

# Qualitative Determination, Quantitative Evaluation and Comparative Insecticidal Potential of *Ruta Graveolens* Essential Oil and Its Major Constituents in the Management of Two Stored Pests *Sitophilus Zeamais* (Coleoptera: Curculionidae) And *Corcyra Cephalonica* (Lepidoptera: Pyralidae)

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## Abstract

Eco-chemical control based on essential oil mediated plant-insect interactions is an alternative method to the unsystematic use of insecticides, due to advanced structural diversity and allelopathic potential of essential oils. In this sense, present work was aimed at qualitative and quantitative investigation of chemical composition and the evaluation of insecticidal activities of *Ruta graveolens* essential oil and its major constituents against *Sitophilus zeamais* and *Corcyra cephalonica* in stored maize. Fresh leaves were subjected to hydrodistillation and the chemical composition of oil was studied. Essential oil and its major constituents were then assessed for their allelopathic activity on test insects. Fifty components were identified, where long chain aliphatic 2-methyl ketones predominated the oil as major constituents. Results revealed strong concentration-, insect species- and time-dependent toxicities, in which oil caused 100 % mortalities at concentrations of 1.52 and 0.46  $\mu\text{L}/\text{cm}^2$  against *S. zeamais* and *C. cephalonica* respectively after 24 hours of exposure. In aliphatic 2-methyl ketone series, respective congeners having odd and even number of carbon atoms were more effective on *S. zeamais* and *C. cephalonica*. These findings provide a scientific basis for the eco-potential of using essential oil of *R. graveolens* and its major constituents in integrated insect pest management programs.

**Keywords:** *Ruta graveolens*, *Sitophilus zeamais*, *Corcyra cephalonica*, contact insecticidal activity, long chain aliphatic methyl ketones

## 1. Introduction

Synthetic insecticides are being extensively used with the view of protecting stored grains in increasing yields which also have triggered many undesirable impacts on entire ecosystems, society and every aspect of life on earth. Thus, the development of green insecticides has been focused as a viable pest management strategy in IPM programs in recent years (Khani and Rahdari, 2012). Particularly, plant essential oils and their constituents have been extensively studied and proposed as being a high potential option (Pumnuan *et al.*, 2015; Guo *et al.*, 2015) due to their specificity in exerting wide array of known biological influences on insects including repellent, deterrent or antifeedant, inhibition of digestion, increasing oviposition or contrarily decreasing reproduction by ovicidal and larvicidal effects (Regnault-Roger, 1997). The high volatility of plant essential oils and their constituents reduce the concern for their residues on stored grains (Kim *et al.*, 2016). Different content ratios among the authentic compounds of essential oils will strongly influence its quality which varies with geographical and seasonal variation (Porel *et al.*, 2014). Even the minor compounds should not be disregarded as they contribute significantly to the quality of oil. Hence the accurate quantification of individual compounds of essential oils is a key element in research or industrial analysis which may be used to understand the relationship not only between the constituent structure and aroma of essential oils (Zhu *et al.*, 2005), but also between the constituent structures and their broad spectrum of biological properties against insect pests.

During our screening process oriented in search of bioactive constituents to replace the use of synthetic insecticides, the essential oil of *Ruta graveolens* was found to possess contact insecticidal activity against the maize weevil,

*Sitophilus zeamais* Motschulsky, and the rice moth, *Corcyra cephalonica* Stainton, which are two key pests of cereal grains in tropical and subtropical regions of the world (Krishna-Ayyar, 1930). Even though Jayaweera (1982) reported the major constituents of *R. graveolens* essential oil as 2-nonanone and 2-undecanone, the entire profile on the chemical composition of the oil of *R. graveolens*, growing in Sri Lanka, has never been established. While, the number of reports the insecticidal properties of essential oil of *R. graveolens* (Jeon *et al.*, 2015) is very limited, literature survey has shown that there is neither a report on quantitative evaluation of the major essential oil constituents nor on their comparative insecticidal activities.

In this phytocentric study, we performed qualitative and quantitative determination of *Ruta graveolens* essential oil and its constituents. Using the results thus obtained, we comparatively evaluated the contact insecticidal effect of the essential oil together with its constituents with the view of identifying and characterizing bio-active constituents of the essential oil to be utilized in the management of *S. zeamais* and *C. cephalonica* under laboratory conditions.

## 2. Materials and Methods

### 2.1 Test Insects

Maize weevils (*Sitophilus zeamais*) and rice moths (*Corcyra cephalonica*) were obtained from the cultures reared on respective media of whole, un-infested maize grains and coarsely ground maize in the Agricultural Insect Pest Management Laboratory of Department of Zoology, Faculty of Applied Sciences, University of Sri Jayewardenepura, Sri Lanka. The relative humidity, light regime and ambient temperature during the experimental period were  $84 \pm 2\%$  RH, 12h:12h light:dark, and  $29 \pm 2^\circ\text{C}$  respectively. One week old, unsexed adult weevils and <24h old rice moths were used in all bioassays.

