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Biological activity and secondary metabolite profile of *Ruta graveolens* leaves against maize weevil infestations

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Abstract
The present phytocentric study provides the first investigation on volatile profile, chemical composition and bioactivity of *Ruta graveolens* (Aruda) leaves against the maize weevil, *Sitophilus zeamais* (Motschulsky). The volatile organic compound (VOC) profile of fresh leaves of *R. graveolens* was analyzed with HS-SPME/GC-MS and phytochemical screening was also performed. In addition, insecticidal and repellent properties of *R. graveolens* were also evaluated in both contact and vapor forms. A total of 45 compounds representing approximately 93% of the total quantum were detected in *R. graveolens* leaves consisting mainly of 2-ketones. Aqueous leaf extract exhibited the presence for majority of phytoconstituents while hexane demonstrating the least. Data on insecticidal potential and repellency against *S. zeamais* indicated strong maize grain protection activities. Present study provides the evidence that *R. graveolens* leaves contain agriculturally important bioactive secondary plant metabolites, justifying the use of plant species against *S. zeamais* in storage pest management programs.

Keywords: *Ruta graveolens*, *Sitophilus zeamais*, secondary metabolites, 2-ketones, repellency

1. Introduction
Plants produce a high diversity of secondary metabolites within their systems with a prominent function in the protection against insect pests on the basis of their insecticidal and repellent nature leading to symptoms ranging from the inhibition of larvae or insect growth to death [1]. After investigating the complexity of their chemical structures and biosynthetic pathways for a long period of time, the secondary metabolites have been assessed for their biological properties against a diverse array of insect pests in post-harvest storage insect pest management programs. Recognition of such biological phenomena involved with the plant chemical composition has increased the current focus towards the search of ecologically more effective novel bio-insecticides in protecting stored grains from insect attack [2]. In that manner, literature reports on phytochemical diversity of insect defenses in tropical and temperate plant families have been significantly proven [3]. From among these characterized secondary metabolites, only a few have been evaluated against a limited number of insect species, thereby leading to an assumption that some plants containing extraordinarily effective phytochemicals may still remain to be discovered. Those studies on plant biological activities carrying out in screening programs can lead to the combat of insect pests by direct application of natural compounds or its derivatives [2].
Hence, botanicals containing bioactive secondary metabolites appear to be promising to address the well-known and serious problems caused by the indiscriminate use of synthetic insecticides including genetic resistance of pest species, toxic residues on stored products, increasing costs of application and many more [4-6]. In that context, botanicals are ecofriendly, economic, target specific and biodegradable. Moreover, unlike synthetic insecticides which primarily rely on a single active ingredient, plant derived insecticides comprise a vast array of secondary metabolites that act concertedly on both behavioral and physiological processes, thus giving least chances for insect pests to develop resistance to such phytoconstituents [4, 7-9].
Accordingly, *Ruta graveolens* which is commonly known as Aruda or garden rue contains a unique reservoir of diverse secondary metabolites that imparts them great medicinal properties as well as a peculiar flavor and taste [10]. It is given internally for hysteria, amenorrhea epilepsy and flatulent colic while it is used externally as a rubefacient. Medicinal oil prepared with its juice is prescribed for convulsions in children, acute bronchitis, pneumonia, odontalgia and otalgia. Leaves are used in cases of rheumatalgia [11].

R. graveolens is used by Arabs in Palestine and Syria as a preventive of the ill effects of water drunk at unaccustomed springs; they either chew the leaves, or soak the plant in water [12]. In South Africa, the bruised herb is placed in hollow teeth and the ears to relieve toothache and earache respectively [12, 13]. In India, honey of the leaf is used for cardiac asthma, and the bruised leaf is given in jaundice and infantile diarrhea. In Central province of Sri Lanka, the leaves pounded with salt and are applied locally for scorpion sting [12].

The bitter leaves of *R. graveolens* are a traditional seasoning in Mediterranean countries where it is used to flavor meats, fish, salads, and egg and cheese dishes. In Africa, especially in Ethiopia, fresh leaves are used to flavor coffee. Leaves of this plant are also used as an aperitif in alcoholic beverages due to its very bitter taste [14].

