnamomum verum* (Lauraceae), *Mentha viridis* (Lamiaceae), *Plectranthus amboinicus* (Lamiaceae) and *Citrus reticulata* (Rutaceae) showed the highest repellent activity. *M. viridis* elicited the highest and the strongest repellency (89.0%) in the weevils when compared with the other three treatments. However, the weevils demonstrated considerably

high rates of repellency to *C. verum*, *O. gratissimum*,, *C. reticulata* and, *P. amboinicus* which were 76.0%, 74.0% ,68.0% and 64% respectively.

When weevils were further tested against 5.0, 10.0 and 15.0 g of leaf powders, all treatments showed significantly ($p < 0.05$) higher repellency of *S. oryzae* than the control. *M. viridis* at 15.0g dose elicited the highest and the strongest repellency (89.0%) in the weevils when compared with the other treatments and concentrations. The overall results indicated that repellency rate increased proportionately with the increase of the dose of the powder. A similar trend of repellency was also observed with time. However, the effects of all plant powders on the insects were highest within 6 hours post treatment.

The overall results of the research provide scientific corroboration for the use of the four plant powders as effective grain protectants against the rice weevil.

Key words : *Sitophilus oryzae,* Rice weevil, Repellency, Plant powders

INTRODUCTION

Rice is the staple food of eighteen million people of Sri Lanka as in many other Asian countries. Every year 30 – 50% of the total paddy production is stored by farmers for their consumption, future sales, wages and seed purposes. In the tropics the suitable temperature and humidity occurring in paddy and rice storages encourage a number of insect pests to become a serious threat to post harvest storages (Nugaliyadde, 2001).

Among the stored grain pests, rice weevil, *Sitophilus oryzae* is considered the most widespread and destructive primary insect pest of paddy and rice in tropics (Hill, 1992). Control of weevil populations worldwide has been provided principally by the use of

synthetic chemical insecticides. Although they are still most effective, their repeated and indiscriminate use has disrupted natural biological control systems and led to resurgence of this insect, often resulting in the development of resistance, and raises environmental and human health concerns. Thus, there is an urgent need to develop cheap, more ecologically friendly, safe alternatives to conventional insecticides for the protection of grain products against insect infestations (Marcio *et al.,* 2007).

One area receiving greater attention is the use of plants rich in bioactive chemicals as grain protectants. Many tropical plants have long been used traditionally as pest control agents for generations throughout the world (Belmain *et al.,* 2001). Farmers often claim successful use of plant materials in storage pest control including plant ash, vegetable oils and powders of plant parts (Dubey *et a.,l* 2008). Talkukder (1995) listed 43 plant species as insect repellents, 21 plants as antifeedants, 47 plants as toxicants and 34 plants as insect reproduction and growth inhibitors.

Insect repellents are desirable chemicals as they drive away the insect pests from the treated materials by stimulating olfactory or other receptors of insects. Repellents from plant origin are considered safe in pest control operations as they minimize pesticide residues, and ensure safety to humans, food, and the environment (Talukder & Howse, 1995).

The present study therefore, was designed to screen thirty seven Sri Lankan plant species to evaluate their repellent properties against the rice weevil *Sitophilus oryzae* with the view of selecting the most effective plants to be used as grain protectants in the management of this peat.

MATERIALS AND METHODS

1 Host Material

Fresh rice grains (white kekulu variety) purchased from the local market was used in the study. The grains were dried under sun light to prevent mouldiness and stored in air tight plastic bottles. Only whole and un-infested grains were used for the experiments.