### 2.2 Plant Material

Fresh, mature and healthy leaves of *Ruta graveolens* were collected from a local market at Colombo Fort, Sri Lanka. These were coarsely powdered by using a domestic electric grinder (Multinational®, 2101, India).

### 2.3 Essential Oil Extraction

The essential oil was extracted by hydro-distillation of the ground plant leaves (250 g of sample in 2.5 L of distilled water) using a modified Clevenger-type apparatus for 5 h with 3 replications. The superior phase was collected from the condenser, dried over anhydrous sodium sulphate and stored in sealed glass vials at  $4^\circ\text{C}$  prior to analysis. The essential oil extraction was carried out in the laboratory at the Ayurvedic Research Institute, Navinna, Maharagama, Sri Lanka.

### 2.4 Reference Compounds and Reagents

Pure commercial standard compounds of 2-octanone (C8), 2-nonanone (C9), 2-decanone (C10), 2-undecanone (C11), 2-dodecanone (C12), 2-tridecanone (C13) representing the 2-ketone series were purchased from Sigma-Aldrich (USA). These reference compounds were selected due to their top-tier presence in the essential oil composition of *R. graveolens*. Acetone (analytical grade quality) for Gas Chromatography-Mass Spectrometry (GC-MS) Analysis was also purchased from Sigma-Aldrich, USA. The other standard compounds were all in GC purities.

### 2.5 Sample Preparation

For the characterization of essential oil composition of *R. graveolens* through GC-MS analysis, 12  $\mu\text{L}$  of accurately measured essential oil sample was dissolved in 1 mL of Acetone. Then 2  $\mu\text{L}$  of the solution was injected into the GC-MS system.

For GC-MS analysis, a standard cocktail comprising long chain aliphatic methyl ketone constituents was prepared separately, in order to detect and quantify the compounds of interest. The cocktail which is a mixed stock solution containing reference standards (C8, C9, C10, C11, C12, and C13) was prepared by dissolving 12  $\mu\text{L}$  of accurately measured standard of each compound in acetone targeting a stock concentration of  $100 \mu\text{g mL}^{-1}$  for the cocktail. Dilutions of the standard cocktail were made in acetone to produce concentrations of the compounds ranged at 10-50  $\mu\text{g mL}^{-1}$ .

### 2.6 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Chemical standards and EO of *R. graveolens* were analyzed using an Agilent Technologies 7890A GC system (Palo Alto, CA) equipped with an Agilent Technologies 5975C inert XL EI/CI mass spectrometer. A DB-5MS fused silica capillary column of  $30\text{m} \times 0.25\text{mm}$  (J & W Scientific, Folsom, CA) was used. The temperature was programmed as follows: initial oven temperature was  $70^\circ\text{C}$ , held for 2 minutes, and then ramped at  $100^\circ\text{C}/\text{min}$  up

to 2800C, where it was held for 7 minutes. The injector and detector temperatures were set at 2750C using helium as the carrier gas, at a flow rate of 1mL/min. The injection volume of the sample was 2  $\mu$ L using the split flow mode (split ratio, 5:1).

The identification of EO components was based on the NIST reference mass spectral library provided with the GC-MS equipment. The composition was reported as a relative percentage of the total peak area. EO constituents were ranged into 2 molecular framework categories of aliphatic and aromatic compounds.

### 2.7 Quantification of Key Essential Oil Constituents

Based on the prolific percentage in the functional group composition (56.16 %) of essential oil, long chain aliphatic methyl ketones were selected for their quantitative determination of the essential oil of *R. graveolens*. Calibration graphs were constructed using the series of standard solutions of varying nominal concentrations containing known amounts of 2-methyl ketone constituents prepared with acetone. Concentration and absolute percentage of each chemical constituent in *R. graveolens* EO were evaluated by comparison of the chromatographic peak areas of reference compounds in standard solutions with those of authentic compounds present in the EO extract of *R. graveolens*.

### 2.8 Insecticidal Effect by Contact With Treated Glass

Preliminary range finding studies were conducted in order to determine the appropriate testing concentrations of the EO of *R. graveolens* to be applied against *S. zeamais* and *C. cephalonica*. A series of concentrations for the EO and its major long chain aliphatic 2- methyl ketone constituents were prepared in acetone, where each concentration of individual compounds was prepared according to their persisting authentic proportions in the EO composition of *R. graveolens*.