Despite being widely used and studied especially regarding the pharmacological properties, and even if *R. graveolens* extracts and essential oil claim to suppress stored insect pests, a scarce data for a clear validation is available. As there is no previous report about the volatile organic compounds (VOCs) of *R. graveolens*, the present study provides the first investigation on the volatile profile of *R. graveolens* fresh leaves. In addition, preliminary phytochemical screening and investigation on the biological potential of *R. graveolens* fresh leaf powders against the maize weevil (*Sitophilus zeamais*) infestations were conducted.

2. Materials and Methods

2.1 Collection of Plant Material

Fresh, mature and healthy leaves of *Ruta graveolens* (Aruda) were collected from Bandarawela region. The 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. These were shade dried and coarsely powdered by using a domestic electric grinder (Multinational®, 2101, India). Then the powder was packed in glass containers with tight lids and stored in a refrigerator at 4 ^{0}C prior to use.

2.2 Sample Preparation for Phytochemical Screening

The coarse powders were subjected to successive extraction in various solvents such as n-hexane, ethyl acetate, methanol and water by using Soxhlet apparatus. The collected extracts were then taken up for further investigations.

2.3 Maintenance of Insect Cultures

Sitophilus zeamais used for the study were obtained from infested stock of maize in local market and then reared on wholly, un-infested maize grains in glass jars covered with muslin cloth held in place with rubber bands for the passage of air at 29 ± 2 ^{0}C and 84 ± 2% relative humidity. One week old adult weevils were used for all experiments.

2.4 Contact Insecticidal Activity

Contact insecticidal action was assayed by admixing 30 g of uninfested maize grains with powdered leaves of plants weighing 1.0, 3.0, 5.0, 7.0 and 10.0 g in the plastic containers (height 8 cm, diameter 7.5 cm). Twenty adult weevils (one week old) were introduced into each cup. The containers were covered with muslin cloth held firmly with rubber bands to prevent the escape of the weevils and to ensure the adequate aeration. Maize grains with no plant powders were included to serve as the control. Experiment was replicated five times. The contact mortality of the weevils was recorded at 3 hour intervals up to 24 hours of weevil exposure.

2.5 Fumigation Insecticidal Activity

The bio apparatus for the fumigation toxicity test was consisted of a small plastic container (height 4 cm, diameter 5 cm) attached to a plastic cup (height 8 cm, diameter 7.5 cm). The bottom of the plastic cup was removed and replaced by a nylon cloth to allow the vapor 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 put in the separate plastic containers while 30 g maize grains were placed in the plastic cups. Twenty adult weevils (one week old) were introduced into each plastic cup. The bio apparatus was covered with muslin cloth held in place with rubber bands. Bio apparatus without the leaf powders was kept as the positive control. The experiment was replicated 5 times. Fumigant mortality of the maize weevils was evaluated after every 3 hours up to 24 hours of weevil exposure**.**

2.6 Contact Repellency

Contact repellency was tested according to the standard method adopted by Mohan and Fields (2002) with some modifications [15]. 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 30 g of maize grains. 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 are 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 are moving out through the holes of the small plastic cup. One week old, 20 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. The experiment was replicated five times.

2.7 Fumigant Repellency

The bio apparatus used was somewhat similar to the setup in the contact repellency bioassay but with some alterations [15]. The bottom of the small plastic cup was removed and replaced by 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 vapor of leaf powders inside the container to pass through the cloth and reach the weevils. Leaf powder was then put in the plastic container, and 30 g of maize grains were placed in the small plastic cups. One week old, 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 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. The experiment was replicated five times.

2.8 Preliminary Qualitative Phytochemical Screening

The crude n-hexane, ethyl acetate, methanol and aqueous leaf extracts were subjected to phytochemical screening following the standard methods as described by Harborne (1998) [16] and Ayoola *et al*. (2008) [17].

2.9 Isolation of Volatile Organic Compounds (VOCs)

2.9.1 SPME Fibers

SPME holder and fiber assemblies for manual sampling were provided from Agillent Technologies (Palo Alto, CA) and used without modification. The fiber coatings assayed were as follows: polydimethylsiloxane (PDMS 30µm), polyacrylate (PA 85 µm) (Table 1). Before measurements, the fiber was conditioned in the injector for 30 minutes at 250 ^{0}C with split vent open to fully remove any contaminant that might cause high baseline noise and large ghost peaks. Then the fiber was repeatedly injected into the GC until interfering peaks disappeared. During this desorption process the GC column oven temperature was maintained at 250 ^{0}C.

Table 1: SPME fibers used during the microextraction technique.