2 Rearing of *Sitophilus oryzae* Cultures

Rice grains heavily infested with rice weevils were collected from screened in the laboratory for confirmation of the correct insect species. Fifty adult *S. oryzae* (L.) each were introduced into jars (25cm x 10cm) containing 500g of disinfested whole rice grains. The top of each rearing jar was covered with nylon mesh fastened tightly with rubber bands, and the insects were allowed a 7 day period for oviposition. Thereafter, all adults were removed and each jar kept for 25 days during which emerging adult insects were monitored and kept in separate jars (11cm x 5cm) according to their age. Following the same procedure, new stock cultures were started as the new generation of adults emerged. Continuous cultures were reared from initial cultures throughout the study period, with the aim of maintaining a steady and sufficient supply of adult weevils of known age for bioassays. One week old, unsexed adult insects were used in all experiments. The rice weevil cultures were maintained at a temperature of 29 ± 2^0 C and 84±2 % relative humidity.

3 Screening Procedure

Thirty seven plant species collected from Kegalle, Rathnapura, Kurunegala, and Polonnaruwa districts were used in the screening test for their repellency activity against the rice weevil (Table 1). The plant species were identified at the Ayrvedic Research Institute, Navinna. Fresh, healthy, and mature leaves were removed from the plants and cleaned by rinsing thoroughly with tap water. All the leaves were then air dried separately under room temperature for 24 hours. Air dried leaves were ground to a fine powder using a domestic grinder (Multi national, 2101, India).

Table1. Plants screened for repellent activity against *Sitophilus oryzae*

No.	Scientific name	Family	Common name	Activity
1	*Eryngium foetidum*	Apiaceae	Andu	++
2	*Ageratum conyzoide*	Asteraceae	Hulanthala	++
3	*Emilia exserta*		Kadupahara	-
4	*Canarium zeylanicum*	Burseraceae	Kekuna	-
5	*Acalypha indica*	Euphorbiaceae	Kuppameniya	-
6	*Croton laccifer*		Gas keppetiya	++
7	*Clitoria ternatea*	Fabaceae	Katarolu	-
8	*Desmodium triflorum*		Heen undupiyaliya	-
9	*Leucas zeylanica*	Lamiaceae	Getathumba	-
10	*Mentha viridis*		Minchi	++++
11	*Ocimum gratissimum*		Maha Madurutala	+++
12	*Ocimum tenuiflorum*		Heen Madurutala	++
13	*Plectranthus amboinicus*		Kapparawalliya	+++
14	*Plectranthus zatarhendi*		Irievriya	-
15	*Cinnamomum verum*	Lauraceae	Kurundu	+++
16	*Abutilon indicum*	Malvaceae	Beheth Anoda	-
17	*Melastoma malabathricum*	Melastomaceae	Mahabovitiya	-
18	*Osbeckia octandra*		Heenbovitiya	-
19	*Aglaia odoratissima*	Meliaceae	Puwangu	-
20	*Eugenia caryophyllata*	Myrtaceae	Karabu	++
21	*Oxalis corniculata*	Oxalidaceae	Embulambiliya	-
22	*Pandanus amaryllifolius*	Pandanaceae	Rampe	-
23	*Piper longum*	Piperaceae	Thippili	-
24	*Piper nigrum*	Piperaceae	Gammiris	++

25	*Cymbopogon nardus*	Poaceae	Heen pangiri	++
26	*Vetiveria zizanioides*		Savendara	-
27	*Pavetta indica*	Rubiaceae	Pavatta	-
28	*Atalantia rotundifolia*	Rutaceae	Yakinaran	++
29	*Citrus aurantifolia*		Dehi	+
30	*Citrus aurantium*		Embuldodam	-
31	*Citrus madurensis*		Nasnaran	-
32	*Citrus reticulata*		Heen Naran	+++
33	*Murraya koenigii*		Karapincha	-
34	*Solanum melongena*	Solanaceae	Elabatu	-
35	*Lantana camara*	Verbenaceae	Gadapana	+++
36	*Elettaria cardamomum*	Zingiberaceae	Enasal	+
37	*Kaempferia galanga*		Inguru piyali	+

Repellency Very strong activity >80% ++++; Strong 61-80% +++; Moderate 41-60% ++; Weak 20-40% +; Little or No activity <20%