The contact insecticidal effect of the EO and individual compounds against *S. zeamais* adults was measured as described by Kouninki *et al.* (2007) and Betancur *et al.* (2010) with slight modifications. Aliquots of 0.5 mL of the prepared concentrations of EO (0.047, 0.095, 0.190, 0.304, 0.456, 0.760, 1.520, 3.040, 6.079, and 12.158  $\mu$ L/cm<sup>2</sup>) and respective corresponding concentrations of each 2-methyl ketone constituent were applied evenly on the inner surface of glass tubes (diameter 1 cm, height 10 cm, volume 10 mL) and the screw caps. The tubes were agitated for the oil and constituents to uniformly cover the interior surface and then the acetone was allowed to evaporate at ambient temperature for 2 hours. After evaporation of solvents completely, 20 adult maize weevils were introduced into each tube and was then covered with muslin cloth held in place with rubber bands to allow ventilation of weevils. Insect mortality was assessed at 6, 12 and 24 h of exposure to the solutions. Glass tubes treated with acetone alone were used as the control. Each concentration and the control were replicated 5 times.

The contact toxic potential of the *R. graveolens* EO and its individual components against *C. cephalonica* adult moths was investigated using the same bioassay setup of *S. zeamais* with some alterations. After the application of different concentrations of EO (0.007, 0.014, 0.029, 0.046, 0.070, 0.116, 0.232, 0.464, 0.928, and 1.856  $\mu$ L/cm<sup>2</sup>) and respective corresponding concentrations of each 2-methyl ketone constituent, which followed by agitation and evaporation of acetone, 10 adult moths were transferred to both treated and control glass tubes (diameter 4 cm, height 15 cm). Insect mortality was checked after 6, 12 and 24 h of the moth exposure. Five replicates were made in all treatments and control where acetone was used as the control.

### 2.9 Statistical Analysis

Mortality was corrected according to Abbott's formula (Abbott, 1925) and all data were subjected to one-way analysis of variance (ANOVA) using the Minitab software, 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 LC<sub>50</sub> values to determine the lethal concentrations needed to kill 50% of *S. zeamais* and *C. cephalonica*.

## 3. Results and Discussion

### 3.1 Identification of Chemical Composition of *R. graveolens* Essential Oil

The *R. graveolens* essential oil obtained was light yellow in color with a yield of 0.2% v/w. The oil exhibited a more diverse chemical composition where a total of 50 components were identified accounting for 87.59% of the total oil (Table 1). According to the fingerprint chemical profile of essential oil, it is noticeable that the main constituents were 2-undecanone (30.671 %) and 2-nonanone (20.790 %) while long chain aliphatic 2-methyl ketones profusely maintaining the predominance oil composition.

Table 1. Percentage chemical composition of *Ruta graveolens* essential oils produced in Sri Lanka

<b>Molecular Framework</b>	<b>Volatile Organic Compound<sup>a</sup></b>	<b>R<sub>t</sub><sup>b</sup></b>	<b>Percentage (%)<sup>*</sup></b>
Aliphatic	<b>Organooxygen Compounds</b>		<b>2.18</b>
	Diacetone alcohol	2.72	1.64
	Nonanal	6.45	0.37
	Decanal	7.97	0.17
	<b>Long chain aliphatic methyl ketones</b>		<b>56.16</b>
	2-Octanone	4.66	0.49
	2-Nonanone	6.34	20.79
	2-Decanone	7.78	2.71
	2-Undecanone	9.39	30.67
	2-Dodecanone	10.64	1.19
	2-Tridecanone	11.94	0.31
	<b>Fatty Acyls</b>		<b>0.61</b>
	Nonyldichloroacetate	7.45	0.46
	2-Acetoxytetradecane	11.09	0.14
	<b>Fatty alcohols</b>		<b>0.08</b>
	1-Nonanol	9.49	0.05
	Behenyl alcohol	21.73	0.03
	<b>Sesquiterpenoids</b>		<b>1.44</b>
	.alpha-Farnesene	12.10	0.31
	Elemol	12.70	0.05
	$\gamma$ -Eudesmole	13.72	0.19
	$\alpha$ -Eudesmol	13.99	0.99
	<b>Diterpenoids</b>		<b>0.12</b>
	Phytol	18.54	0.12
	<b>Acyclic alkanes</b>		<b>0.33</b>
	Octadecane	23.29	0.33
	<b>Saturated hydrocarbons</b>		<b>0.19</b>
Hexadecane	13.16	0.19	
<b>Lineolic acids and derivatives</b>		<b>0.02</b>	
Linoleic acid	16.45	0.02	
Aromatic	<b>Phenol ethers</b>		<b>2.91</b>
	1-Methoxy-2-methylbenzene	4.99	0.17
	1-Methoxy-4-methylbenzene	5.16	0.11
	2,3-Dimethylanisole	6.54	0.25
	3,5-Dimethylanisole	6.71	0.06
	5,6-Diethenyl-1-methyl cyclohexene	7.08	2.32
	<b>Monoterpene</b>		<b>0.11</b>
	D-Limonene	5.28	0.11
	<b>Arene</b>		<b>0.92</b>
	Indene	5.57	0.05
	Biphenylene	11.49	0.23
	1H-Phenalene	12.94	0.64
	<b>Benzene and substituted derivatives</b>		<b>0.59</b>

