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Utilization of *Ruta graveolens* and *Azadirachta indica* leaf powders and their binary combinations for the management of *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) in stored maize in Sri Lanka

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Abstract

Maize weevil (*Sitophilus zeamais*, Motsch.) is a ubiquitous and cosmopolitan field-to-store pest of maize of economic importance in tropical and subtropical regions of the world. The current renewed interest to replace synthetic insecticides has served as the impetus for the evaluation and intensification of eco-friendly anti-weevil measures such as the use of plant based bio-insecticides. This study was therefore, designed to investigate the insecticidal and repellent potency of leaf powders of *Ruta graveolens* and *Azadirachta indica* in single treatments and combinations of various proportions under ambient laboratory conditions against *Sitophilus zeamais* in both contact and vapour forms. For both plants, 100 g each of maize grains were mixed with leaf powders at different doses of 1.0, 3.0, 5.0, 7.0 and 10.0 g. The bio-potential of three combinations of 1:1, 1:2 and 2:1 of *R. graveolens* and *A. indica* leaf powders incorporating into the dosage level of 10.0g was also evaluated. Maize grains treated with *R. graveolens* leaf powder caused 100 % contact and 68 % fumigation mortalities within 24 hours of exposure whilst repelling 97 % and 96 % weevils within an hour in both contact and vapour forms respectively. *A. indica* powder was less efficacious compared to that of *R. graveolens* causing significantly lower repellency within an hour and mortalities after 10 days of storage. Application of 2:1 combination of *R. graveolens* and *A. indica* produced respective levels of 100 % and 73% contact and fumigation mortalities within 24 hours and highly significant repellent effects on *S. zeamais*, offering greater potential for large-scale storage since such combinations are relatively cheaper than using either of the botanicals singly. Leaf powders of the two plants under present investigation revealed their improved grain protecting capacity when used individually and in mixtures as bio-rational leads in large-scale protection of stored maize.

Keywords: *Sitophilus zeamais*, *Ruta graveolens*, *Azadirachta indica*, repellency, leaf powder, combinations

1. Introduction

Maize (*Zea mays* L), a widely cultivated coarse grain in Sri Lanka is the third most globally important cereal grain after wheat and rice (Golob, *et al*., 2004). It is referred to as the cereal of the future for its nutritional value and utilization of its products and by-products (Lee, 1999) as maize provides an estimated 10 % and 15 % of the world's calories and protein, respectively (Nuss and Tanumihardho, 2010). Maize cultivation in Sri Lanka has expanded over the last several years, becoming a highly commercialized venture for which 69,970 ha of land extent was devoted with the production accounting for 261,000 MT in 2015 (Department of Census and Statistics, 2016). However, there is a significant unmet demand for maize to meet the country's growing requirements especially in the poultry feed industry and for export that can be harnessed in supplying shortages of world's biofuel production requirements, which is an enormous opportunity. Hence, the country's import of maize is bound to be a huge drain on Sri Lanka's foreign exchange. In most of Sri Lanka, the greater proportion of maize production is currently dominated by smallholder farmers who heavily rely on rain fed conditions with limited use of improved seeds, fertilizer, mechanization and post-harvest storage facilities which often make them incur tremendous post-harvest losses and average yields well below attainable levels (Ministry of Agriculture, Animal Production, and Development, Industries, Fisheries and Tourism of Eastern Province, 2016). Globally, it has been observed that the greatest losses of storage grains are due to insects that can damage grains over a short period of time (Suleiman, *et al*., 2013). It is estimated that stored grains of 1 – 5 % and 20 – 50 % are lost in developed and developing countries respectively due to insect damage (Nukenine, 2010). Among the key insects in maize storage, *Sitophilus zeamais* is classified as a primary and major pest due to its ability to destroy a whole grain kernel (Kanyamasoro, *et al*., *2012).*