Fiber Type	Acronym	Full Name	Volume of Coating (mm^3)
Medium-polar	PDMS 30	Polydimethylsiloxane, 30µm	0.132
Polar fiber	PA 85	Polyacrylate, 85 µm	0.521

2.9.2 Headspace Solid-Phase Microextraction Procedure

The HS-SPME extraction after optimization was performed by placing 0.3 g of freshly ground neem leaf powder in 12 mL crimp-top headspace vial (diameter 2 cm, height 6.7 cm), capped with porous poly-tetrafluoroethylene (PTFE) silicon rubber septum. The sample in the headspace vial was heated by supporting them with a clamp in a hot water bath (60 ^{0}C). After 10 minutes, needle of the SPME device was pierced the septum of the vial and the fiber was immersed to the headspace of the sample for 30 minutes, 1 cm above the leaf powder, which was kept at 60 ^{0}C. After extraction, the fiber was inserted into the hot injector of the GC systems for analysis.

2.9.3 Gas Chromatography–Mass Spectroscopy (GC-MS) Analysis Conditions

Chromatographic analysis was performed using an Agilent Technologies 7890A gas chromatograph (Palo Alto, CA) equipped with an Agilent Technologies 5975C inert XL EI/CI mass selective detector. A DB-5MS fused silica capillary column of 30 m × 0.25 mm i.d. × 0.25 µm film thickness (J & W Scientific, Folsom, CA) was used. Helium was used as the carrier gas at a flow rate of 1 ml/min and detector gases were hydrogen and air. The temperature was programmed as follows: initial oven temperature was 70 ^{0}C for 2 minutes, and then was increased at 10 ^{0}C/min up to 280 ^{0}C, where it was held for 7 minutes and maintained constant for 30 minutes. The injection port was in the split less mode. SPME fiber was introduced in the injector port, held at 250 ^{0}C for chromatographic analysis and remained in the inlet for 30 minutes.

Identification of components in the sample was based on the chromatographic criteria (retention times) and MS spectral library at Chemistry Department of University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka.

2.10 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 LD_{50} values to determine the lethal concentrations needed to kill 50% of maize weevils.

3. Results and Discussion

3.1 Contact Insecticidal Activity

The contact toxic effect of leaf powders on the survival of maize weevil adults are presented in Table 2. The effect of the *R. graveolens* leaf powders on adult mortality was significantly different (p<0.05) among the treatments and between them and the untreated positive control thus, corroborating that the contact insecticidal effect depends on the dose of the plant leaf powder and affects the endurance of *S. zeamais* adults. The highest dose (10.0 g) produced 100% contact toxicity within 21 hours, while the lowest dose (1.0 g) offering 44% within 24 hours of weevil exposure. On the other hand, an intermediate dose of 7.0 g the complete mortality of maize weevils within 24 hours of post treatment. It was noted that the number of maize weevils rendered sluggish, observed becoming worsened till the death when they were in contact with the leaf powders as the exposure time period of the maize weevils to the treatment proceeds (from 3 to 24 hours) registering an appreciable contact mortality level of *Sitophilus zeamais* in all treatments. Moreover, the rapid knockdown of the insects characterized during the observations within 21 hours justifies the well-known fact that a considerable number of plant species used as medicines locally, pose noticeable insecticidal activities [18, 19] without leading to the future progenies of maize weevils.

Table 2: Contact insecticidal effect of *R. graveolens* leaf powders on *S. zeamais* after every 3 hrs up to 24 hrs.