2,3-Dimethoxytoluene	8.49	0.29
Biphenyl	10.54	0.20
Benzenebutanol	14.11	0.11
<b>Pyridines and derivatives</b>		<b>6.73</b>
4-Hydroxypyridine1-oxide	9.62	6.73
<b>Methoxybenzenes</b>		<b>0.78</b>
4-Ethyl-1,2-dimethoxybenzene	9.70	0.45
Methyleugenol	10.79	0.33
<b>Naphthalenes</b>		<b>2.22</b>
2-Ethenyl naphthalene	11.22	0.87
2-Phenyl naphthalene	17.40	0.15
Fluoranthene	18.30	1.20
<b>Phenols and derivatives</b>		<b>1.57</b>
Acetosyringone	11.76	1.57
<b>Benzodioxoles</b>		<b>0.32</b>
4-(3,4-Methylenedioxyphenyl)-2-butanone	13.29	0.27
5-(2,2-dimethylethyl) 1,3-benzodioxole	15.66	0.05
<b>Phenanthrenes</b>		<b>6.30</b>
Phenanthrene	14.50	0.77
1-Methylphenanthrene	16.64	0.11
4H-Cyclopenta[def]phenanthrene	16.88	0.05
Pyrene	18.81	5.37
<b>Anthracenes</b>		<b>3.07</b>
Anthracene	15.43	3.07
<b>Other</b>		<b>0.95</b>
1-Ethyl-4-methoxybenzene	6.63	0.07
Dibenzofuran	12.34	0.83
n-Heptanoicacid,methyl(tetramethylene)silyl ester	19.30	0.05
<b>Total</b>		<b>87.59</b>

<sup>a</sup>Compounds listed primarily on the basis of class category percentage and secondarily on the individual percentage within the respective category,  $R_t^b$ – Retention time of the essential oil solution, \*Data are expressed as percentage of the total peak area.

The results of the analysis of the present study were to some extent in agreement with the other literature that reported 2-undecanone and 2-nonanone as major constituents in the essential oil of *R. graveolens* grown in Egypt, Hong Kong, Tunisia and Iran (Aboutab *et al.*, 1988; Zhu *et al.*, 1993; Fredj *et al.*, 2007; Soleimani *et al.*, 2009) with a slight exception where in Jordan the major compounds of *R. graveolens* essential oil were reported to be 2-nonanone and undecanal (Al-Shuneigat *et al.*, 2015). However, there were overall differences in each oil compositions with respect to the relative proportions of each compound and the presence or absence of the other minor components. These variations may be attributed to plant part, season, method of harvesting, geographical zone, and isolation method of plant product (Khani and Rahdari, 2012).

However, several identified essential oil constituents could be formed by autoxidation during the sample preparation procedures. Moreover, there are some limitations for volatile compounds as structural alterations of thermally labile compounds may occur during analysis due to high temperature of injector or columns in GC method (Porel *et al.*, 2014). According to Adam (1995), essential oils are comprised of vast number of compounds and like materials, thus, similarity of retention indices of many related compounds will result in overlapping peaks in chromatograms. The presence of unsaturated bonds, various branched and cyclic compounds, and oxygenated analogues including alcohols and ketones would further complicate this issue (Zhu *et al.*, 2005).

### 3.2 Quantitative Determination of Key Long Chain Aliphatic 2-methyl Ketone Constituents of *R. Graveolens* Essential oil

Experimentations on the structure-activity relationship of some essential oil constituents, have revealed that the oil constituents with ketone functional groups are usually stronger in their insecticidal potential, than those of hydrocarbons (Kim *et al.*, 2016), thus emphasizing the fact that assessing the bioactivity of chemical constituents belonging to such functional groups to be of high priority. Accordingly, based on both prolific percentages in the functional group composition (56.16 %), long chain aliphatic methyl ketones were selected for their quantitative determination of the essential oil of *R. graveolens*. Calibration curves were thus constructed for the major congeners of 2-methyl ketone series (C8-C13) which followed linear relationships. The detailed results of the regression equations, corresponding correlation coefficients and original percentage of each compound in *R. graveolens* essential oil are shown in Table 2.

