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Research Article

Effect of collection time on phytochemical profile and insecticidal activity of essential oil from leaf of *Ocimum gratissimum* grown in Nigeria

¹Lamidi A. Usman, ¹Bello S. Olatayo, ²Samuel A. Babarinde, ¹Ismaeel R. Olanrewaju, ³Victor A. Ogundele.

¹Department of Chemistry, University of Ilorin, P.M.B. 1515, Ilorin, Kwara State, Nigeria. ²Department of Crop and Environmental Protection, Ladoke Akintola University of Technology, P.M.B.4000, Ogbomoso, Oyo State, Nigeria. ³Department of Chemical, Geological and Physical Science, Kwara State University, P.M.B 1530, Malete, Nigeria.

Correspondence. Lamidi Usaman; usmanlamidi@unilorin.edu.ng; +2348035032378.

ABSTRACT: Leaves (500g) of *Ocimum gratissimum* harvested at interval of three hours (7:00 am-7:00 pm) in a day were separately hydrodistilled for three hours. Oil yields from the leaves ranged from 0.33 - 0.94%. GC and GC-MS analyses revealed abundance of hydrocarbon monoterpenoids in the oils (47.6-67.9%). Principal constituents of the oils were; γ -terpinene (17.6 – 26.9), m-cymene (18.1–26.0%), thymol (18.3–32.6%), α -thujene (3.6–5.0%), β -myrcene (3.7-4.7%), β -selinene (2.7-8.2%) and β -caryophyllene (1.7-3.6). Fumigant toxicity and repellence bioassays of the oils revealed that the oils exhibited insecticidal properties against *Callosobruchus maculatus*. The oil from 10:00 am harvest demonstrated stronger efficacy than other oils. The LT_{50} {1.78 h} of the oil against *C. maculatus* was significantly lower than the oils from other harvests. Percentage mortality (83.33%) observed in the oil was significantly greater than the oil from other harvests. Percentage repellency (90.00%) observed in the oil was significantly greater than the PR of the oils from other harvests. With the insecticidal properties, the oils can be used as alternative to synthetic insecticide against *Callosobruchus maculatus*.

KEYWORDS: *Ficus capensis*, antibacterial, folk medicine, Igala, Flash chromatography

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INTRODUCTION

Ocimum gratissimum family lamiaceae has diverse classes of phytochemicals such as; flavonoids, alkaloids, steroids, tannins, phlobatannins, terpenoids and cardiac glycosides (Edeoga *et al.*, 2006; Adeniyi *et al.*, 2010). The phytochemicals are responsible for the therapeutic potentials of the plant, as revealed from the biological activity of its extracts. The ethanolic extract was active against *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Shigella*, *Salmonella* (El-said *et al.*, 1969; Begum *et al.*, 1993). The anti-fungal activity of the extract against *Trichophyton rubrum*, *T. mentagrophytes*, *Cryptococcus neoformans*, *Penicillium islandicum*, and *Candida albicans* have also been reported (Nwosu *et al.*, 1995; Akinyemi *et al.*, 2004). Hence the plant extracts can be used to cure diseases caused by the tested pathogens. Investigation on insecticidal activity of leaf ethanolic extract of the plant on beans weevil (*Acanthscelid esobtectus*) also revealed that, the extract was active against the insect (Morales *et al.*, 1996).

Earlier work on essential oil of the plant from different part of the world revealed the existence of oil of different chemotypes. For instance, essential oils of Nigerian grown *Ocimum gratissimum* from different locations were of the 1,8-cineole, eugenol, γ -terpinene, chemotypes; other principal constituents of the oils were; ocimene, β -pinene, , neral, geranial, fenchone, linalool, borneol, α -terpeneol, nerol, geraniol and linalyl acetate, β -caryophyllene, γ -muurolene, α -humulene, β -selinene, and α -selinene (Ekundayo., 1986; Saliu *et al.*, 2011; Owokotomo *et al.*, 2012). However, the oils of Rwanda and Indian grown *Ocimum gratissimum* were of thymol chemotype, other major constituents of the oils were; eugenol, p-cymene, γ -terpinene. Similarly, the oil from the leaf of Togolese and Brazilian grown *Ocimum gratissimum* were of thymol and eugenol chemotypes respectively (Rabelo *et al.*, 2003; Koffi *et al.*, 2009). Variation in chemotypes of the oil may be due to differences in environmental factors that determine the physiological condition of the plant at various locations. The condition dictate the enzyme (Terpene synthase) that mediate the biosynthesis of the terpenoids from their respective precursors. Hence, causes variations in phytochemical profile and biological activity of the oil. At a particular plant location, the physiological condition of a plant can change from time to time in a day. Hence, could affect the composition pattern and biological activity of its essential oil. It is on the basis of this that this work aimed at investigating the variability of phytochemical profile and insecticidal activity of essential oil of leaves of *Ocimum gratissimum* harvested at different time in a day against *Callosobruchus maculatus*.