Insect pest control in stored grain products heavily relies on the use of gaseous fumigants and residual contact insecticides. Moreover, given that maize is used as human food and feed for livestock renders, the use of potentially toxic synthetic insecticides is unacceptable since these would definitely lead to serious problems such as toxic residues, health and environmental hazards, increasing cost of application and erratic supply in developing countries due to foreign exchange constraints (Obeng-ofori, *et al*., 1997; Aslam, *et al*., 2002 Udo, 2005; Ntonifor, *et al*., 2010). Due to the complications of synthetic insecticides, resource poor farmers in developing countries like Sri Lanka would be more inclined to mix their grains with protectants made up of aromatic local plants that are long thought to possess insecticidal and repellent properties prior to storage (Ntonifor, *et al*., 2010; Ntonifor, *et al*., 2011). Plant products are being used to control many insect pests in the field and also in storage since time immemorial (Yohannes, *et al*., 2014). This accentuates the importance of seeking alternative pest management technologies such as the use of plant derived natural insecticides for effective protection over stored maize, that are readily available, affordable, and non-toxic to humans and to the environment (Niber, 1994; Talukder and Howse, 1995; Aslam, *et al*., 2002).

Hence, the present phytocentric study was undertaken to evaluate leaf powders derived from garden rue (*Ruta graveolens*) and neem (*Azadirachta indica*) for their insecticidal and repellent activities against *Sitophilus zeamais* as well as to test the potency of various combinations of plant powders for potential large-scale exploitation in integrated insect pest management programs.

2. Materials and Methods

A series of experimental designs were conducted at the insect pest management laboratory of Department of Zoology, Faculty of Applied Sciences, University of Sri Jayewardenepura, Sri Lanka from June to October 2015. The relative humidity and ambient temperature during the experimental period were 84 ± 2%RH and 29 ± 2^0C respectively. All the assessments carried out in this study were replicated ten times.

2.1 Collection of Plant Material

Fresh, mature and healthy leaves of *Ruta graveolens* (Sapindales: Rutaceae) and *Azadirachta indica* (Sapindales: Meliaceae) were collected from Bandarawela region and university premises respectively. These were shade dried and coarsely powdered by using a domestic electric grinder (Multinational®, 2101, India). Then the powders were packed in glass containers with tight lids and stored in a refrigerator at 4^0C prior to use.

2.2 Culturing of Insects

Sitophilus zeamais used for the study were obtained from infested stock of maize in the local market and then reared on un-infested maize grains in glass jars covered with muslin cloth held in place with rubber bands for the passage of air and prevention of the weevil escape. One week old adult weevils were used for all experiments.

2.3 Assessment of Contact Mortality

Contact toxic action was assayed by admixing 100 g of un-infested maize grains with powdered leaves of plants weighing 1.0, 3.0, 5.0, 7.0 and 10.0 g corresponding to respective levels of 1.0, 3.0, 5.0, 7.0, 10.0 % w/w in the plastic containers (height 8cm, diameter 7.5cm) and 20 adult weevils were introduced into each. The containers were then covered with muslin cloth held firmly with rubber bands to prevent the escape of the weevils and to ensure adequate aeration. Maize grains without any plant powder were served as the control. The contact mortality of the weevils was recorded at 3 hour intervals up to 24 hours and at 24 hour intervals up to 10 days of post treatment for *R. graveolens* and *A. indica* respectively.

2.4 Assessment of Fumigation Mortality

The bio apparatus for the fumigation toxicity test consisted of a small plastic container (height 4cm, diameter 5cm) attached to a plastic cup (height 8cm, diameter 7.5cm). The bottom of the plastic cup was cut off and replaced with a nylon cloth to allow the vapour of plant powders in the container to pass through the cloth and reach the weevils. Leaf powders (1.0, 3.0, 5.0, 7.0 and 10.0 g) were placed in the separate plastic containers while 100 g maize grains were placed in the plastic cups. Twenty adult weevils were then introduced into each plastic cup. The bio apparatus was covered with muslin cloth held in place with rubber bands. A similar set up without the leaf powders was used as the positive controls. Fumigant mortality of the maize weevils was evaluated after every 3 hours up to 24 hours and every 24 hours up to 10 days of weevil exposure for leaf powders of *R. graveolens* and *A. indica* respectively.