Time/ HAT	*Mean % Contact Mortality ± *SD*					
	Dose/ g					
	Control	1.0	3.0	5.0	7.0	10.0
3	0.00 ± 0.00^a	0.00 ± 0.00^a	3.00 ± 3.54^b	13.00 ± 2.74^c	33.00 ± 2.74^d	37.00 ± 2.74^d
6	0.00 ± 0.00^a	0.00 ± 0.00^a	10.00 ± 3.54^b	18.00 ± 2.74^c	43.00 ± 2.74^d	47.00 ± 2.74^e
9	0.00 ± 0.00^a	2.00 ± 2.74 ^{a}	19.00 ± 5.48^b	22.00 ± 4.47^b	48.00 ± 2.74^c	62.00 ± 2.74^d
12	0.00 ± 0.00^a	8.00 ± 7.58^b	33.00 ± 2.74^c	40.00 ± 3.54^d	58.00 ± 2.74^e	74.00 ± 2.24^f
15	0.00 ± 0.00^a	23.00 ± 2.74^b	35.00 ± 5.00^c	50.00 ± 3.54^d	68.00 ± 2.74^e	83.00 ± 2.74^f
18	0.00 ± 0.00^a	28.00 ± 2.74^b	40.00 ± 0.00^c	55.00 ± 3.54^d	73.00 ± 2.74^e	91.00 ± 2.24^f
21	0.00 ± 0.00^a	33.00 ± 2.74^b	47.00 ± 4.47^c	70.00 ± 0.00^d	86.00 ± 2.24^e	100.00 ± 0.00^f
24	0.00 ± 0.00^a	44.00 ± 4.18^b	64.00 ± 2.24^c	89.00 ± 4.18^d	100.00 ± 0.00^e	100.00 ± 0.00^e

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

*Mean Percentage Contact Toxicity ± *SD* for five replicates (n = 100)

*HAT – Hours after Treatment

3.2 Fumigation Insecticidal Activity

According to the results of analysis of variance (ANOVA) test, leaf powders of *R. graveolens* were observed to possess fumigation insecticidal potential, significantly affecting the longevity of *S. zeamais* adults compared to the untreated control indicating the influential effectiveness of *R.*

graveolens as fumigants (Table 3). Least adult mortality (17%) was noted when the maize grains were treated with leaf powders at 1.0 g, while the highest dose of 10.0 g demonstrating the highest in that respect accounting for 76% within 24 hours of post treatment time period. Thus, it is observable that although the leaf powders produced clear dose dependent fumigation mortalities of adult maize weevils, the end-point mortalities were slightly less than 80% after the 24 hour exposure time interval. As evidenced by the probit regression analysis of dose response (Table 4), the treatments were found to be significantly different with respect to the median lethal dose offering the respected LD_{50} values of 2.194 and 5.947 g in both contact and vapor forms that require killing 50% of the maize weevil populations within 21 hours of exposure. Studies have not been reported previously concerning the activity of the fresh leaves of *Ruta graveolens* as fumigant on insect pests.

Table 3: Fumigation insecticidal effect of *R. graveolens* leaf powders on *S. zeamais* after every 3 hrs up to 24 hrs.

Time/ HAT	*Mean % Fumigation Mortality ± *SD*					
	Dose/ g					
	Control	1.0	3.0	5.0	7.0	10.0
3	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	1.00 ± 2.24^a	4.00 ± 2.24^b
6	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	8.00 ± 2.74^b	13.00 ± 2.74^c
9	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	12.00 ± 2.74^b	24.00 ± 4.18^c
12	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	17.00 ± 2.74^b	27.00 ± 2.74^c	29.00 ± 4.18^c
15	0.00 ± 0.00^a	0.00 ± 0.00^a	3.00 ± 2.74^b	29.00 ± 2.24^c	42.00 ± 2.74^d	53.00 ± 2.74^e
18	0.00 ± 0.00^a	8.00 ± 2.74^b	14.00 ± 4.18^c	35.00 ± 5.00^d	54.00 ± 2.24^e	64.00 ± 4.18^f
21	0.00 ± 0.00^a	11.00 ± 2.24^b	16.00 ± 4.18^b	45.00 ± 3.54^c	63.00 ± 2.74^d	66.00 ± 4.18^d
24	0.00 ± 0.00^a	17.00 ± 2.74^b	28.00 ± 2.74^c	50.00 ± 3.54^d	70.00 ± 3.54^e	76.00 ± 4.18^e

*Means followed by the same letters in each row are not significantly different according to the Tukey's test at $P<0.05$
*Mean Percentage Contact Toxicity ± *SD* for five replicates (n = 100)
*HAT – Hours After Treatment

Table 4: Median lethal doses (LD_{50}) of *S. zeamais* due to *R. graveolens* leaf powders after 21 hours of exposure.