MATERIALS AND METHODS

Plants Material

Fresh leaves of *Ocimum gratissimum* were collected at interval of three hours (7:00 am – 7:00 pm) in a day from Ilorin West Local Government Area of Kwara State, Nigeria. Identification of the plant was carried out at Herbarium of Forest Research Institute of Nigeria (FRIN), Ibadan where voucher specimen was deposited (FHI 108361).

Oil Isolation

Pulverized leaves of *Ocimum gratissimum* (500g) of various harvests were separately hydrodistilled for 3 hrs according to the British Pharmacopoeia Specification (British Pharmacopoeia., 1980). Extracted oil from each harvest was collected, preserved in a sealed sample tube and stored under refrigeration until analysis.

Gas Chromatography

GC analysis was performed on an Orion micromat 412 double focusing Gas Chromatography system fitted with two capillary column coated with Cp-sil5 and Cp-sil 19 (fused silica, 0.15 film thickness) and flame ionization detector (FID). The volume injected was 0.2 μ l and the split ratio was 1:30. Oven temperature was programmed from 50-230 °C at 5 °C/min, using hydrogen as carrier gas. Injection and detector temperatures were maintained at 200 and 250 °C respectively. Quantitative data were obtained by electronic integration of FID area percent without the use of correction factor.

Gas Chromatography /Mass Spectrometry

A Hewlett-Packard HP5890A GC, interfaced with a VG analytical 70-250s double focusing mass spectrometer was used. Helium was the carrier gas at 1.2ml/min. The MS operating conditions were: ionization voltage 70eV, ion source 230 °C. The GC was fitted with a 25 m $\times$ 0.25 mm, fused silica capillary column coated with CP-sil 5. The film thickness was 0.15 μ m. The GC operating conditions were identical with those of GC analysis. The MS data were acquired and processed by on-line desktop computer equipped with disk memory. The percentage compositions of the constituents of the oil were computed in each case from GC peak areas. The identification of the components was based on the comparison of retention indices (determined relative to the retention time of series of n-alkanes) and mass spectral with those of authentic samples and with data from literature (Jennings *et al.*, 1980).

Fumigant Toxicity

Portions of the oils (0.01 ml and 0.02 ml) were dissolved in 0.2 ml acetone. Ten active 1-3 day old adult *Callosobruchus maculatus* were introduced into fumigation chamber. The control was treated with 0.2 ml acetone. The experiment was replicated 3 times. Data on mortality were collected at 30 minute interval for 48 hours. Probit analysis was used to determine LT_{50} for each of the oils.

Repellence bioassay

The method used for testing repellence of *O. gratissimum* essential oil extract against *C. maculatus* adult was based on the area preferred test described by Babarinde and Adeyemo (Adedire., 2005). The test was performed by dissolving different concentrations of essential oils (0.01 ml, 0.02 ml, 0.03 ml and 0.04 ml) in 0.2 ml acetone. Ten beetles were introduced at the centre of each repellence chamber and then covered. The experiment was replicated 3 times. The number of insects present in the oil treated and the acetone treated was recorded at 30 minutes, 1 hour, 3 hours and 24 hours after experimental set up. Percentage repellence was determined using the formula:

$$PR = [N_C - N_t / N_C + N_t] \times 100$$

Where N_C = number of insects in acetone treated disc

N_t = number of insects in oil treated disc

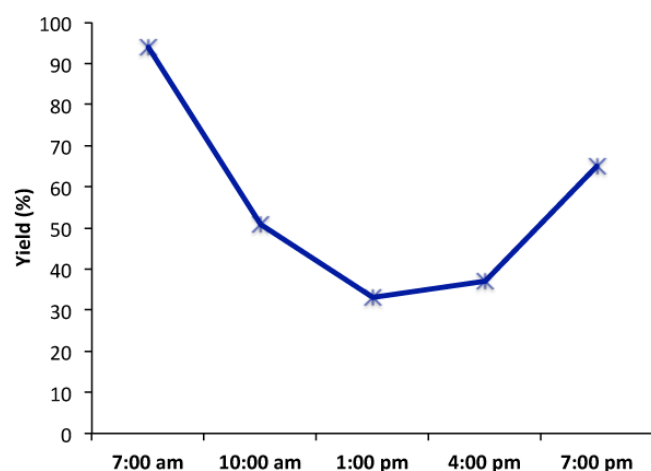


Figure 1: Variation in oil yields (%) from the leaves of *Ocimum gratissimum* harvested at different time in a day.
*Percentage yields were estimated from mean of the yields of the oils in gram determined in triplicate.