2.5 Assessment of Contact Repellency

Contact repellency was tested according to the standard method adopted by Mohan and Fields (2002) with some modifications. Portions of 1.0, 3.0, 5.0, 7.0 and 10.0 g of leaf powders were weighed and each added into a small plastic cup (height 8 cm, diameter 7.5 cm) which contained 100 g of maize grains which correspond to 1.0, 3.0, 5.0, 7.0 and

10.0 % w/w respectively. The grains in the controls contained no leaf powders. The top ¼ height of the small plastic cup was perforated using a soldering gun (220V/240V, 40W, China). Those holes were made to allow the weevils to escape from the plastic cup if they were repelled by the leaf powders. This small plastic cup was then placed inside a large plastic bottle (height 15 cm, diameter 7.5 cm) to trap the weevils that were moving out through the holes of the small plastic cup. Twenty adult weevils were introduced into each small plastic cup. The bio-apparatus was then covered with muslin cloth held in place with rubber bands to allow the air passage for weevils. The number of repelled weevils in the large plastic bottle was counted one hour after their introduction.

2.6 Assessment of Fumigation Repellency

The bio apparatus used for this study was somewhat similar to the setup used in the contact repellency bioassay but with some alterations (Mohan & Fields, 2002). The bottom of the small plastic cup was removed and replaced with a nylon cloth which was then fitted with a small plastic container (height 4 cm, diameter 5 cm) to place the leaf powders inside the latter. This adjustment allowed the vapour of leaf powders inside the container to pass through the cloth and reach the weevils. Leaf powder was then put in the plastic container, 100 g of maize grains were placed in the small plastic cups and 20 adult weevils were introduced into the small plastic cups. 1 .0, 3.0, 5.0, 7.0 and 10.0 g of leaf powders correlative to 1.0, 3.0, 5.0, 7.0 and 10.0 % w/w respectively, were tested separately. The grains in the control test contained no leaf powders. Number of repelled weevils in the large plastic bottle was counted one hour after introduction.

2.7 Bioassay with binary combinations of leaf powders

Bioassays were conducted based on the procedure described by Ntonifer, *et al*., (2010, 2011) with minor modifications. In the light of *Azadirachta indica* is often used by traditional farmers to protect their grains from stored insect pests and previous experiments with 10.0 g of *Ruta graveolens* caused around 100 % weevil mortalities and repellencies, binary combinations with proportions of 1:1, 1:2, 2:1 of *Ruta graveolens* and *Azadirachta indica* leaf powders incorporating into 10.0 g were then evaluated. Each of the proportion was then mixed with 100 g of un-infested maize grains in the bioassay chambers and 20 weevils were introduced. Experiment was conducted as in the previous assessments, in both contact and vapour forms evaluating the number of dead and repelled insects accordingly.

2.8 Analysis of Data

All data were subjected to one-way analysis of variance (ANOVA) using the "Minitab", version 14.0. Tukey's multiple comparison test was used to separate mean values of the experiments, where significant differences existed ($p<0.05$). Probit analysis was used to estimate LT_{50} values to determine the lethal time needed to kill 50% of maize weevils.

3.0 Results and Discussion

3.1 Assessment of Contact Mortality

The cumulative contact mortality of *R. graveolens* exerted on maize weevils within 24 hours of exposure is presented in Figure 1. Leaf powders elicited clear dose dependent mortality of adult *S. zeamais* producing end-point mortalities amounting to 100% after 21 and 24 hours for the dosage levels of 10.0 g and 7.0 g respectively. It was notable that after 24 hours of post treatment period, maize seeds mixed with 3.0 g of *R. graveolens* leaf powder showed over 50 % contact insecticidal activity.