Treatment	LD_{50} (g)	Confidence Interval		Slope (± *SE*)
		Lower	Upper	
Contact	2.194	1.828	2.552	0.183
Fumigation	5.947	5.149	7.011	0.457

95% lower and upper fiducial limits are shown in parenthesis
LD_{50} – Lethal dosage that kills 50% of the population

3.3 Contact and Fumigation Repellent Activity

Mean percentage repellent results of *S. zeamais* adults to maize grains treated with varying leaf powder doses of *R. graveolens* are presented in Figure 1. It was evident that the magnitude of repellent level of maize weevils was significantly influenced ($P<0.05$) by bioassay form and the applied dose of leaf powder over the one hour of exposure time period. Leaf powders of *R. graveolens* elicited the highest mean percentage repellency of 97% at the highest dosage of 10.0 g within an hour of exposure when the powders were in contact with the maize weevils. On the other hand, significantly similar, but a bit low repellent effects (96%) were elicited by vapor form of the leaf powders, at the same dosage within an hour. Accordingly, leaf powders exhibited relatively high influence on the pest in the contact repellency test compared to fumigation repellency test in each dosage level. Moreover, the dose-dependent repellent activity of the leaf powders increased ranging from moderate to strong levels with the increase in dose. Furthermore, it could be observed that even lower doses of leaf powders caused significantly superior maize weevil repellent levels in comparison with the corresponding untreated controls thus implicitly proving the fact that the leaf powder and its smoke of *R. graveolens* which have long been used in daily life, can be utilized as promising and eco-socio-friendly repellents in reducing post-harvest losses in contrast to synthetic insecticides.

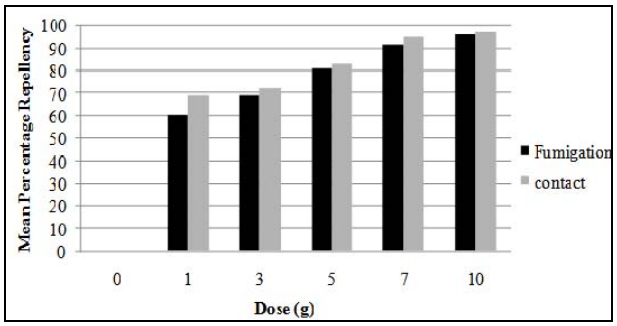

Fig 1: Contact and fumigation repellency effect of *Ruta graveolens* leaf powders on *S. zeamais* after 1 hour of exposure in the repellency bioassays.

Being successful in producing over 60% repellent values is a major achievement in the tropical agricultural research showcasing the rationalized use of botanicals for long-term protection of stored, durable agricultural products, such as maize grains against the maize weevils and other insect pests of stored products [20]. Even though the components of *Ruta graveolens* are renowned due to their potential and diverse pharmacological activities, limited published work is available on the broad spectrum of its biological activities in the field of insect pest management programs.

3.4 Preliminary Qualitative Phytochemical Screening

Table 5 provides the list of classes of plant secondary metabolites present in *Ruta graveolens* leaves that emphasize some concrete evidences on their defensive roles against insect pests.

Eleven bioactive classes of secondary metabolites/phytochemical constituents in each leaf extract were tested, namely, alkaloids, saponins, flavonoids, steroids, tannins, terpenoids, anthraquinones, glycosides, coumarins and phenols. Aqueous leaf extract exhibited the presence for majority of phytochemicals while hexane demonstrating the least presence.

Table 5: Phytochemical constituents of *Ruta graveolens* in Aqueous, Hexane, Ethyl acetate and Methanol extracts.

Phytochemical Constituent	Aqueous	n-Hexane	Ethyl Acetate	Methanol
Alkaloids	+	+	+	+
Saponins	+	+	-	+
Flavonoids	+	-	+	+
Tannins	+	-	-	+
Steroids	-	+	+	-
Terpenoids	+	-	+	-
Anthraquinones	+	+	+	-
Glycosides	+	+	+	+
Phlobatannins	-	-	-	-
Coumrins	+	+	+	+
Phenols	+	-	-	+

+ = Presence; - = Absence

It was found that alkaloids, glycosides and coumarins were present in each while phlobatannins were in none of the leaf extracts. Saponins, flavonoids and anthraquinones were reported in every extract but except in ethyl acetate, n-hexane and methanol respectively. Moreover, tannins and phenols were found to present only in the aqueous and methanol solvents. Steroids tested positive in the n-hexane and ethyl acetate solvents whereas terpenoids showcasing its presence in aqueous and ethyl acetate leaf extracts of *R. graveolens*.