Statistical Analysis

Data were subjected to analysis of variance and where there significant treatment effect, mean was separated using Tukey Honestly Significant Difference at 5% probability level.

RESULTS AND DISCUSSION

Oil yields from the leaves of *Ocimum gratissimum* harvested at interval of 3 hours (7:00 am, 10:00 am, 1:00 pm, 4:00 pm 7:00 pm) in a day ranged from 0.33 to 0.94% (Figure 1).

The yields decreased steadily from 7:00 am to 1:00 pm harvests which later increased from 4:00 to 7:00 pm harvest. Highest and lowest quantities of the oils were obtained from 7:00 am (0.94%) and 1:00 pm (0.33%) harvests. The lowest yield from 1:00 pm harvest might be linked to the volatilization of the oil due to high temperature at the time of harvest. However, the yields compared favorably with the yields from the leaves of the plant grown in different part of the world (Atuboyedia *et al.*, 2010; Benedicta *et al.*, 2012).

Table 1 shows the percentage composition, retention indices and identities of the constituents of essential oils from leaf *O. gratissimum* harvested at different time in a day. In the Table, a total of 24-34 compounds that represents 97.3-99.0% of the oils were identified from their mass spectra. The oils were predominated by hydrocarbon monoterpene (47.6-67.9%). Oxygenated monoterpene constituted 22.2-36.0% of the oils.

The percentage composition of hydrocarbon sesquiterpene in the oils was 5.5-16.4%. The principal constituents of the oils were; γ -terpinene (17.6 – 26.9%), thymol (18.3 – 32.6%) and m-cymene (18.1 – 26.0%). Other notable compounds in the oils include; α -thujene (3.5 – 5.0%), β -myrcene (3.7 – 4.7%), p-cymene (1.9 – 2.7%), β -selinene (2.7- 8.2%), terpinen-4-ol (1.1- 2.6%), α -selinene (0.6- 2.1%), β -caryophyllene (1.7-3.6%), limonene (1.2 – 1.6%), α -pinene (1.0 – 1.2%), β -pinene (0.4 – 4.8%), sabinene (0.7 - 1.0%), β -ocimene(E) (0.8 – 1.0%) and β -phyllandrene (0.7 – 0.9%). Compounds that were present in minor quantities include; camphene (0.1 – 0.2%), α -phyllandrene (0.3 – 0.4%), α -terpinolene (0.3%), 1,3,8-p-menthatriene (0.2%) thymolmethylether (0.5-0.8%), carvacrol (0.2-1.2%), umbellulone (0.1-0.5%). Borneol (0.1-0.4%), linalool (0.2-0.4%), caryophyllene oxide (0.2-0.4%), α -caryophyllene (0.2-0.7%), elixene (0.2-0.5%), α -panasinsen (0.2- 0.7%), and β -ocimene (0.2 -0.3%) were also found as minor constituents of the oils.

Table 1: Chemical compositions (%) of leaf essential oils of *Ocimum gratissimum* obtained from different harvest periods in a day.

