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EFFICACY OF ESSENTIAL OIL OF *RUTA GRAVEOLENS* LEAVES AGAINST *SITOPHILUS ORYZAE* (LINNAEUS) AS A BIORATIONAL PESTICIDE IN POST-HARVEST PEST MANAGEMENT

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Abstract: Essential oil was isolated from leaves of *Ruta graveolens* (Arudha in Sinhala) by hydro-distillation to evaluate its repellent and toxic activities against the rice weevil, *Sitophilus oryzae*. Essential oil at concentrations of 50, 100, 150 and 200 µl/ 10 ml acetone were tested against one week old adults using un-infested white raw rice as the food medium. Repellency rate of insects increased with the increase in dose and 100% adult repellent activity was observed at the highest dose (200 µl) after 30 minutes. Relatively high level of repellency was also recorded at the lowest dose (50 µl) amounting to 89%. In contact and fumigation toxicity bioassays, highest doses of the oil tested (150 and 200 µl) were extremely effective in inducing 100% mortality of weevils within an hour and over 90% within half an hour compared to that of the control which gave no mortality. The efficacy in respect to contact and fumigation toxicity after one hour was found to be 93% and 91% respectively at the lowest dose. Present study reveals that essential oil present in leaves of *R. graveolens* has very high potential as a repellent, contact toxicant and a potent fumigant in controlling *S. oryzae* infestations in pest management programmes.

Keywords: *Ruta graveolens*, *Sitophilus oryzae*, essential oil, repellency, toxicity.

Introduction

Prodigious post -harvest losses sustaining in the developing world due to physical, nutritional and quality deterioration of stored grains are caused by insects and the detrimental impact of these losses on food security are well known [1]. Of all the coleopteran pests of stored grains, the most damaging species of storage insects are found in the genus *Sitophilus. Sitophilus oryzae,* an utterly ubiquitous pest of economic importance, is an internal feeding insect pest that bores into stored grain [2]. Direct damage is caused by the consumption of cereal grain. Usually one larva hollows out small grain during its development. Adults cause further damage by feeding, mainly by attacking previously damaged grain. Rice weevil infestations may also produce a considerable amount of heat and moisture which encourage extensive quality loss, mould growth and growth of other insect populations [3].

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The control of rice weevil infestations has been primarily through the use of fumigants and residual chemical insecticides to augment the more obvious approach of hygiene. Treatment of rice with these synthetic insecticides is not recommended because of direct and indirect health hazards to humans [2]. In addition, most of them are expensive and thus farmers and medium scale traders cannot afford them. Furthermore, the use of these expensive chemicals has led to a considerable increase of cost of the production of rice and other grains [4]. Recognition of such detrimental effects of synthetic insecticides has prompted the development of new alternatives as less obtrusive management strategies that must be ecologically safer with no residual and noxious effects on non target animals [2].

In this regard, plant essential oils have been proved effective suppressing stored insect pests which possess a broad spectrum of activities ranging from repellent, insecticidal, antifeedant, growth regulatory and oviposition deterrent actions [5]. These properties are becoming much more popular among farmers for the protection of stored product commodities, due to the volatile nature, limited persistence in field conditions and edible properties of the essential oils [5]. Essential oils are also known to be more readily biodegradable, ecologically safer [6] [7] and many oils themselves or products based on them are nontoxic to mammals [5] [8]

The present work, therefore, was to investigate the possibility of utilizing the essential oil extracted from the leaves of *Ruta graveolens* as a repellent or/and a toxicant to control the rice weevil, *S. oryzae* in stored-product protection programs.

Materials and Methods

Isolation of Essential Oils

Fresh, healthy and mature leaves of *Ruta graveolens* collected from Bandarawela region were taxonomically identified at the Herbarium of Department of Botany at the Ayurvedic Research Institute, Maharagama, Sri Lanka. The 450g of air-dried and powdered leaf material was hydro-distilled in Clevenger type apparatus continuously for 3 hours to yield essential oil. Essential oil thus obtained was kept in a sealed glass tube at 4^0C until experimental use. A series of concentrations was prepared by diluting a precise volume of essential oil (50, 100, 150, and 200 µl) in 10ml of acetone.