Different solvents alone and in combinations are generally used for the maximum recovery of bioactive compounds, because different plants constitute different compositions of bioactive compounds [13].Though the results of present bioactive phytochemical screening are also comparable to those of other researchers who investigated in this respect, there were considerable changes that somewhat not strictly in line with the nature of chemical composition of *R. graveolens* grown in Sri Lanka [10, 13, 21, 22]. Discrepancy in this regard with the present study might be due to locality, geographical, climatic, seasonal and genetic differences of the plant, *Ruta graveolens*. In addition, more than 120 phytochemical compounds are commonly known to be present in *Ruta graveolens*; such as acridone alkaloids, coumarines, flavonoids, furoquinoline [23], saponin, tannins and glycosides [24].

Phytochemicals are the vast repository of compounds that have long been used to control insect pest damages in agricultural crops that possess wide range of biological activities such as insecticidal, repellent, antifeedant and growth retardant properties [25]. Glycosides are good sources of insect repellents [26], whilst alkaloids, phenols, flavonoids, tannins, steroids, anthraquinones and terpenoids signify the richness of *Ruta graveolens* leaves with insecticidal properties [27, 28, 29, 30]. The mode of action of alkaloids varies, but many affect acetylcholine receptors in the nervous system or membrane sodium channels of nerves. Important phenolics in terms of repellent and insecticidal functions are the flavonoid groups that can act as potential grain protectants. Accordingly, flavonoids possess a catecholic B-ring that seemisto be responsible for the toxicant activity to insects pests [31, 32]. Although, the large size of the phenols denotes that they have little significance as repellents due to their lack of volatility [33, 34], they impart negative effects on insects decreasing fertility as well as shortening their life span [35, 36].Tannins are inhibitors of proteases, affecting insect growth and survival, since they inactivate digestive enzymes and form a tannin-protein complex that is not easily degradable [37, 38]. Moreover, a slowly developing paralysis is a major feature of insect poisoning by coumarins [31, 39]. On the other hand saponins have clear insecticidal potential; they exert a strong and quick action against a broad range of insect pests which is different from neurotoxicity which includes increased mortality levels, retardation in development and decreased reproduction. The main hypotheses in literature underlying those observed effects are repellent/detterent activities, digestive problems, insect molting defects and cellular toxicity effects caused by saponins [40]. In that manner, bioactive secondary metabolites present in the leaves of *Ruta graveolens* may independently or jointly contribute to cause the observed repellent and insecticidal effect against *S. zeamais.*

3.5 Analysis of Volatile Organic Compounds (VOCs)

Table 6 presents the area values and the number of volatile organic compounds extracted from headspace of the leaves of *R. graveolens* using different fibers. Two fibers of PDMS 30 and PA 85 µm were chosen because both combine the best signal-to-noise ratio with maximum extraction of compounds. At present, this study represents the first ever report on the characterization of volatile compounds from the leaves of *R. graveolens* by using HS-SPME technique. The HS-SPME technique provides advantage of minimizing the sample handling and consequently decreasing the loss in any of the volatile compounds. It is rather a simple and fast modern tool used to characterize the volatile fingerprint of aromatic and medicinal plants while offering a valid alternative to traditional methods such as hydro-distillation, steam distillation and solvent extraction[41, 42].

Overall, 45 volatile organic compounds were identified in leaf fragrances of *R. graveolens* with different proportions amongst the two SPME fibers. However, the number of compounds detected using the medium-polar fiber (PDMS 30 µm) was higher and were better extracted exhibiting comparatively higher area percentages/ relative abundances except for 2-nonanone and 2-undecanone whereas the responses of polar fiber (PA 85 µm) in that context was sensitively lower. And yet, as a rule in general, major components extracted by the fibers remain being the same ones, only varying in their concentration levels.

The major extracted compounds were found to be 2-nonanone and 2-undecanone, each occupying approximately 35 % of the total spectrum followed by 2-decanone, 2-tridecanone, 3,4-diethenyl-3-methylcyclohexene, 4-(3,4-methylenedioxyphenyl)-2-butanone,cyclopropane carboxylic acid, dodecyl ester, 2-octanone and 2-dodecanone.

Table 6: SPME headspace analysis of volatile compounds from leaves of *Ruta graveolens* using PA 85 µm and PDMS 30 µm fibers.