Compounds ^a	RI ^b	Percentage Composition					Mass spectra data
		<u>OG1</u>	<u>OG2</u>	<u>OG3</u>	<u>OG4</u>	<u>OG5</u>	
2- hexenal	854	0.2	-	0.1	0.3	0.3	41,55,69, 83, 98
3- hexen-1-ol	857	0.1	-	-	-	-	41,55,67,82,100
α - thujene	931	4.4	3.5	5.0	3.6	4.0	77, 91, 93, 105, 121
α - pinene	937	1.1	1.0	1.2	1.1	1.2	77, 91, 93, 105, 121
Camphene	953	0.1	0.1	0.2	-	0.1	77, 79, 91, 93, 107
Sabinene	976	0.7	-	1.0	-	-	69, 77, 91, 93,105
β - pinene	980	-	0.4	4.8	-	-	79,91, 93, 107, 121
β -myrcene	991	4.2	4.0	-	3.7	4.7	79,91,93, 107,121
α -phellandrene	1005	-	0.4	0.3	0.3	0.3	77,91,93, 105,119
Limonene	1031	1.1	1.6	1.4	1.2	1.4	68,93,107,121,136
β -phellandrene	1033	-	0.7	-	0.8	0.9	77,91, 93,105,121
β - ocimene (Z)	1040	-	0.3	-	0.2	0.2	79,91,93, 105,121
β - ocimene (E)	1050	0.9	1.0	1.0	0.8	0.8	79,91,93, 105,121
γ - terpinene	1062	24.8	24.2	26.9	19.5	17.6	91,93,105,121,136
p- cymene	1080	2.1	2.0	2.2	1.9	2.7	91,102,115,117,132
m- cymene	1082	21.0	18.1	23.8	21.2	26.0	91,103,119,126,134
α - terpinolene	1088	0.3	0.3	-	0.3	0.3	79,91, 105,119,134
Linalool	1098	0.3	0.2	0.3	0.3	0.4	71, 80, 93, 107,121
α - thujone	1102	0.1	-	0.2	0.1	0.2	67,79,81,95,110
1,3,8-p-menthatriene	1111	-	0.2	-	-	-	77,91,105,119,134
Borneol	1165	0.3	0.1	0.3	0.3	0.3	93,95,110,121,139

Table 1 continued.

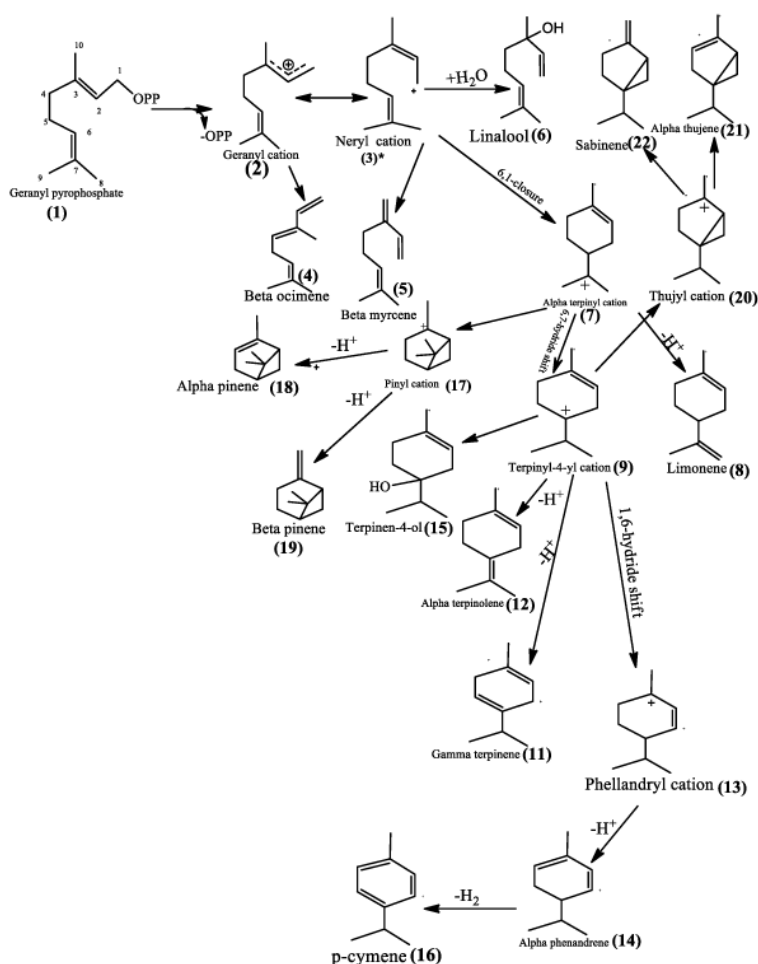
Compounds ^a	RI ^b	Percentage Composition					Mass spectra data
		<u>OG1</u>	<u>OG2</u>	<u>OG3</u>	<u>OG4</u>	<u>OG5</u>	
Unbellulone	1171	0.3	0.5	-	0.1	0.4	91,108,122,135,150
Terpinen-4-ol	1177	1.7	1.1	2.1	1.7	2.6	86,93,111,136,154
Thymolmethylether	1235	0.7	0.8	0.5	0.6	0.7	105,119,134,149,164
Thymol	1290	22.5	18.3	21.4	32.6	25.8	91,107,117,135,150
Carvacrol	1298	-	1.2	0.2	0.3	0.7	91,107,117,135,150
α - cubebene	1351	0.1	-	-	-	-	105,147,163,189,204
Eugenol	1356	-	0.4	-	-	-	103,121,131,149,164
β - elemene	1375	-	0.3	-	-	-	68,81,93,107,121,147
α - copaene	1376	-	0.1	-	-	-	105,119,161,189,204
β - caryophyllene	1418	3.1	3.6	1.8	2.0	1.7	79,93,105,133,147
Precocene I	1440	-	0.2	-	-	-	115,132,160,175,190
α - caryophyllene	1454	0.5	0.7	0.2	0.3	0.3	80,93,107,121,147
β - selinene	1485	5.6	8.2	2.7	4.0	3.8	105,121,161,189,204
α - selinene	1494	1.5	2.1	0.6	1.0	1.0	133,147,161,175,189
Elixene	1514	0.2	0.5	-	-	-	68,81,93,107,121,147
Caryophellene Oxide	1581	-	0.4	-	-	0.2	43,55,69,79,93, 109
α - cadinol	1653	-	0.1	-	-	-	43, 79, 95, 105, 121
α - panasinsen	1840	0.4	0.7	0.2	0.3	0.3	107,122,133,161,204
Total	98.3	97.3	98.8	98.5	99.0	98.9	