Table 2. Regression equations, correlation coefficients and original percentages of key long chain aliphatic 2-methyl ketone constituents identified in the *Ruta graveolens* essential oils

Compounds	Molecular Formula	Regression Equation	r <sup>2</sup>	Original Percentage in Essential oil (%)
2-Octanone	C <sub>8</sub> H <sub>16</sub> O	y = 444,504.67x + 3,033,841.15	0.9654	0.447
2-Nonanone	C <sub>9</sub> H <sub>18</sub> O	y = 431,260.77x + 95,125.927	0.9882	22.684
2-Decanone	C <sub>10</sub> H <sub>20</sub> O	y = 576,824.01x - 1,312,092.54	0.9838	2.230
2-Undecanone	C <sub>11</sub> H <sub>22</sub> O	y = 802,213.27x - 4,180,855.08	0.9724	17.760
2-Dodecanone	C <sub>12</sub> H <sub>24</sub> O	y = 745,619.20x + 1,962,139.23	0.9870	0.727
2-Tridecanone	C <sub>13</sub> H <sub>26</sub> O	y = 826,175.07x + 8,085,014.92	0.9628	0.786
<b>Total</b>				<b>44.643</b>

In the regression equation  $y = mx + c$ , x refers to the sample injection amount, y to the peak area, r<sup>2</sup> - Correlation coefficient of the equation

Quantitative analysis produced better and confirmatory proof for long chain aliphatic methyl being the foremost functional group in the *R. graveolens* essential oil as evidenced in the qualitative determination. These ketones solely established 44.643 % of the total oil content with 2-undecanone and 2-nonanone being occupying over 40 % of the total content.

### 3.3 Insecticidal effect on *S. zeamais* and *C. cephalonica* by contact with treated glass

The contact insecticidal effects of *R. graveolens* essential oil and its principle long chain 2-methyl ketone constituents applied onto a glass surface against *S. zeamais* are shown in Figure 1. In all instances, considerable differences in insecticidal activity of essential oil and its ketone constituents on insects were observed with the increase in concentrations and time periods after application. Results indicated that the oil itself produced relatively the highest insecticidal effects than any of its ketone constituents at each concentration level during each time period. The oil produced 100% mortality of *S. zeamais* after 24 h exposure at an intermediate concentration of 1.52 µL/cm<sup>2</sup>. The most abundant constituents of 2-nonanone and 2-undecanone exhibited significantly similar protection efficacy over *S. zeamais* at each concentration, exerting 100% weevil mortalities at the corresponding concentrations of 1.38 and 2.15 µL/cm<sup>2</sup> after 24 h of post treatment. 2-Tridecanone showed only moderate insecticidal potential with all corresponding test concentrations at each time interval, while 2-octanone followed by 2-dodecanone produced the least insecticidal effect only after 24 hours of weevil introduction. On the contrary, 2-decanone was not effective against *S. zeamais* notwithstanding the concentrations and exposure periods.

