Volume 3, issue 1, (2016), pp. 1-18. **Research article**

Journal homepage: http://dergipark.ulakbim.gov.tr/jotcsa

e-ISSN: 2149-0120

EFFECT OF DRYING ON YIELD, CHEMICAL COMPOSITION, AND INSECTICIDAL ACTIVITY OF LEAF ESSENTIAL OIL OF SWEET ORANGE (*Citrus sinensis*)

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Abstract: Pulverized fresh and dried leaves of *Citrus sinensis* (500 g) that were dried for five consecutive days during dry season were separately hydro-distilled for 3 hours. Oil yields from the samples ranged from 0.10 - 0.37% (w/w). Characterization of the oils using Gas chromatography - mass spectrometry showed that the oils were predominated by monoterpenoids (56.7 – 90.2%). Car-3-ene was the most abundant compound in the oils from fresh and the leaves dried for four days. Interestingly, the oils from other dried leaves had α-fenchene, α-terpinolene and β-pinene as their major constituents. The insecticidal activities of the oils against *Callosobruchus maculatus* were also determined via contact toxicity bioassay. Regardless of the level of dryness, the oils were observed to be toxic to *C. maculatus*. Oil obtained from the leaves dried for five days was found to be more active against the insect than other oils.

Key words: Drying, Essential oil, *Citrus sinensis*, terpene synthase, *Callosobruchus maculatus*.

Submitted: September 4, 2015. **Revised:** November 27, 2015. **Accepted:** December 14, 2015.

Cite this: Usman L, Watti O, Ismaeel R, Ojumoola A. EFFECT OF DRYING ON YIELD, CHEMICAL COMPOSITION, AND INSECTICIDAL ACTIVITY OF LEAF ESSENTIAL OIL OF SWEET ORANGE (*Citrus sinensis*). JOTCSA. 2016;3(1):1–18.

DOI: 10.18596/jotcsa.98689.

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Introduction

Postharvest drying of plant materials is an accepted practice in isolation of natural products. This is because it improves extract yield by increasing loading capacity of the sample due to the loss of moisture. Drying techniques in used include air-drying, sun-drying and oven-drying. Choice of the technique depends on the nature of targeted compounds. Oven drying is not suitable for sample preparation in the isolation of thermolabile compounds. Hence, the other methods of drying are preferred. For essential oils, several workers have monitored the effect of drying methods on the yields and constituents of the oils. For instance, Ashafa *et al*., [1] hydrodistilled air-dried, sun-dried and oven-dried (at 40^{o}C) leaves of *Felicia muricata*. Air-dried sample yielded more oil than other dried samples. However, there was no significant difference in the composition pattern of the oils. Meanwhile, the highest yield was obtained in the leaves of *Satureja hortesis* dried in an oven at 50^{o}C. The oil was also richer in carvacrol than the oils from other samples [2]. Similarly, leaves of *Origanum vulgare* that was oven-dried at a temperature of 45^{o}C yielded more oil than samples dried at other temperatures (30^{o}C and 40^{o}C). The highest amount of carvacrol was detected in the oil of the sample dried at 30^{o}C [3].

Oven-dried leaves of *Mentha longifolia* yielded more oil at a lower temperature (30^{o}C) than samples dried at higher temperatures (40^{o}C and 45^{o}C) [4]. Meanwhile, the highest amount of carvacrol was found in the oil of the sample dried at 30^{o}C. Furthermore, oil from air dried sample was richer in p-cymene, γ-terpinene and thymol than oils from other samples. Similarly, hydrodistilled air dried aerial parts of *Lipia citriodoral* afforded more oil than oven- (60^{o}C) and sun-dried samples [5]. The quantities of limonene, neral and geranial were higher in the oils from air- and oven-dried (60^{o}C) samples. Variations in the quantities of some constituents of the oils may be due to their volatilization during drying [6, 7]. Hence, it may affect their biological activities.

Fruit peel of *Citrus sinensis* is rich in essential oil. The effect of different drying methods on the yields and constituents of oil from the plant have been documented. For instance, oven-dried (40^{o}C) peels of the plant grown in Pakistan yielded more oil and contained more oxygenated monoterpenes than the fresh and air dried samples [8]. However, fresh peels of Algerian grown *C. sinensis* afforded more oil than dried samples [9]. It has been established that drying of plant material affects the yield, composition patterns and biological activity of some essential oils [1-4, 7]. It is on this basis that, this research aimed at monitoring the effect of length of drying at ambient temperature on the yield, chemical composition and contact toxicity of essential oil from leaves of *Citrus sinensis* collected during dry season against *Callosobruchus maculatus*.