a- Compounds are listed in order of elution from silica capillary column coated with Cp-Sil 5. b- Retention indices on fused capillary column coated with Cp-Sil 5. OG1- Oil of 7.00am harvest. OG2- Oil of 10.am harvest. OG3- Oil of 1.00pm harvest. OG4- Oil of 4.00pm harvest. OG5- Oil of 7.00pm harvest.

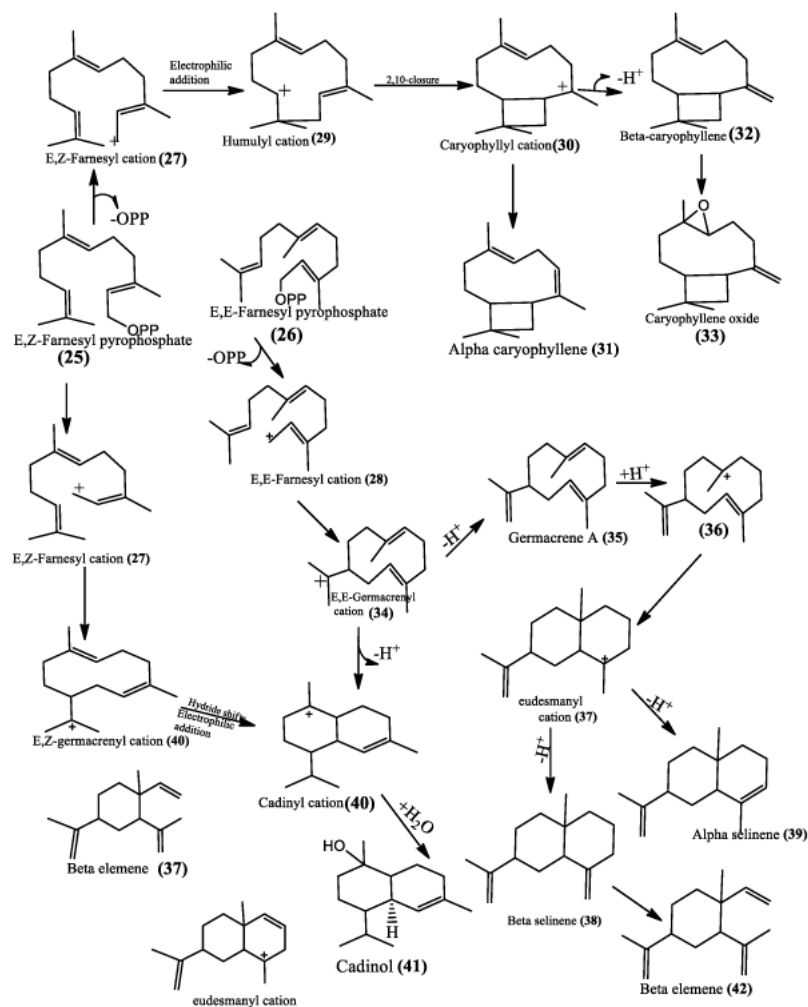
Comparison of the oils showed that their phytochemical profiles varied significantly. Qualitatively, carvacrol and α -phellandrene were detected in the oils except oil from 7:00 am harvest. Unbellulone, α -terpinolene and β -myrcene were also identified in the oils except oil of 1:00 pm harvest. In addition, β -phellandrene and β -ocimene were found in the oils except oils of 7:00 am and 1:00 pm harvests. Meanwhile, oil of 10:00am harvest had α -cadinol, precosene I, β -elemene, α -copaene, eugenol and 1,3,8-p-menthatriene that were not detected in other oils. Similarly, oil of 7:00am harvest was characterized by 3-hexen-1-ol and α -cubebene that were not identified in other oils. Quantitatively, β -caryophyllene, α -selinene and β -selinene were found to be