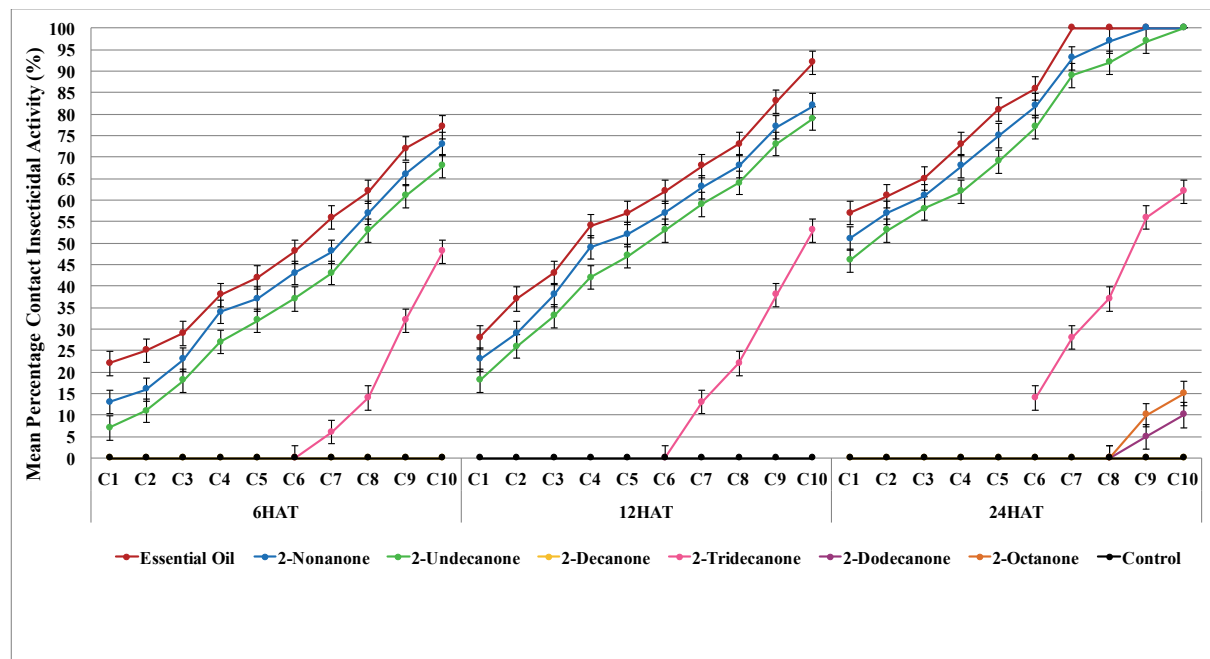


Figure 1 Mean percentage contact insecticidal activity ( $\pm$  SD) of *R. graveolens* essential oil and its principle long chain aliphatic 2-methyl ketone constituents against *S. zeamais* at 6, 12 and 24 hours of weevil exposure to treated glass. C= Concentration based on 10 levels of essential oil content (0.047, 0.095, 0.189, 0.303, 0.455, 0.758, 1.517, 3.033, 6.066, 12.132  $\mu\text{L}/\text{cm}^2$ ); HAT= Hours After Treatment; n=100

Results in terms of contact insecticidal effectiveness of *R. graveolens* essential oil and its principle long chain 2-methyl ketone constituents applied on a glass surface at different concentrations against adult *C. cephalonica* are illustrated in Figure 2. Cumulative insect mortalities increased with the increase of concentrations and exposure time. Essential oil was found to be greatly effective on rice moths, achieving 100 % adult mortalities after an exposure period of only 12 hours at the highest concentration of 1.86  $\mu\text{L}/\text{cm}^2$ . The insecticidal activity of 2-undecanone followed by 2-nonanone, were comparable to that of essential oil at all concentrations. 2-Undecanone and 2-nonanone revealed 100% *C. cephalonica* mortalities at the corresponding concentrations of 0.16 and 0.45  $\mu\text{L}/\text{cm}^2$  after 24 h of exposure respectively. 2-Decanone produced strong maize protection efficacy accounting for 88% while, 2-dodecanone and 2-tridecanone inducing moderate potency against *C. cephalonica* at the corresponding concentrations of the highest essential oil concentration at 24 h of post treatment. The 2-octanone exerted the weakest insecticidal action on rice moths only after 24 hours compared to the other compounds in aliphatic methyl ketone series.

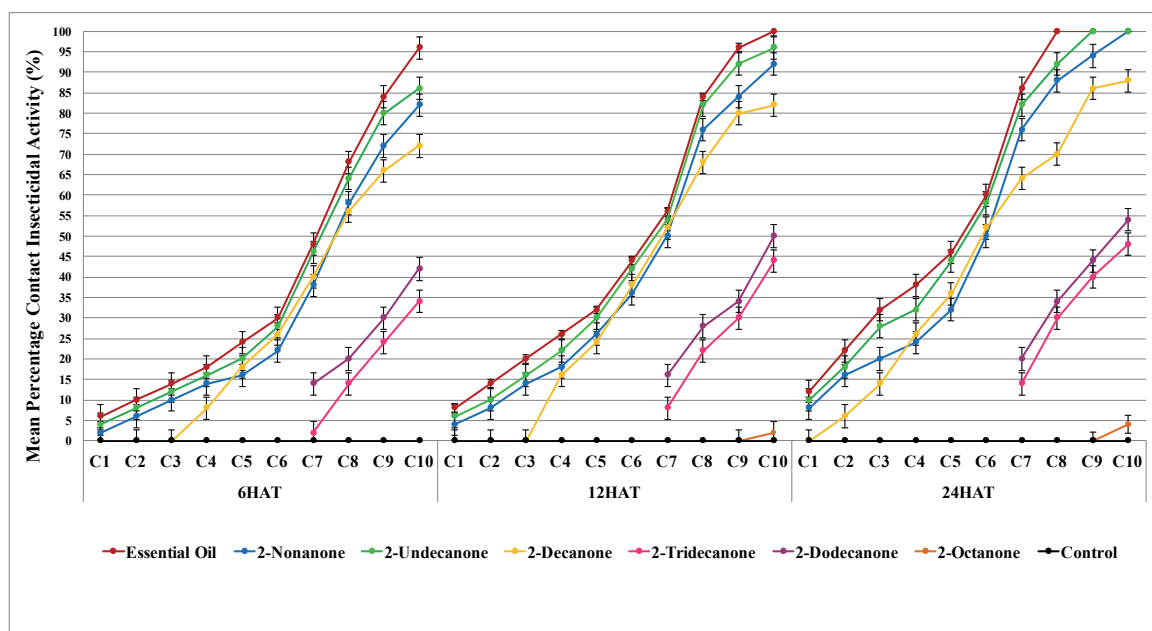


Figure 2 Mean percentage contact insecticidal activity ( $\pm$  SD) of *R. graveolens* essential oil and its principle long chain aliphatic 2-methyl ketone constituents against *C. cephalonica* at 6, 12 and 24 hours of weevil exposure to treated glass. C= Concentration based on 10 levels of essential oil content (0.007, 0.014, 0.029, 0.046, 0.070, 0.116, 0.232, 0.464, 0.928, 1.856  $\mu\text{L}/\text{cm}^2$ ); HAT= Hours After Treatment; n=50