Maintenance of *Sitophilus oryzae* Cultures

S. oryzae was reared on un-infested white raw rice (white kekulu) grains in the Pest Management laboratory, USJ, Sri Lanka at 29 ± 2^0C , $84 \pm 2\%$ RH and 12h:12h light: dark regime. Unsexed adult weevils of 3-7 days old were used for all experiments.

Repellent Effect

Repellent effect of the oil on the rice weevil was carried out using the area preference method. The test area consisted of Filtermann® (125mm) paper was cut into 2 equal parts. One half of filter paper was treated with 1ml of each prepared concentration of essential oil as uniformly as possible while the other half (control) was only treated with 1ml of acetone. These two halves were then air dried to evaporate the solvent completely. A full disc was carefully remade by attaching the treated half to the control half with the help of an adhesive tape. Each remade filter paper was placed in a petridish and 10g of rice grains was uniformly distributed over it with the purpose of providing the natural condition for the rice weevils. Then 10 adult rice weevils were released on to the center of each filter paper disc and cover was placed over the petridish and kept in the dark. Each concentration of essential oil solution and the control were replicated 5 times. The number of insects present on each strip was recorded after 30minutes.

Contact Toxic Effect

Aliquots of 0.5ml of the essential oil concentrations were applied evenly on the inner surface of glass vials (diameter 1.5cm, height 10cm, volume 10ml) and the screw caps. Acetone was used as the control. After evaporating the solvents completely, 20 adult rice weevils were introduced into each vial containing culture media and the cap was screwed tightly on to the vial and then kept in the dark. Mortality of insects was recorded for every 30 minutes up to an hour after the introduction of weevils. Five replicates were set for each concentration of essential oil and the control.

Fumigant Toxic Effect

A Filtermann® (125mm) paper strip (diameter 2cm) was impregnated with 0.5ml of essential oil concentrations. After allowing the solvent to evaporate for 1 minute, the filter paper was placed on the underside of the screw cap of a glass vial. Twenty adult rice weevils were introduced into each vial containing the culture medium, the cap was screwed tightly and the vials were kept in the dark. Acetone was used as control. After 30 and 60 minutes of fumigation, adult mortality was recorded and each essential oil concentration and the control were replicated 5 times.

Analysis of Data

One-way analysis of variance (ANOVA) test was applied to analyze the data obtained for both repellency and toxicity bioassays using Minitab 14.0. Tukey's multiple comparison test was used to separate mean values of the experiments, where significant differences existed

($p \leq 0.05$). Comparisons between concentrations of leaf extracts were analyzed using the General Linear Model (GLM) procedure.

Results and Discussion

Data in Table1 demonstrate the repellency effect of the essential oil on rice weevils after 30 minutes of exposure in the area preference bioassay. Even though the highest concentration (200µl/10ml Acetone) produced the highest repellency (100%), all the other treatments were not significantly different from each other indicating that considerably very low concentrations could also be used effectively as repellents against rice weevils even within short time durations. The repellent effect of essential oils against *S. oryzae* adults may be due to the suffocation and inhibition of different biosynthetic processes of the insect metabolism [9]. The repellent effect of essential oils may have an important implication in post harvest storage system and potential activity for their local availability, making it an attractive candidate in management of rice weevil infestations [6].

Table 1. Repellent action of essential oil of *Ruta graveolens* on *Sitophilus oryzae* after 30 minutes in area preference bioassay

Concentration (µl oil/10ml acetone)	*Mean % Repellency ± SD
50	89.00 ± 4.18^{a}
100	95.00 ± 3.54^{b}
150	97.00 ± 2.47^{b}
200	100.00 ± 0.00^{b}

*Means followed by the same letters (a,b) are not significantly different according to the Tukey's test at P<0.05
*Mean Percentage Repellency ± SD for five replicates (n = 100)

Insecticidal effect of the essential oil on the rice weevil in contact toxicity bioassay is presented in Table 2.