more abundant in the oils of 7:00 am and 10:00 am harvests than other oils. However, thymol was more abundant in the oil of 4:00 pm harvest than other oils. Furthermore, terpinen-4-ol and m-cymene were of greater abundance in the oil of 7:00 pm harvest than other oils.

Variation in composition pattern of the oils is attributable to changes in environmental factors at various time of harvest of the leaves. The factors determine physiological condition that dictate the activity of the enzymes require to mediate the biosynthesis of mono- and sesquiterpenoids from their respective precursors. Interestingly synthesis of the most abundant terpenoids are favoured as mediator for the



Scheme 1: Biosynthesis of major monoterpenoids catalyzed by gamma-terpinene, thymol and m-cymene synthases in the leaves of *Ocimum gratissimum* at various time



Scheme 1: Biosynthesis of major sesquiterpenoids catalyzed by beta-selinene, thymol and m-cymene synthases in the leaves of *Ocimum gratissimum* at various time of harvest.

biosynthesis of the compounds (Wise *et al.*, 1998). In the biosynthesis, the synthase aid the ionization of the precursors to cationic intermediates and subsequent reactions such as; wagner-meerwing rearrangement, cyclisation, deprotonation and hydration to form various terpenoids via cationic mechanism (Croteau, 1987; Wise and Croteau, 1999).

Reaction Mechanisms

The predominance of γ -terpinene, thymol and m-cymene indicate that their synthases mediated the formation of all monoterpenoids in the oils where each of the compounds predominates (**Reaction scheme 1**). In the scheme, the

synthases aided transformation of geranyl and neryl pyrophosphates (**1 and 2**) to geranyl(**3**) and neryl cations (**4**) that isomerized to linalyl cation, (**5**). Deprotonation of the ion (**5**) at C₄ was aided by γ -terpinene synthase in 10.00am harvest and formed β -ocimene(Z) (**6**) in the oil of the harvest. Interestingly physiological conditions in the leaves at 7.00am and 1.00pm did not favour the formation of the compound by γ -terpinene synthase. However, the enzymes aided the formation of β -ocimene (E) via C₄ deprotonation of linalyl ion (**5**) in the oils of 7.00 am to 1.00pm harvests. Similarly, thymol and m-cymene synthases facilitated the formation of β -ocimene(E) from the ion (**5**) in the oils of 4.00 pm and

7.00pm harvests respectively. Hydration of the ion (5) was mediated by γ -terpinene synthase in 7.00 am to 1.00pm harvests and formed linalool (6) in the oils. Formation of the compound (6) in the oils from 4.00 pm and 7.00 pm harvests was aided by thymol and m-cymene synthase respectively. Formation of α -terpinyl cation(7) via electrophilic attack of the neryl cation (4) on C₆-C₇ double bond was catalysed by γ -terpinene synthase in 7.00am to 1.00 pm harvests. Thymol and m-cymene synthases mediated the formation of the ion (7) in 4.00pm and 7.00pm harvest respectively. Deprotonation of the ion (7) at C₈ was aided by γ - terpinene synthase and produced limonene (8) in the oils of 7.00 am to 1.00 pm harvests. However, the formation of the compound (8) in the oils of 4.00pm and 7.00 pm harvests was initiated by thymol and m-cymene synthase respectively. 6,7-hydride shift of the ion (7) that formed terpinen-4-yl cation (9), which deprotonated at C₅ and C₇ to form γ -terpinene(10) and α -terpinolene(11) was initiated by γ -terpinene synthase in oils of 7.00 am- 1.00 pm harvests. For the oils from 4.00pm and 7.00pm harvests, the formations of the compounds (10 and 11) were facilitated by thymol and m-cymene synthase respectively.