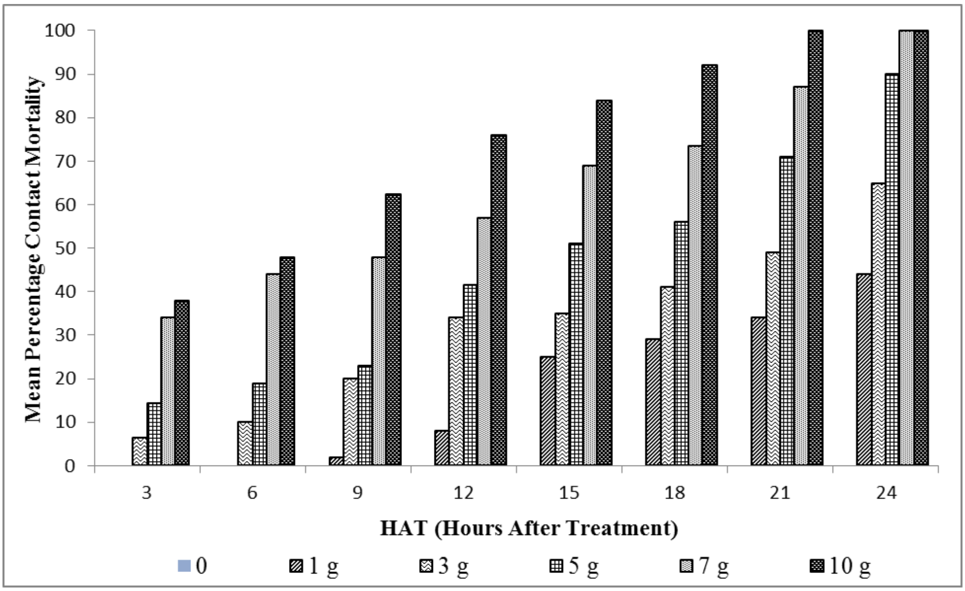

Figure 01. Contact mortality of *S. zeamais* caused by treating stored maize grains with varied doses of *R. graveolens* leaf powders after every 3 hrs up to 24 hrs of weevil exposure.

Insecticidal activity of *A. indica* leaf powders tested against *S. zeamais* after 10 days of exposure period is presented in Figure 2. Although the leaf powders produced clear and complete weevil management (100 %) within 9 days at the highest dose level, the mortality rate was not as fast as the mortality produced by *R. graveolens.* It is evident according to the results of both botanicals that the mortalities of crude powders were significantly ($P < 0.05$) influenced by plant, dose applied and the contact duration. In addition, this phenomenon suggests the presence of pungent secondary metabolites in the plant that act as insecticides influencing insect locomotion, oviposition, feeding behavior, developmental and physiological processes as well as behavioral patterns (Udo, 2015). However, as the exposure time increased, there was progressive increasing in the toxicities of both plants to test insects showcasing appreciable mortality of *S. zeamais.*

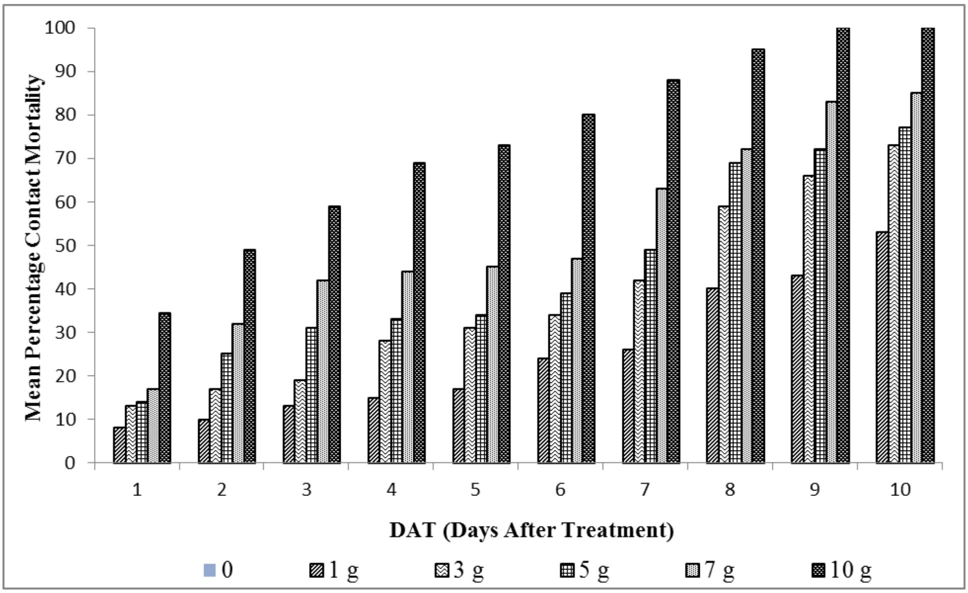

Figure 02. Contact mortality of *S. zeamais* caused by treating stored maize grains with varied doses of *A. indica* leaf powders at 24 hour interval up to 10 days of weevil exposure.

3.2 Assessment of Fumigation Mortality

The percent mortality of weevils to the volatiles originated from different doses of *R. graveolens* and *A. indica* leaf powders is presented in Figures 3 and 4 respectively. At dose levels of 7.0 and 10.0 g, both plant materials caused more cumulative insecticidal effects on *S. zeamais*, while untreated control giving no weevil mortalities over the exposure time period. However, *R. graveolens* leaf powder was comparatively more effective than *A. indica* as a fumigant by eliciting satisfactory results at a faster rate.