Results of the probit regression analysis shown in Table 3 revealed that *S. zeamais* is more tolerant to *R. graveolens* essential oil and its 2-ketone analogues than *C. cephalonica*. Regarding  $\text{LC}_{50}$  values of 2-methyl ketone series, it was observed that the odd-chained congeners were more effective than those of the even-chained congeners on *S. zeamais* with the exception of 2-decanone being not effective against *S. zeamais* within the corresponding measure range to essential oil concentrations, hence its  $\text{LC}_{50}$  value could not be calculated ( $\text{LC}_{50} > 0.095$  mortality 0 %). In respect to *C. cephalonica*, an inverse pattern could be observed, of which the insecticidal potency of even-chained congeners over the odd-chained congeners was apparent.

Table 3.  $\text{LC}_{50}$  values of *R. graveolens* essential oil and its major constituents against the adults of *S. zeamais* and *C. cephalonica*

Insect	Treatments*	$\text{LC}_{50}^{\text{a,b}}$	Confidence Interval <sup>b</sup>		Slope $\pm$ SE
			Lower	Upper	
<i>S. zeamais</i>	Essential oil	0.059	0.041	0.468	0.51 $\pm$ 0.04
	2-Octanone	0.145	0.091	94.370	0.94 $\pm$ 0.21
	2-Nonanone	0.017	0.012	0.098	0.46 $\pm$ 0.04
	2-Decanone	>0.095 (Mortality 0 %)	-	-	-
	2-Undecanone	0.017	0.012	0.126	0.42 $\pm$ 0.03
	2-Dodecanone	0.325	0.172	343.732	0.93 $\pm$ 0.27
	2-Tridecanone	0.044	0.034	6.635	0.50 $\pm$ 0.06
<i>C. cephalonica</i>	Essential oil	0.055	0.187	0.245	0.72 $\pm$ 0.04
	2-Octanone	0.011	-	-	-
	2-Nonanone	0.022	0.019	0.026	0.68 $\pm$ 0.04
	2-Decanone	0.006	0.005	0.007	0.60 $\pm$ 0.03
	2-Undecanone	0.022	0.019	0.026	0.70 $\pm$ 0.04
	2-Dodecanone	0.009	0.007	0.017	0.45 $\pm$ 0.09
	2-Tridecanone	0.016	0.013	0.023	0.89 $\pm$ 0.11

<sup>a</sup>Units  $\text{LC}_{50}$  =  $\mu\text{L}/\text{cm}^2$  applied for 24 h time period; <sup>b</sup>95 % lower and upper confidence limits are shown in parenthesis; \* Mortality of the control (acetone) was 0  $\mu\text{L}/\text{cm}^2$  for both *S. zeamais* and *C. cephalonica*



Jeon *et al* (2015) outlined the insecticidal capacity of *R. graveolens* essential oil and its commercial phenolic analogs against *Sitophilus zeamais*, *Sitophilus oryzae* and *Lasioderma serricorne*. Ali *et al* (2013) gave a detailed account on the larvicidal potential of 2-nonanone followed by 2-undecanone against *Anopheles quadrimaculatus* and *Aedes aegypti*. 2-Undecanone was found capable of causing larval mortality in *Heliothis zea* (Robert *et al.*, 1987). Meanwhile, it has been reported that normal odd-chained alkanones of 2-undecanone and 2-tridecanone are acutely toxic to neonate larvae of *Keiferia lycopersicella* and *Spodoptera exigua* (Lin *et al.*, 1987). In addition, 2-tridecanone seemed to have a remarkable role in insecticidal resistance to *Manduca sexta*, *Heliothis zea* and *Leptinotarsa decemlineata* (Kennedy and Dimock, 1982). Moreover, 2-dodecanone was also effective against tobacco hornworm, the *M. sexta* with LC<sub>50</sub> value of 0.028  $\mu\text{m}^2/\text{cm}^2$  (Antonious *et al.*, 2003). 2-Dodecanone and 2-tridecanone were about equal in toxicity towards green peach aphid (*Myzus persicae*) which required a significantly lower dose than 2-undecanone. The spider mite (*Tetranychus urticae*) was more tolerant to 2-undecanone and 2-dodecanone than 2-tridecanone thus delineating that there are differences in sensitivity among arthropods in their sensitivity to constituents of potential bio-pesticides (Antonious *et al.*, 2003; Antonious and Snyder, 2008).