Formation of phellandryl cation(13) from terpinen-4-yl cation (9) occurred via 1, 6 –hydride shift and delocalization of C₂-C₃ pie electrons to C₁-C₂ . The formation was initiated by γ -terpinene synthase in 7.00am – 1.00pm harvest. The same enzyme aided the deprotonation of the ion at C₄ to form α -phellandrene (14) in the oils with exception of oil of 7.00am harvest. Thymol and m-cymene synthases also aided the hydration of the ion (9) to form terpinen-4-ol (15) in the oils. Dehydrogenation of α -phellandrene(14) at C₅ and C₆ formed p- cymene and m-cymene (16) in the oils. γ -Terpinene synthase catalysed the formation of the compounds in 7.00am to 1.00pm harvests. However the biosynthesis of the compounds was mediated by thymol and m-cymene synthases in 4.00pm and 7.00pm harvests respectively. Folding of α -terpinylcation(7) towards C₂-C₃ double bond and its electrophilic attack on the bond via C₂ to produce pinyl cation (17) was aided by all the synthases. But deprotonation of the ion at C₄ to form α -pinene(18) was mediated by γ -terpinene synthase in the oils of 7.00am to 1.00pm harvests. While, thymol and m-cymene sythases facilitated the formation of the compound in the oils of 4.00pm and 7.00pm harvests. However, only γ -terpinene synthase facilitated the formation of β -pinene(19) in the oils of 10.00am and 1.00pm harvests via deprotonation of the ion (17) at C₁₀.

The sythases aided the electrophilic attack of terpinenyl-4-cation on C₂-C₃ double bond to form thujyl cation (20). Deprotonation of the ion (20) at C₄ was aided by γ -terpinene synthase in 7.00am to 1.00pm harvests to form α -thujene

(21) in the oils. Similarly, thymol and m-cymene synthases facilitated the formation of the same compound (21) in the oils of 4.00 and 7.00pm harvests. Deprotonation of the ion (20) at C₁₀ was aided by γ -terpinene synthase to form sabinene (22) in the oils of 7.00am and 1.00pm harvests respectively.

The predominance of β -selinene indicated that its synthase facilitated the formation of all sesquiterpenoids in the oils (Reaction scheme 2). In the scheme, E E and E Z farnesyl pyrophosphates (23 and 24) ionized to (E-E)- and (E-Z)-farnesyl cations (25, 26). Electrophilic attack of E,E-farnesyl cation on C₁₀-C₁₁ double bond formed humulyl cation (26). Caryophyllyl cation (27) was formed by electrophilic attack of humulyl cation on C₂-C₃ double bond. Deprotonation of the ion (27) at C₁₅ gave β -caryophyllene (28) in the oils. Epoxidation of β -caryophyllene gave β -caryophyllene oxide (29) in the oils. Electrophilic attack of the ion (24) on C₁₀-C₁₁ double bond via C₁₀ formed germacradienyl cation (30). Subsequent deprotonation of the ion(30)at C₁₂ produced germacrene A (31) which protonated to form an intermediate ion. that subsequently undergo electrophilic attack on C₂-C₃ double bond to form eudesmyl cation (32). Deprotonation of the ion (32) at C₄ formed α - selinene (33) in the oils. β -selinene (34) was formed via deprotonation of the ion (31) at C₁₅. 10,11 and 1,10 hydride shift of ion (30) formed an intermediate ion (35). Electrophilic attack of the ion on C₆-C₇ double bond formed cadinyl cation (36). Deprotonation of the ion at C₁₄ produced γ -cadinene (37). Hydration of C₆-C₇ double bond in γ -cadinene (37) formed cadinol(38). 2,7-cyclization of the ion (30) follow by subsequent hydride shifts gave eudesmanyl cation(39). 4,5-cleavage of the ion (39) and its deprotonation at C₁₅ formed β -elemene (40).

The result of fumigant toxicity of leaf essential oils of *O. gratissimum* obtained from various harvests against *C. maculatus* are presented in Tables 2 and 3. Percentage mortality increased with increase in exposure time. This result agreed with the previous works where toxicity of botanical products was dependent on exposure period (Adedire *et al.*, 2005; Salunke *et al.*, 2005; Kordali *et al.*, 2008; Lucia *et al.*, 2009; Babarinde *et al.*, 2010).The results of probit analysis showed significant difference in the LT₅₀ (lethal time for 50% of treated adult) of the oils obtained from 10:00 am, 4:00 pm and 7:00 pm, since their 95% fiducial limit did not overlap (Babarinde *et al.*, 2014). With LT₅₀ of 1.78 (0.49 – 3.39) hour for oil of 10:00 am harvest, it is adjudged to be the best, since the lower the LT₅₀, the better the efficacy of the oil.

The oils were repellent against *C. maculatus* adults, but the percentage repellency (90.00%) of the oil from 10:00 am harvest was significantly higher than the percentage repellency of other oils.