Table 2. Mortality of essential oil from *Ruta graveolens* on *Sitophilus oryzae* in the contact toxicity bioassay

Concentration (µl oil/10ml acetone)	*Mean % Mortality ± SD	
	½ HAT	1 HAT
Control	0.00 ± 0.00[a]	0.00 ± 0.00[a]
50	76.00 ± 2.24[b]	93.00 ± 2.74[b]
100	84.00 ± 4.18[c]	98.00 ± 1.74[c]
150	91.00 ± 2.24[d]	100.00 ± 0.00[c]
200	95.00 ± 3.54[d]	100.00 ± 0.00[c]

*Means followed by the same letters (a,b,c,d) in each column are not significantly different according to the Tukey's test at P<0.05
*Mean Percentage Repellency ± SD for five replicates (n = 100)
*HAT – Hours After Treatment

It is of interest to note that all treatments exhibited extremely high insecticidal action against rice weevils after an hour in comparison to the control which gave no mortality at all. Essential oils drew out the highest mean percentage mortality (100%) of rice weevils after an hour of exposure from the intermediate concentration (150µl/10ml Acetone) onwards. It is noteworthy that the mean percentage mortality at the lowest concentration (50µl/10ml Acetone) within 30 minutes was also higher than 75%, thus indicating the strong insecticidal action of essential oil of *R. graveolens*.

Table 3. Mortality of essential oil from *Ruta graveolens* on *Sitophilus oryzae* in the fumigation toxicity bioassay

Concentration (µl oil/10ml acetone)	*Mean % Mortality ± SD	
	½ HAT	1 HAT
Control	0.00 ± 0.00[a]	0.00 ± 0.00[a]
50	77.00 ± 2.74[b]	91.00 ± 2.24[b]
100	87.00 ± 2.74[c]	97.00 ± 2.74[c]
150	92.00 ± 2.74[d]	100.00 ± 0.00[d]
200	94.00 ± 2.24[d]	100.00 ± 0.00[d]

*Means followed by the same letters (a,b,c,d) in each column are not significantly different according to the Tukey's test at P<0.05
*Mean Percentage Repellency ± SD for five replicates (n = 100)
*HAT – Hours After Treatment

Essential oil extracted from leaves of *R. graveolens* produced the highest mortality rate in rice weevils (100%) after an hour of exposure to 150μl and 200μl treatments of the essential oil which were also similar to the mortality rates obtained for the contact toxicity study (Table 2) in fumigation toxicity bioassay (Table 3). Even the lowest concentration (50μl/10ml Acetone) was able to produce over 75% and 90% fumigation toxic activities within 30 and 60 minutes respectively thus indicating the strong effectiveness of the essential oils of *R. graveolens* as fumigants.

Moreover, according to the results, it was evident that the repellent effect together with the contact and fumigant toxic effects have progressively increased with the increase of oil concentration.

Essential oils are complex mixtures of volatile organic compounds produced as secondary metabolites whose functions are other than the nutrition in plants; they are constituted by terpenoids (monoterpenoids, diterpenoids and sesquiterpenoids), benzene derivatives, hydrocarbons and other miscellaneous compounds[10]. They frequently are responsible for the distinctive odour of plants[11]. Essential oil constituents are primarily lipophilic compounds, and their prospective protective effects against insect pests range from repellency expressed as feeding and oviposition deterrence, to interference with growth and development or ultimately, acute contact and fumigation toxicity [12].

Essential oils are presumed to interfere with basic metabolic, biochemical, physiological and behavioral functions of insects. However, little is known about the true mode of action of essential oils on insects[12]. The rapid onset of contact and fumigation toxic signs suggests a neurotoxic mode of action involving competitive inhibition of acetylcholinesterase, competitive activation of octopaminergic receptors[13] or interference with GABA-gated chloride channels. Findings of the present study explicitly revealed that the volatiles emitted in the form of essential oils extracted from the leaves of *R. graveolens* would be strongly effective in controlling *S. oryzae* infestations, with evidence in their superior fumigant, repellent and contact toxic activities. Hence, this essential oil with its highly effective fumigant action could particularly be useful in controlling not only the rice weevil but also the other coleopteran pests living in food storage bins, warehouses and various other enclosed places.

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