Materials and Methods

Sample Collection: Leaves of *Citrus sinensis* were collected during dry season at Tanke, Ilorin, Nigeria. The plant was identified at the Herbarium of Plant Biology Department, University of Ilorin, Ilorin, Nigeria, where voucher specimens were deposited [UIL/001/996]. The leaves were air-dried at room temperature for five consecutive days.

Oil Isolation: Pulverized fresh and dried leaves (500 g) of *Citrus sinensis* were separately hydro-distilled for 3 hours in a Clevenger-type apparatus, according to British Pharmacopoeia specification [10]. The resulting oil from each sample was collected, preserved in a sealed sample tube and stored under refrigeration until analysis.

GC-MS Analysis: Analysis of the oils was carried out using GC (Agilent 19091S) coupled with a quadruple focusing mass spectrometer (433HP-5). Helium was used as the carrier gas at a flow rate of 1.5 mL/min. The GC was fitted with a 30m x 0.25 mm fused silica capillary column coated with phenyl methyl siloxane in split ratio of 1:50. The film thickness was 0.25 µm. Oven temperature was initially at 100 ^{0}C for 5 min and then programmed to 150 ^{0}C at a rate of 4 ^{0}C/min for 8 min and increased to 250 ^{0}C at a rate of 20 ^{0}C/min. The MS operating conditions were as follows: Transfer line temperature, 300 ^{0}C, ionization potential, 70 eV. The percentage composition of the oils was computed in each case from GC peak areas. The identification of the components was done based on comparison of retention indices (determined relative to the retention times of series of n-alkanes) and mass spectra with those of authentic samples and with data from literature [11-13].

Insecticidal Properties

Insect Culture: Cowpea beetles (*Callosobruchus meculatus*) were obtained from heavily infested cowpea. They were reared on clean beans and maintained under ambient environmental condition (28±2 ^{0}C). The rearing jars were covered with muslin fabric to allow aeration and prevent escape of the insects. The jars were placed inside a wire-netted shelf in the laboratory.

Experimental procedure

The toxicity of essential oils from *Citrus sinensis* leaves to adult *C. maculatus* was tested for 42 hours. 0.1 mL of oils from fresh leaves, leaves dried for one, two, three, four and five days, were separately mixed with 10 g of clean, uninfested cowpea grains inside a 4 cm diameter plastic container. Ten newly emerged *C. maculatus* adults were then introduced into the essential oil coated cowpeas. Each treatment was replicated 3 times. A control experiment without the oils was also set up. Adult mortality was observed at 6-hour intervals.

Data Analysis

The adult toxicity experiments were laid out in a Completely Randomized Design. Percentage mortality data from the experiment was subjected to a one way Analysis of Variance (ANOVA). Where there was a significant difference, mean separation was done using the Duncan Multiple Range Test at 5% level of significance. Statistical analysis was done using SPSS software, version 21.

Results and Discussion

Fresh leaves of *Citrus sinensis* afforded oil in the yield of 0.10% (w/w). Oil yields from the leaves that were dried for five consecutive day range from 0.15 - 0.37% (w/w). The yields increased steadily from fresh to the leaves dried for three days and later decreased in the leaves dried for four days before it increases after five days of drying (Fig. 1). Increase in oil yields is attributable to the loss of moisture content in the leaves. The decrease in the yield from the leaves dried for four days may be due to increase in ambient temperature at the fourth day of drying which may lead to the volatilization of the oil. However, the yields compared favourably with the yields from previous work on the leaves of the plant [14-16].