To evaluate the repellent effect of the plant leaf powders, a modified cup-bioassay technique (Kumar et al., 2004) was used. First, powdered plant leaves were admixed with clean and un-infested rice grains at the rate of 15g per hundred grams (100g) and placed in separate plastic containers (11cm x 5cm). Each container was perforated using a thick needle and 50 holes were made (Plate 1). These were made to allow the insects to escape from the container if they are repelled by the plant powders. Batches of twenty, one week old, unsexed adult insects were introduced into each container. This was then placed inside a larger container (25cmx10cm) to trap the insects that moved our through perforations of the smaller container (Figure 1)

Before the onset of each experiment these holes were covered with a sticky tape for five minutes to let the introduced insects settle down inside the container. Five replications each for treated and five for untreated rice grains (control) were made. The number of escaped insects in the large container was counted after 9 hrs. The repellent effect of each tested powder was expressed as the percentage of insects moving out from the treated rice after 9 hours.

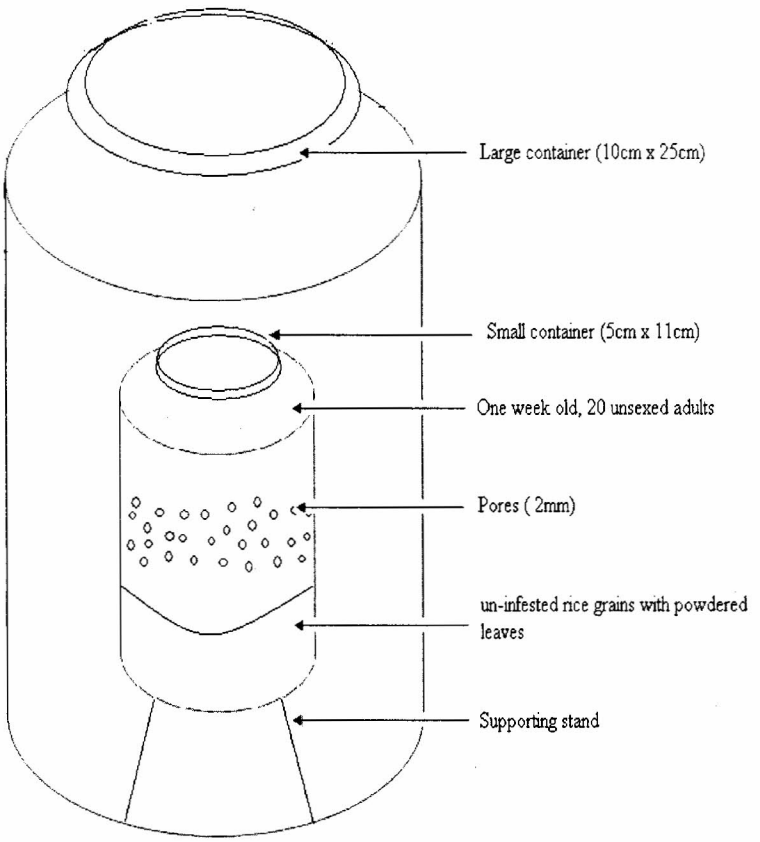

Figure 1. - Schematic diagram of Bioassay apparatus for testing repellency of *S. oryzae*

Percentage repellency of the weevils was classified using the following scale:

Activity	Percentage Repellency	Symbol
Very strong	>80%	++++
Strong	61-80%	+++
Moderate	41-60%	++
Weak	20-40%	+
Little or no activity	<20%	-

4 Repellent Effects of the Selected Plant Leaf Powders on Rice Weevil

For this study, plant leaf powders that elicited the highest repellency in the screening test were selected. Experiments were conducted using the same procedure described in the previous screening test. However, in this study, powdered leaves were admixed with un-infested rice grains separately at the rates of 5g, 10g, and 15g per hundred grams. The number of escaped individuals was counted 1, 3, 6, and 9hrs after the introduction of insects. Each treatment together with the control was replicated five times.