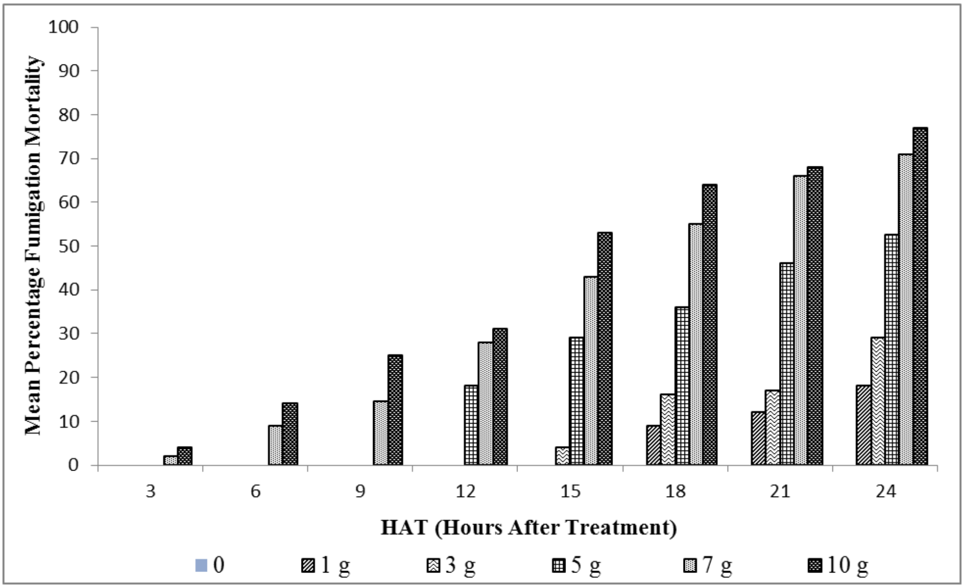

Figure 03. Fumigation mortality of *S. zeamais* caused by treating stored maize grains with varied doses of *R. graveolens* leaf powders after every 3 hrs up to 24 hrs of weevil exposure.

Though none of the plants offered complete protection over stored maize from maize weevils, there were significant differences amongst the doses of *R. graveolens* and *A. indica* leaf powders used, from which the level of damage is quite high compared to untreated control. Moreover, fumigation mortality of adult weevils increased with the increase in dose level over the time. However the level of mortality observed could be tolerable and useful as successful fumigants in traditional small holder storage systems.

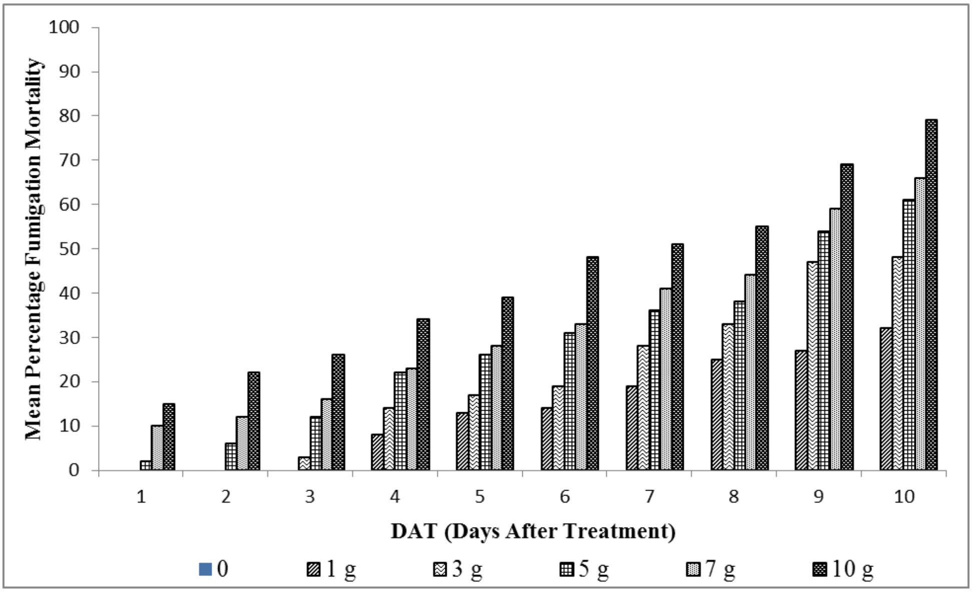

Figure 04. Fumigation mortality of *S. zeamais* caused by treating stored maize grains with varied doses of *A. indica* leaf powders at 24 hour interval up to 10 days of weevil exposure.