Table 1 shows the identities, kovats indices and percentage composition of the constituents of essential oils from fresh and dried leaves of *Citrus sinensis*. In the Table, compounds 34 - 45 that represent 85.9-98.5% of the oils were identified from their mass spectra. Hydrocarbon monoterpenes constituted 34.2- 54.4% of the oils. The percentage composition of oxygenated monoterpenes ranged from 14.2 - 38.8%. Meanwhile, hydrocarbon sesquiterpenes represented 5.6 - 21.5% of the oils. 0.4 – 3.1% of the oils were oxygenated sesquiterpenes. The major constituents of the oils were: α-fenchene (0 – 13.7%), β-pinene (0 – 11.4%), 3-carene (2.5 – 9.9%), limonene (4.4 – 7.0%), cis-β-ocimene (0 – 11.4%), γ-terpinene (0.5 – 6.7%), α-terpinolene (2.7 – 13.3%), citronellal (0.6 – 10.5%), citral (0.7 – 4.1%), citronellyl acetate (0 – 4.4%), β-elemene (0.2 – 5.8%) and β-caryophyllene (1.3–4.5%). α-Pinene (0.9 - 1.6%), β-myrcene (0 - 2.5%), α-phellandrene (0.3 – 1.3%), o-cymene (0 – 1.4%), 1, 3, 8-p-menthatriene (0 – 3.9%), terpinen-4-ol (0 – 3.3%), citronellol (1.2 – 3.6%), neral (0 – 4.4%), 8-hydroxyneomenthol (0 – 3.8%), humulene (0.5 – 1.8%), cis-β-farnesene (0.4 – 2.7%), β-guaiene (0 – 4.1%), eremophilene (0 – 1.3%), α-sinensal (0 – 2.7%) and phytol (0 – 3.3%) were also detected in appreciable quantities.

Qualitatively, there were variations in the constituents of the oils. For instance, para-α-dimethyl styrene, 1, 5-dimethyl cyclooctadiene and isoborneol that existed in the oil from fresh leaves were not identified in the other oils. Also, neryl acetate that was identified in the oil from leaves dried for a day was not found in oils from other samples. Ladene oxide, trans-α-bergamotene, citronellyl propionate, bornylene, trans-farnesol, β-myrcene and thymol that were detected in the oil from leaves dried for two days were absent in the oils from other samples. Similarly, sulcatone, supraene, sabinene hydrate, benzene methanol- 3, 5- dimethyl, isothujene, epicamphor, phytolacetate, β-selinene and caparratriene that were found in the oil from leaves dried for three-days were not identified in the oils from other samples. In addition, oil of the leaves that were dried for four days had 3-octen-5-yne-2, 7-dimethyl and alloaromadendrene that were not found in the oils from other samples. Meanwhile, linalool and geranyl acetate were detected in the oil of leaves dried for five days but were not identified in the oil from other samples.

Quantitative variations were also observed in some of the constituents of the oils. For instance, γ-terpinene, 1,3,8-p-menthatriene and α-sinensal were of greater abundance in the oil from fresh leaves than oils from other samples. Furthermore, α-terpinolene and citronellal were more abundant in the oil from leaves dried for one day than other oils. Similarly, the quantities of β-elemene and β-caryophylene were higher in the oil from the leaves dried for four days than oils from other samples. Meanwhile, oil from the leaves dried for one day was richer in α-terpinolene than other oils. Citronellal and citral were of greater abundance in the oils except in the oil from leaves dried for four days. With the abundance of 3-carene, α-terpinolene, α-fenchene and β-pinene in the oils, the oils were of 3-carene, α-terpinolene, α-fenchene and β-pinene chemotypes. Previous studies revealed the existence of essential oil of limonene chemoype from fresh leaves of Indian, Kenyan and Iranian grown *Citrus sinensis* [17-19]. However, this study showed that the oil from fresh sample is of car-3-ene chemotype without limonene. Interestingly, the monoterpene was found in appreciable amounts in the oils from the dried leaves. Absence of limonene in the oil of the fresh leaves signify that the physiological condition in the fresh leaves did not favor the biosynthesis of limonene.

Reaction mechanisms

It has been established that, the enzymes of the most abundant mono- and sesquiterpenoids facilitate the transformation of their precursors (geranyl pyrophosphate/ farnesyl pyrophosphate) to various cationic intermediates (linalyl, geranyl, farnesyl, nerolidyl and humullyl cations) in the presence of divalent metal ions [20,21]. The ions subsequently undergo series of cyclizations, hydride shifts and other rearrangements until the reaction is terminated by proton loss or hydration to give various terpenic products [22].