	Volatile Organic Compound[a]	PA 85 µm		PDMS 30 µm	
		RT[b]	Relative Peak Area (%)*	RT[b]	Relative Peak Area (%)*
1	Cis-2-nonene	ND	-	3.739	0.289
2	2-Octanone	4.778	0.903	4.758	1.498
3	1-Methoxy-2-methylbenzene	ND	-	4.995	0.267
4	2-Nonanone	6.409	39.752	6.446	33.304
5	2,6-Dimethylanisole	ND	-	6.682	0.331
6	3,5-Dimethylanisole	ND	-	6.812	0.069
7	3,4-Diethenyl-3-methylcyclohexene	7.111	1.194	7.151	3.627
8	Cycloheptane	7.481	0.406	ND	-
9	2-Decanone	7.806	3.615	7.845	3.506
10	Pentylcyclopropane	ND	-	8.063	0.344
11	Cyclopropanecarboxylic acid, dodecyl ester	8.414	1.245	8.440	2.312
12	2-Undecanone	9.412	36.410	9.441	33.790
13	Acetic acid, nonyl ester	9.504	0.467	ND	-
14	1,2,3-Trimethoxybenzene	9.585	0.154	9.675	0.176
15	4-Ethyl-1,2-dimethoxybenzene	ND	-	9.778	0.338
16	Biphenyl	10.547	0.111	ND	-
17	2-Dodecanone	10.637	0.924	10.672	1.320
18	1,2,3-Trimethoxy-5-methylbenzene	10.799	0.214	ND	-
19	1,2-Dimethoxy-4-(2-propenyl)benzene (Methyleugenol)	ND	-	10.805	0.266
20	2,7-Dimethyl naphthalene	ND	-	11.362	0.054
21	Biphenylene	11.499	0.343	11.513	0.614
22	2,6,6-Trimethyl-1-cyclohexene-1-propanoic acid	11.765	0.222	ND	-
23	1-(4-Hydroxy-3,5-dimethoxyphenyl)ethanone	ND	-	11.771	0.264
24	1-Pentadecene	ND	-	11.868	0.193
25	2-Tridecanone	11.937	1.358	11.965	2.113
26	alpha-Farnesene	12.102	0.302	12.118	0.796
27	1,2-Dimethoxy-4-(2-methoxyethenyl)benzene	ND	-	12.238	0.032
28	Dibenzofuran	ND	-	12.356	0.135
29	Cyclohexanemethanol,4-ethenyl-alpha.,.alpha.,4-trimethyl-3-(1-methylethenyl)-,[1R-(1.alpha.,3.alpha.,4.beta.)] (Elemol)	12.705	0.115	ND	-
30	3,7-Cyclodecadiene-1-methanol,.alpha.,.alpha.,4,8-tetramethyl (Hedycaryol)	ND	-	12.711	0.222
31	4-(3,4-Methylenedioxyphenyl)-2-butanone	13.286	2.329	13.296	1.275
32	Hexadecanal	14.508	0.147	14.507	0.091
33	Anthracene	15.448	0.077	15.440	0.047
34	Cycloteradecane	ND	-	15.504	0.022
35	3-Adamantanecarboxylic acid, phenyl ester	15.649	1.600	ND	-
36	5-(2,2-Dimethylethyl)-1,3-benzodioxole	ND	-	15.660	1.488
37	2H-Furo[2,3-H]-1-benzopyran-2-one (Angelicin)	15.945	0.710	15.947	0.208
38	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	16.081	0.148	ND	-
39	5-Nonadecen1-ol	ND	-	16.083	0.055
40	6-Tetradecyne	ND	-	16.377	0.011
41	Cis,cis-7,10-hexadecadienal	16.379	0.037	ND	-
42	9,12,15-Octadecatrienoic acid	16.448	0.071	16.444	0.013
43	Phytol	18.553	0.043	18.532	0.024
44	1-(3-Aminopropyl)azacyclotridecan-2-one	19.266	0.314	ND	-
45	2,6,10,,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexanene (Squalene)	24.466	0.081	ND	-
	Total		93.292		89.094

[a]Compounds listed in order of elution; [b]RT – Retention Time;
*Data are expressed as percentage of the total peak area
ND – Not Detected

Chemical class composition of the volatile compounds in the headspace from *Ruta graveolens* leaves are illustrated in Table 7. The leaf volatile profile was dominated by 2-ketones, esters, monoterpenoids, sesquiterpenoids, fatty aldeyhydes and benzodioxoles. The highest composition of long chain alphatic methyl 2-ketones, notably 2-undecanone, 2-nonanone, with the low abundances of 2-decanone, 2-tridecanone, 2-octanone and 2-dodecanone constituted approximately 83 % of the fragrant headspace of *R. graveolens* whilst other constituents present in influential amounts were making a significant contribution in the leaf volatiles of *R. graveolens*.