Table 2: Median lethal time (LT₅₀) of *Callosobruchus maculatus* adult exposed to essential oil from the leaves of *Ocimum gratissimum* harvested at different time in a day

Harvest time	LT ₅₀ (95% FL) (h)	Slope $\pm$ SEM	X ²	Df	Pr.
7:00 am	15.44 (3.12-8.27)	15.61 $\pm$ 0.12	25.01	5	0.000
10:00 am	1.78 (0.49-3.39)	10.69 $\pm$ 0.10	16.78	5	0.005
1:00 pm	4.36 (2.74-6.19)	7.52 $\pm$ 0.09	7.04	5	0.218
4:00 pm	71.16 (48.34-125.99)	8.35 $\pm$ 0.11	3.53	5	0.619
7:00 pm	8.48 (4.19 -15.81)	8.48 $\pm$ 0.09	11.49	5	0.042

FL- fiducial limits; SEM - standard error of mean; X² - chi square; df - degree of freedom; Pr –probability.

Table 3: Percentage mortality of leaf essential oils of *Ocimum gratissimum* obtained from different time of harvest in a day against *Callosobruchus maculatus*

Time (h)	OG1	OG2	OG3	OG4	OG5
1	7.33 $\pm$ 2.56a	33.33 $\pm$ 12.30a	10.00 $\pm$ 5.77a	30.00 $\pm$ 10.00a	6.67 $\pm$ 3.33a
3	44.00 $\pm$ 3. 72a	60.00 $\pm$ 20.82a	33.33 $\pm$ 12.90a	30.33 $\pm$ 15.28a	6.67 $\pm$ 3.33a
6	71.00 $\pm$ 19.86ab	83.33 $\pm$ 16.67b	54.00 $\pm$ 13.57ab	50.00 $\pm$ 5.77a	23.33 $\pm$ 8.82a
12	74.33 $\pm$ 20.87a	81.00 $\pm$ 15.63a	59.00 $\pm$ 19.52a	56.67 $\pm$ 12.02a	30.00 $\pm$ 11.55a
24	83.33 $\pm$ 12.02a	86.33 $\pm$ 16.67a	70.00 $\pm$ 20.00a	73.33 $\pm$ 13.33a	40.00 $\pm$ 15.28a
36	90.33 $\pm$ 3.33a	93.33 $\pm$ 13.33j	76.67 $\pm$ 18.56a	76.67 $\pm$ 12.02a	46.67 $\pm$ 11.28a
48	96.67 $\pm$ 2.33a	96.67 $\pm$ 3.33a	76.67 $\pm$ 18.56a	76.67 $\pm$ 12.02a	46.67 $\pm$ 11.28a

Interestingly, essential oils with repellent properties have the tendencies to prevent non-resident population of *C. maculatus* from infesting cowpea seeds (Obeng-Ofori *et al.*, 1997a; Adedire *et al.*, 2005). Since the oils exhibit repellent property, irrespective of their source they can be used to protect cowpea from *C. maculatus* infestation. Bioactivities of the oils from *Ocimum* species against insect pests have been attributable to the phytochemical components identified in the oils (Obeng-Ofori *et al.*, 1997b; Kpoviessi *et al.*, 2012).

Earlier study on the insecticidal activity of the essential oils obtained from the aerial parts of *O. gratissimum* harvested at three daytimes of harvest in a day (7:00 am, 1:00 pm and 7:00 pm) against *Artemiasalina* revealed that the oils were active against the insect. But the oil of 7:00am harvest exhibited higher activity than other oils. This was attributed to the presence of some compounds in the oil that were not found in the oils of other harvests (Isman *et al.*, 2000). Similarly, oil from 10:00 am harvest in this study showed

significant insecticidal activity against *C.maculatus* than other oils. Interestingly, the insecticidal activities of the oil containing eugenol, β -elemene and caryophyllene oxide have been reported in the previous studies (Bozin *et al.*, 2006; Prieto *et al.*, 2011). So, higher activity of the oil of 10:00am harvest against the insect is attributable to the presence of 1, 3, 8-p-menthatriene, eugenol, β -elemene, α -copaene, precocene 1, caryophyllene oxide and α -cadinol in the oil. In addition, the abundance β -caryophyllene, β -selinene and α -panasinsen in the oil from 10:00 am harvest could be responsible for its stronger efficacy than other oils. Irrespective of the time of harvest of the leaves, the oils exhibited insecticidal properties against *C.maculatus*. Hence the oils can be used as insecticide for *C.maculatus*.

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