RESULTS AND DISCUSSION

Table2. Repellent activity of the *Sitophilus oryzae* to powders (15 g) of selected plants

Plant Species	Repellency (%)
Eryngium foetidum	45.0 b
Ageratum conyzoide	58.0 b
Croton laccifer	50.0 b
Mentha viridis	82.0 d
Ocimum gratissimum	78.0 d
Ocimum tenuiflorum	45.0 b
Plectranthus amboinicus	66.0 c

Cinnamomum verum	73.0 cd
Aglaia odoratissima	18.0 a
Eugenia caryophyllata	45.0 b
Oxalis corniculata	18.0 a
Piper nigrum	53.0 b
Cymbopogon nardus	55.0 b
Atalantia rotundifolia	55.0 b
Citrus aurantifolia	24.0 a
Citrus madurensis	13.0 a
Citrus reticulata	70.0 cd
Lantana camara	65.0 c
Elettaria cardamomum	33.0 b
Kaempferia galanga	37.0 b
Control	4.0 a

In the present study, leaf powders of thirty seven local plant species were screened for their effectiveness as repellents. Repellent effects of these plants as shown in Tables 1 and 2, varied with the plant species and the range of percentage repellent activity varied from 0.0 to 82. Of the thirty seven (37) plant leaf powders tested for repellency against *S. oryzae*, 1, 5, 8 and 3 plant powders exhibited very strong ++++, strong +++, moderate ++ and weak + activities respectively. Repellent effects of all these seventeen plants were significantly higher compared to that of the untreated control (Table 2). Little or no activity was recorded from the other twenty plant powders. Very strong repellency was observed only with *Mentha viridis* (89.0%) whereas strong repellent activity was recorded from *Ocimum gratissimum* (74.0%), *Cinnamomum verum* (76.0%), *Citrus reticulata* (68.0%), *Plectranthus amboinicus* (64.0%), and *Lantana camara* (63.0%). Also, of the six tested plants belonging to the family Lamiaceae, four plants found to have very strong (++++) and strong (+++) activities. According to Dethier *et al.* (1960) an insect repellent is a chemical stimulus, which causes the insect to make oriented movements away from the source of stimulus. Therefore, such repellents are contemplated to be desirable chemicals as they drive away insect pests from the treated materials by stimulating olfactory or other receptors of

insects and also as they offer protection with minimal impact on the ecosystem.

Some families like Asteraceae, Meliaceae, Rutaceae, Lauraceae, Euphorbiaceae, Poaceae, Zingiberaceae, Apiaceae, and Verbenaceae showed repellent activity. However plants belonging to the family Lamiaceae elicited the strongest repellency in weevils than with the other families. According to Talukder (2006), tissues of some higher plants contain arrays of biochemicals, known as 'secondary plant chemicals', which are defensive in function. They include alkaloids, steroids, phenolics, saponins, resins, essential oils, various organic acids, and other compounds. Therefore different plant species shows different mode of action. However, it is noteworthy that some plant species showed extremely different results even they belonged to the same family.

After selecting the five highly effective plant species based on the repellency of adult weevils, these plants were further evaluated to study their repellent properties using different concentrations at different time intervals. The bioassays revealed that repellent activities of all five plant species which were selected from the screening test were significantly different from their untreated control. With the increase of the time of exposure, the number of adults repelled also increased in all treatments. The highest repellent effect was observed after 6 hours of exposure. Thereafter the repellent effect was found to be at a constant level.

Table 3. Repellent effects of five plant powders at different concentrations after 6 hours of exposure to *Sitophilus oryzae*

* Means followed by the same letters in each column and each row are not significantly different (Tukey's test at p=0.05)

* Mean value for 5 replicates (Mean: ±SD)