A methyl ketone, 2- undecanone is a known insect repellent in natural plant defense mechanism and the active ingredient (7.75 %) of the latest arthropod repellent of BioUD, registered for the use by U.S. Environmental Protection Agency [43, 44] that has been convinced to be comparable to DEET in its activity against insect pests [45]. The repellent properties of 2-nonanone and 2-tridecanone have also been well-documented [46, 47]. Moreover, 2-undecanone, 2-tridecanone and 2-

nonanone are three major insecticidal volatile compounds with confirmed adverse toxic effects on the larvae of *Keiferia lycopersicella*, *Spodoptera exigua* [48], larvae of *Anopheles quadrimaculatus*, *Aedes aegypti* [49], parasitoids and predators of *Heliothis zea* [50] and many more insect pests in every respect of the world. Accordingly, current researches have better proven the long chain aliphatic methyl ketone analogies as the potential sources of insect repellents [51] and toxicants against *Sitophilus sp.* as evidenced in fumigant and contact assays [52] where the presence of carbonyl groups of ketones augment the biological activities [53]. On the other hand, monoterpenoids have been considered as potential stored-insect pest control agents because they are acutely toxic to insects and possess repellent [54] and antifeedant properties [55]. The volatile fraction of *R. graveolens* leaves contains three sesquiterpenids, such as, alpha-farnesene, elemol and hedycaryol, with the latter being reported to possess insecticidal, antifeedant [56] and repellent activities against insects [57].

Table 7: Chemical class composition of volatile organic compounds of leaves of *Ruta graveolens*.

Molecular Framework	Class	Percentage (%)	
		PA 85 µm	PDMS 30 µm
Aliphatic	2-Ketones	83.276	75.531
	Esters	3.312	2.312
	Monoterpenoids	1.600	3.627
	Diterpenoids	0.191	0.024
	Triterpenoids	0.081	-
	Sesquiterpenoids	0.417	1.018
	Biphenyls and derivatives	0.343	0.614
	Hydrocarbon derivatives	0.222	-
	Fatty aldehydes	0.184	0.091
	Fatty alcholos	-	0.055
	Cycloalkanes	-	0.366
	Acyclic olefins	-	0.493
	Lineolic acid and derivatives	0.071	0.013
	Other	0.077	0.047
Aromatic	Benzodioxoles	2.329	2.763
	Methoxybenzenes	0.368	0.812
	Furanocoumarins	0.710	0.208
	Phenol ethers	-	0.667
	Phenols and derivatives	-	0.264
	Naphthalenes	-	0.054
	Biphenyls and derivatives	0.111	0.135
Total		93.292	89.094

Although the simple coumarins are toxic and detterent to insects and being found in many plant families, furanocoumarins are more restricted to a few like Rutaceae and Umbelliferae. Linear furanocoumarins such as angelicin have a unique phyto-toxicity which can crosslink both strands of DNA by binding to the pyrimidine bases under UV radiation leading to the inhibition of DNA replication and transcription, and eventually to cell death, thus transforming furanocoumarins highly toxic to generalist insects [58, 59].

4. Conclusion

It implicitly emerges from the above arguments that the biological activities in terms of repellent and insecticidal effects exerted against the *Sitophilus zeamais* infestations on stored maize, could be attributed to the presence of plant secondary metabolites tracked during the preliminary phytochemical screening and headspace solid phase microextraction (HS-SPME) the latter of which is a novelty designed for the VOC profiling of secondary metabolites. A synergistic phenomenon among these bioactive secondary metabolites might have resulted higher biological activities in both contact and vapor forms during the experimentation. Correspondingly, the present study suggestively demonstrates the importance of identifying the intraspecific chemical composition for the evaluation of biological activities of the leaves of *Ruta graveolens* to be exploited in commercial scale. Hence, the present study refurbished the current state of knowledge compiling broad spectrum of bioactivity of the plant leaves with its rich pool of bioactive secondary constituents, with the view of linking the traditional worthiness of *Ruta graveolens* with its scientifically evidenced proof of efficacy for the inclusion in integrated insect pest management programs as potential protectants of stored-durable-agricultural products against the coleopteran insect pests.

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