3.3 Assessment of Repellency

Data in Figures 5 and 6 depict the repellent activities emitted by *R. graveolens* and *A. indica* leaf powders admixed with 100 g of maize seeds within an hour of weevil exposure period in both contact and vapour forms. Among the leaf powders tested, maximum percentage of repellency of 97 % was recorded with 10 g/100 g of maize seeds, in *R. graveolens* treatment and a lesser amount (74%) in *A. indica* when the leaf powders were in contact with *S. zeamais*. In the sense of vapour form, maize grains treated with crude *R. graveolens* powders had the highest percent repellent value of 96 %, while *A. indica* caused 47 % repellence of adult *S. zeamais*. It is very remarkable that *R. graveolens* produced quite a considerable repellent effect over *A. indica* whereas in the control bio-assay, the weevils were more or less dispersed among untreated maize grains exhibiting no repellence at all. Repellency percentage produced by both plants was found to be dose dependent showing statistical significance at 5 % confidence level.

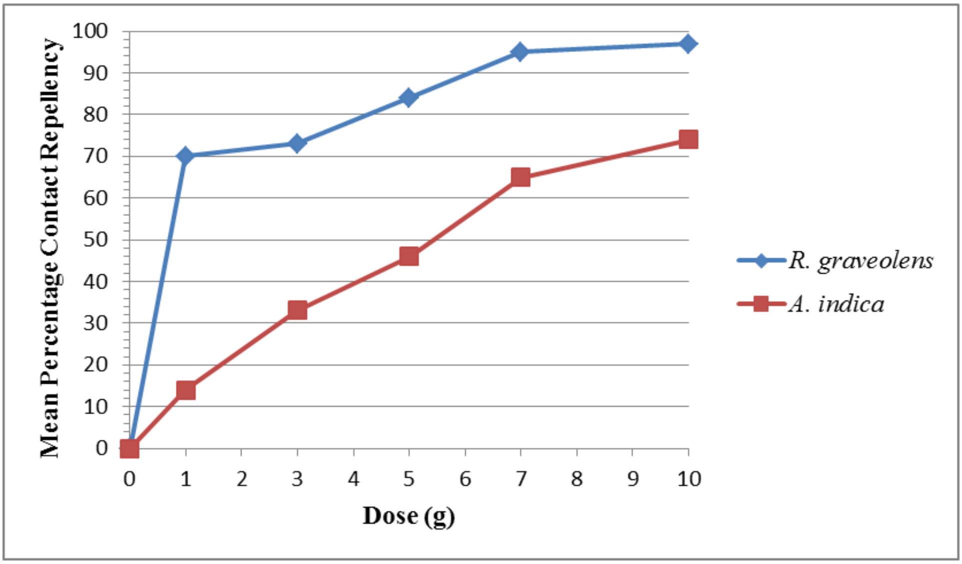

Figure 05. Contact repellency of *S. zeamais* caused by treating stored maize with different doses of *R. graveolens* and *A. indica* leaf powders tested singly within an hour of exposure