Leaf Preparation (g)	***Cinnamomum verum*** **Mean±SD**	***Citrus reticulata*** **Mean±SD**	***Mentha viridis*** **Mean±SD**	***Ocimum gratissimum*** **Mean±SD**	***Plectranthus amboinicus*** **Mean±SD**	**Probability**
Control	5.0 ± 0.00^{a}	5.0 ± 0.00^{a}	5.0 ± 0.00^{a}	4.0 ± 2.23^{a}	5.0 ± 0.00^{b}	NS
5	34.0 ± 6.51^{b}	33.0 ± 2.73^{b}	41.0 ± 4.18^{b}	41.0 ± 4.18^{b}	31.00 ± 4.18^{b}	NS
10	38.0 ± 2.73^{b}	53.0 ± 2.73^{c}	53.0 ± 2.73^{c}	53.0 ± 2.73^{c}	43.00 ± 2.74^{b}	$p<0.05$
15	76.0 ± 2.23^{e}	68.0 ± 2.73^{d}	89.0 ± 2.23^{f}	74.0 ± 4.18^{e}	63.00 ± 2.74^{d}	$p<0.05$
Probability	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$	

With different concentrations of plant preparations and time of exposure, the number of adults repelled increased in all five treatments. On the whole, *Mentha viridis* at 15.0g dose elicited an extremely high repellency (about 89.0%) after 6h of exposure of the weevils which was in fact the highest of all the plants tested. The strong repellency of *M. viridis* in this study was reflected by the reduced numbers of insects on treated milled rice. Also, the weevils demonstrated considerably high rates of repellency to the other four plants, *Cinnamomum verum, Ocimum gratissimum, Citrus reticulate* and *Plectranthus amboinicus* which were 76.0%, 74.0%, 68.0% and 64.0% respectively. One of the interesting findings of the current study is the similar efficiency of *C. verum, O. gratissimum,* and *C. reticulata* shown at the concentration of 10.0g. According to these results, either of these three plant species can be utilized to repel similar number of rice weevils at this concentration. At the concentration of 5.0g, all the four plant species were not significantly different in their repellency ($p<0.05$) (Table 03).

The overall results indicated that repellency rate increased proportionately with the increase of the dose of the powders. A similar trend of repellency was also observed with time. However, the effects of all plant powders on the insects were at their highest within 6 hours post treatment. Present results in this study show that these botanical powders are in fact, very effective against the rice weevil on milled rice grains, but its effectiveness is highly determined by the characteristics of the plant, dose rate, and the time of exposure.

Similar observations of the effect of other plant extracts on *S. oryzae* have been reported. Pretheep Kumar *et al* (2004) found that an extract of protein enriched bean flour had a high level of repellency on *S. oryzae.* They found 76.3% and 91.2% of repellency with a concentration of 0.1% and 1% of the extract, respectively, after 48h after the assay was initiated. Also, the present results corroborate with the findings of Papovic *et al* (2006) who reported that, *Ocimum brasilicum* was highly effective in

reducing and repelling populations of *Sitophilus oryzae.* Similarly, Paranagama *et al* in 2004 reported that *Cinnamomum zeylanicum* was a highly potent fumigant and contact toxicant against *Sitotroga cerealella.* Saljoqi *et al* (2006) reported the repellent and fatal effects of extracts of six common plants in Pakistan on *S. oryzae.* The ethanol extract of *Mentha longifolia* had the 24.20 % repellency and 47.40% fatal effect on the rice weevil. On the contrary, no work has been reported so far on the repellent effects of *C. reticulata* on the rice weevil.

In conclusion, the results from the present study signify that *Menta viridis* can be utilized as a grain protectant very effectively to protect stored rice from rice weevil infestations. Similarly the other four plants, *Ocimum gratissimum, Cinnamomum. verum*, *Citrus reticulata* and *Plectranthus amboinicus* are also considerably effective in storage pest management of the rice weevil.

Today, the environment safety of an insecticide is considered to be of paramount importance. The world of flora has a variety of plant species and in order to increase the number of plants used for pest control, more studies should be carried out. Thus, a variety of effective substances found in different plant species could be discovered. Consequently, substances alternative to many chemical pesticides, that pollute our natural sources and threaten our future, can be found. In addition, pesticides that are easily affordable to the farmer can be obtained and environmental pollution will eventually decrease.

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