Although the two active major constituents (2-nonanone and 2-undecanone) of tested essential oil influenced comparable and strong protection efficacy, the insecticidal potential of essential oil may be ascribed in part, but not exclusively, to the total content (40.44 % of the total oil) of those active major constituents. For the reason that essential oils always represent a complex mixture of many chemical compounds and thus it is rather difficult to reduce the effect of the total oil to a few active components (Guo *et al*, 2016). Besides, some compounds identified in the *R. graveolens* essential oil also have demonstrated bioactivities against wide array of insect pests. Linoleic acid was found to be toxic against the larval stages of *Spodoptera littoralis* (Heba *et al.*, 2013) and 4<sup>th</sup> larvae of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* (Rahuman and Venkatesan, 2008). It has been reported that d-limonene was toxic to *Rhyzopertha dominica*, *Sitophilus oryzae* and *Tribolium castaneum* (Tripathi *et al.*, 2003). In addition, methyleugenol is known to have strong contact insecticidal properties on *S. zeamais* with LD<sub>50</sub> value being approximately 30  $\mu\text{g}/\text{mg}$  insect. Moreover, methyleugenol reported to have negative impact on the RGR (Reduced Growth Rate) and RCR (Reduced Food Consumption) of *Sitophilus zeamais* and *Tribolium castaneum* with decreased ECI (Food Consumption and Food Utilization) of *S. zeamais* adults and *T. castaneum* larvae (Huang *et al.*, 2002). In that manner, inactive constituents which imposed relatively lower or no insecticidal activity may have some synergistic effect on active constituents, and though not active individually, their presence would be necessary for the essential oil of *R. graveolens* to achieve its comprehensive and complete insecticidal potential. Active constituents, on the other hand, might have an antagonistic effect on each other, since their insecticidal level is significantly greater when tested individually than when they are in an essential oil mixture with other active constituents (Miresmailli *et al.*, 2006). Hence, contact insecticidal property of the essential oils may be related to the synergistic effects of its diverse array of major and minor constituents (Guo *et al*, 2016).

Due to the observed trend in results, it was evidenced that contact insecticidal activity was the result of differential ability of essential oil and its major authentic constituents in penetrating to more than one active site (Innocent *et al.*, 2008) of the test insects, *S. zeamais* and *C. cephalonica*. Thus, having mentioned that, presented results would make a constructive characterization in investigating the relationship between structure and insecticidal activity of congeners in aliphatic 2-methyl ketone series which varying in their chain lengths and carbonyl position. Odd-chained and even-chained congeners demonstrated comparable or significantly greater contact insecticidal activities against *S. zeamais* and *C. cephalonica* respectively. This contingent nature lies in the structure-activity relationship strived against test insects, has been previously explained by Kennedy and Dimock, 1982, assuming that the insecticidal capacity of a congener depends upon the probability of that molecule in reaching the site of action of an insect. In addition, they further clarified that, this probability depends at least in part on the ability of the congener which is determined by the compound's lipophilicity, to pass through lipid barriers such as the cuticle and the cell membranes of the insects. Many studies done for the recognition in the respect of mode of action of natural insecticides have shown that treating insect pests with natural essential oils or their pure compounds may cause symptoms indicating neurotoxic activity that could affect insects through acetyl cholinesterase enzyme inhibition in their central nervous system (Keane and Ryan, 1999), including hyperactivity, seizures and tremors followed by paralysis which are very similar to those produced by the insecticidal pyrethroids (Kostyukovsky *et al.*, 2002; Khani and Rahdari, 2012). It has also been discovered that the ketone compounds augment the inhibitory effect on acetylcholinesterase due to the presence of the double bond of the carbonyl group (Dambolena *et al.*, 2016).

#### 4. Conclusion

Data of the present study clearly indicated that the essential oil of *R. graveolens* possessed significantly strong insecticidal efficacy, followed by its major functional component of long chain aliphatic 2-methyl ketones constituting C8-C13 congeners against *Sitophilus zeamais* and *Corcyra cephalonica*. In that manner these constituents and their parent essential oil of *R. graveolens* leaves play an important role in stored grain protection, thus having the potential for being developed into natural insecticides, reducing the risks associated with the use of conventional chemical control strategies. It might also be useful to create various blends comprising authentic constituents in different ratios based on their activity and effect on the insect pests. The current study clearly supports the use of *R. graveolens* essential oil and its long chain aliphatic 2-methyl ketones in the post-harvest control of *S. zeamais*, *C. cephalonica* as well as other insect pests of stored maize in the light of recent interest in developing green insecticides in integrated pest management programs. Additionally, for the practical use of essential oil of *R. graveolens* and its major constituents as novel green insecticides, further studies concentrating on their safety to humans are necessary to improve efficacy, potency and stability in the environment.

#### Abbreviations

GC-MS: Gas Chromatography-Mass Spectrometry; C8: 2-Octanone; C9: 2-Nonanone; C10: 2-Decanone; C11: 2-Undecanone; C12: 2-Dodecanone; C13: 2-Tridecanone

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