The predominance of 3-carene, α-terpinolene, α-fenchene and β-pinene signified that, their synthases mediate the formation of all monoterpenoids in the oils (Reaction scheme 1). In the scheme, the monoterpenoid synthases facilitated transformation of geranyl pyrophosphate (1) to geranyl (2) and linallyl cations (3). Hydration of geranyl cation followed by subsequent hydrogenation at C2 and C3 gives citronellol (4). Dehydrogenation of the ion at C1 forms β-ocimene (5). α-Terpinyl cation (6) intermediate is formed by electrophyllic attack of geranyl cation (3) on C6-C7 double bond. Deprotonation of the ion (6) at C8 produces limonene (7). 6,7-hydride shift of the ion (6) forms terpinyl-4-yl cation (8). Subsequent deprotonation of the ion at C1 and C7 forms α-phellandrene (9) and α-terpinolene (10) respectively. Electrophillic attack of the ion (6) on the deprotonated C5 forms car-3-ene (11). Folding of the ion (6) towards C2-C3 double bond followed by its electrophillic attack on C2 gives pinyl cation (11). Loss of proton by the ion (11) at C4 and C10 form α-pinene (12) and β-pinene (13) respectively. Wagner-Meerwein rearrangement of the pinyl cation follow by 2,3-methyl shift forms fenchyl cation (14). Deprotonation of the ion (14) at C10 forms α-fenchene (15). Oxidation of citronellol gives citronellal (16). Nucleophillic attack of C6-C7 double bond on the carbonyl group, follow by deprotonation at C8 forms isopulegol (17).

The predominance of α-sinensal, β-elemene and β-caryophyllene indicates that their synthases facilitate the formation of all sesquiterpenoids in the oils (Reaction scheme 2). In the scheme, farnesyl pyrophosphate (18) ionizes to form farnesyl (19) and nerolidyl (20) cations. The ion (20) undergoes 1,3-hydride shift followed by loss of proton at C15 to form farnesene (21). Electrophillic attack of the ion on C10-C11 double bond gives humulyl cation (22) which subsequently deprotonate at C9 to form humulene (23). Similarly, the ion (22) undergoes electrophillic attack on C2-C3 double bond to form caryophyllyl cation (24). The ion (24) is deprotonated at C15 to give β-caryophyllene (25). 5,7-epoxidation of β-caryophyllene leads to the formation of caryophyllene oxide (26). Electrophillic attack of the ion (19) on C10-C11 double bond forms germacrenyl cation (27). The cation (27) also undergoes 2,7-ring closure to form β-selinyl cation (28). Deprotonation of the ion (28) at C12 gives β-selinene (29). The cation (28) initially undergoes 3,7-ring closure, follow by 7,11-hydride shift and then deprotonation at C3 to form α-copaene (30). δ-cadinene (31) is formed via 1,6-cyclization of (27) followed by loss of proton at C8. 2,11-hydride shift of the ion (27) and then 2,7-cyclization gives eudesmanyl cation (32). The cation (32) subsequently undergoes 4,5-cleavage followed by loss of proton at C15 to form β-elemene (33).

Insecticidal Activity

In this study, essential oil from the leaves of *C. sinensis* showed contact toxicity against adult *C. maculatus* as presented in Table 2. At the end of 42 hours of exposure, oils obtained from the fresh leaves of *C. sinensis* caused 50.0% mortality of *C. maculatus*. The oil from the leaves dried for one day (DSA1) caused a mortality of 45.0% over the same period. On the other hand, higher mean percentage mortalities (80.0%, 90.0%, 100.0%) of the *C. maculatus* were caused by the oils from the leaves dried for two, three, four and five days, respectively. No mortality was observed in the control experiment after 42 hours of exposure. The percentage adult mortality caused by oils from the leaves dried for 2 to 5 days were significantly different ($P < 0.05$) from those caused by oils from the fresh leaves and the leaves dried for 1 day. Oils from the leaves dried for five days caused the highest mean adult mortality (60.0%) within 6 hours of treatment and may thus be regarded as the most active oil. The toxicity of essential oils to stored products insect pests had been linked to the separate and synergistic actions of the constituents of the oils [23] . Such constituents includes; ß – pinene, limonene, citronellal, geraniol, linalool, myrcene, phellandrene, α-pinene and γ-terpinene [24-28]. The presence of these compounds in the oil may be responsible for their activity against *C. maticulatus*. However, the activity of the oil compared favorably with the activity of the oils from previous studies on the leaves of the plant [29-31].

Conclusion

The yields of essential oil from the leaves of *Citrus sinesis* increases as the day of drying increases except in the leaves dried for three days where the yield decreases. In addition, there were variations in contact toxicity of the oil against *C. maticulatus* which was attributable to qualitative and quantitative variations in the constitutients of the oil.

Table 1. Chemical composition (%) of essential oils from fresh and dried leaves of *Citrus sinensis*.