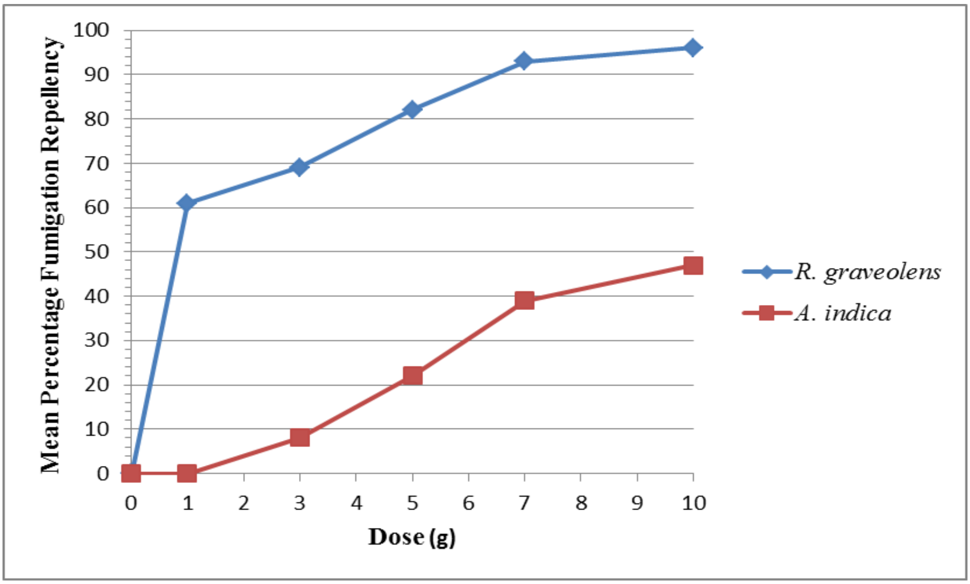

Figure 06. Fumigation repellency of *S. zeamais* caused by treating stored maize with different doses of *R. graveolens* and *A. indica* leaf powders tested singly within an hour of exposure

3.4 Bioassay with binary combinations of leaf powders

The combined effect of botanicals on mortality of *S. zeamais* estimated from pooled data in both contact and vapor forms are illustrated in Table 1. The percentage mortality recorded in each proportion of each form was significantly higher than the mortality for the experimental control that had zero percent weevil mortalities.

Table 01. Mortality of *S. zeamais* caused by treating stored maize grains with various proportional combinations of *R. graveolens* and *A. indica* leaf powders

Time/ HAT	*Mean % Mortality ± SD						
	Proportional Combinations (*Ruta graveolens* : *Azadirachta indica*)						
	Control	Contact			Fumigation		
	0:0	1:1	1:2	2:1	1:1	1:2	2:1
3	0.00 ± 0.00[a]	20.00 ± 4.08[a]	11.00 ± 3.94[a]	40.00 ± 3.33[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	3.00 ± 2.58[a]
6	0.00 ± 0.00[a]	24.00 ± 3.94[ab]	15.00 ± 3.33[a]	49.00 ± 3.94[b]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	11.00 ± 4.59[b]
9	0.00 ± 0.00[a]	28.00 ± 3.49[b]	25.00 ± 4.71[b]	53.00 ± 2.58[c]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	19.00 ± 3.94[b]
12	0.00 ± 0.00[a]	46.00 ± 3.16[c]	39.00 ± 3.16[c]	62.00 ± 2.58[d]	19.00 ± 2.11[b]	0.00 ± 0.00[a]	33.00 ± 3.49[c]
15	0.00 ± 0.00[a]	56.00 ± 3.94[d]	40.00 ± 4.71[c]	74.00 ± 3.16[e]	34.00 ± 2.11[c]	9.00 ± 2.11[b]	46.00 ± 3.16[d]
18	0.00 ± 0.00[a]	61.00 ± 3.94[d]	46.00 ± 2.11[d]	80.00 ± 0.00[f]	40.00 ± 4.08[d]	19.00 ± 2.11[c]	59.00 ± 4.59[e]
21	0.00 ± 0.00[a]	76.00 ± 2.11[e]	54.00 ± 6.58[e]	93.00 ± 2.58[g]	51.00 ± 3.16[e]	22.00 ± 3.49[d]	70.00 ± 4.08[f]
24	0.00 ± 0.00[a]	92.00 ± 2.58[f]	70.00 ± 3.33[f]	100.00 ± 0.00[h]	58.00 ± 5.87[f]	34.00 ± 2.11[e]	73.00 ± 3.49[f]

*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 Contact Toxicity ± SD for ten replicates (n = 200); *HAT – Hours After Treatment

Proportionate combination of 1:2 incorporating into 10.0 g of *R. graveolens* and *A. indica* (3.33: 6.66 g) killed 70 % and 34 % of *S. zeamais* adults at 24 hours after treatments in contact and fumigation assays respectively. An increased dose of *R. graveolens* in the mixture 2:1 of *R. graveolens* and *A. indica* (6.66: 3.33 g) produced 100 % and 73 % of respective contact and fumigation weevil mortalities after 24 hours of post treatment. This clearly discernible that each plant individually influenced weevil mortalities while in proportional combinations, thus offering good grain protective activity in contrast to what prevailed in their single assays.

The LT_{50} values obtained from the probit regression analysis of time-response for each plant when they were tested singly and in combinations are given in Table 2.

Table 02. Comparative median lethal time (LT_{50}) of *S. zeamais* due to leaf powders of *R. graveolens*, *A. indica* and their binary combinations at the dosage level of 10.0 g

Treatment		Bioassay Form	LT_{50} (Hours)	Confidence Interval	
				Lower	Upper
Ruta graveolens		Contact	5.31	4.62	5.95
		Fumigation	14.52	13.46	15.73
Azadirachta indica		Contact	44.73	39.23	49.61
		Fumigation	119.53	107.87	133.72
Binary combination (*R. graveolens:A. indica*)	**1:1**	Contact	11.38	10.29	12.55
	1:2		17.82	15.81	20.62
	2:1		5.83	4.91	6.67
	1:1	Fumigation	20.39	19.23	21.87
	1:2		27.74	25.34	32.06
	2:1		15.53	14.43	16.79

95% lower and upper fiducial limits are shown in parenthesis

LT_{50} – Lethal time that kills 50% of the population

R. graveolens leaf powders caused 50 % of contact and fumigation weevil mortalities within 5 and 14 hours of weevil exposure respectively, while the proportional combination of 2:1 of *R. graveolens* and *A. indica* producing the similar mortalities also within 5 and 15 hours of post treatment at the 10.0 g of dosage level.

The proportion of 2:1 of *R. graveolens* and *A. indica* leaf powders was the most effective binary combination that caused significantly higher repellent rates amounting for over 90 % in both contact and fumigation bioassays within an hour (Table 3). Although differences in effectiveness of leaf powders tested alone and in combinations were relatively small, 2:1 proportional mixture was found to be the most effective.

Table 03. Repellency of *S. zeamais* caused by treating stored maize grains with various proportional combinations of *R. graveolens* and *A. indica* leaf powders within an hour of weevil exposure

Proportional Combinations (*Ruta graveolens* : *Azadirachta indica*)	*Mean % Repellency ± SD	
	Bioassay Form	
	Contact	Fumigation
1:1	88.00 ± 3.49[b]	84.00 ± 4.59[b]
1:2	79.00 ± 3.94[a]	75.00 ± 5.27[a]
2:1	97.00 ± 2.58[c]	94.00 ± 2.11[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 Contact Toxicity ± SD for ten replicates (n = 200)

Single and binary mixtures of leaf powders of *R. graveolens* and *A. indica* caused varying degrees of mortalities and repellencies on maize weevils. The bio-potential of leaf powders was based mainly on the dose and the exposure period. The present study revealed that *R. graveolens* leaf powder when used singly was more effective against *S. zeamais* than when it was combined with the leaf powder of *A. indica*. However, binary combinations considerably reduced the quantity of *R. graveolens* used in the mixtures significantly reducing the cost of the preparations, while maintaining their potency almost at the level when *R. graveolens* leaf powder was used alone. In fact, at 10.0 g, 2:1 mixture of *R. graveolens* and *A. indica* was almost on par with R. graveolens in the exertion of potent lethal and repellent activities in both contact and vapour forms. Using a combination rather than using *R. graveolens* alone would be a cost effective advantage since leaves of *A. indica* are much easier to obtain. It is also advantageous to use combinations of plant powders, as it may delay the development of resistance in insects to these plant powders due to the complexities of the chemicals.

As evidenced by the results, availability of *Ruta graveolens* and *Azadirachta indica* locally and their potentials as bio-pesticides make them candidates in upgrading post-harvest protection practices of stored maize in Sri Lanka. In the extended exploration of effectiveness of those botanicals, binary combinations offer a cheaper and easy control method for farmers and small scale enterprises thus encouraging them to develop their own plant-based and eco-friendly post-harvest insecticide formulations as supplements to more toxic synthetic insecticides. Further studies are still being underway to elucidate the active ingredients, mode of action and their interactions of these plants to ensure a steady supply of good quality maize grains enabling their resourceful use in large scale stored product protection.

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