S/N	Compounds	KI	% Composition					
			Fresh	DSA1	DSA2	DSA3	DSA4	DSA5
1	α-thujene	931	0.3	1.0	0.4	0.7	0.3	N/A
2	α-pinene	939	0.9	1.6	1.4	1.0	0.9	1.2
3	α-fenchene	951	9.8	N/A	13.7	12.6	N/A	N/A
4	β-thujene	971	0.3	N/A	0.6	N/A	N/A	1.2
5	β-pinene	980	2.4	2.9	N/A	1.8	2.6	11.4
6	Bornylene	980	N/A	N/A	2.9	N/A	N/A	N/A
7	Sulcatone	985	N/A	N/A	N/A	0.2	N/A	N/A
8	β-myrcene	991	N/A	N/A	2.3	N/A	N/A	2.5
9	2-carene	1001	N/A	5.0	N/A	N/A	N/A	0.6
10	α-phellandrene	1005	0.9	1.3	1.0	0.8	0.9	0.3
11	3-octen-5-yne-2,7-dimethyl		N/A	N/A	N/A	N/A	2.2	N/A
12	3-carene	1011	9.9	8.0	8.7	2.5	9.3	8.2
13	α-terpinene	1018	1.1	N/A	N/A	N/A	1.0	N/A
14	Ortho-cymene	1020	N/A	1.4	0.5	0.4	1.5	0.3
15	p-cymene	1026	N/A	N/A	0.5	N/A	N/A	1.3
16	D-limonene	1031	N/A	5.9	5.1	4.4	5.5	7.0
17	Cis-β-ocimene	1040	9.2	11.4	4.7	N/A	6.1	5.9
18	Isocarvestrene	1047	2.5	N/A	N/A	N/A	2.6	N/A

Table 1 (Contd)

S/N	Compounds	KI	% Composition					
			Fresh	DSA1	DSA2	DSA3	DSA4	DSA5
19	Trans- β-ocimene	1050	0.5	0.6	0.5	0.5	0.5	0.5
20	γ-terpinene	1062	6.7	1.6	0.5	0.9	1.9	1.1
21	Sabinene hydrate	1068	N/A	N/A	N/A	0.4	N/A	N/A
22	m-cymene	1082	0.6	0.4	N/A	N/A	0.5	N/A
23	α-Terpinolene	1088	3.8	13.3	3.7	2.7	3.5	3.3
24	p-α-dimethylstyrene	1096	0.2	N/A	N/A	N/A	N/A	N/A
25	Linalool	1098	N/A	N/A	N/A	N/A	N/A	5.5
26	1,3,8-p-menthatriene	1111	3.9	N/A	2.0	1.1	0.6	N/A
27	cis-p-mentha-2,8-dien-1-ol	1128	N/A	N/A	3.4	3.7	N/A	N/A
28	Benzenemethanol-3,5-dimethyl	N/A	N/A	N/A	N/A	2.7	N/A	N/A
29	Isothujene	N/A	N/A	N/A	N/A	5.7	N/A	N/A
30	Isopulegol	1145	0.4	0.4	0.5	0.6	0.2	0.4
31	1,5-dimethylcyclooctadiene	N/A	5.6	N/A	N/A	N/A	N/A	N/A
32	Citronellal	1153	5.1	10.5	5.1	5.9	0.6	8.2
33	Isoborneol	1156	5.4	N/A	N/A	N/A	N/A	N/A
34	2-p-tolylpropene	N/A	N/A	N/A	0.5	N/A	0.2	N/A
35	Terpinen-4-ol	1177	N/A	3.3	N/A	N/A	3.2	2.1
36	α-terpineol	1189	0.3	0.3	0.4	0.2	N/A	N/A

Table 1 (Contd)

S/N	Compounds	KI	% Composition					
			Fresh	DSA1	DSA2	DSA3	DSA4	DSA5
37	Decanal	1204	N/A	N/A	N/A	0.2	N/A	N/A
38	Citronellol	1228	3.7	3.6	3.1	3.0	1.2	3.4
39	Neral	1240	N/A	3.9	N/A	N/A	N/A	4.4
40	Citral	1240	2.8	4.1	3.7	4.3	0.7	3.8
41	Geranyl linalool	1244	N/A	0.4	0.7	N/A	N/A	N/A
42	Geraniol	1255	N/A	0.9	N/A	N/A	N/A	3.1
43	Thymol	1290	N/A	N/A	0.2	N/A	N/A	N/A
44	Methyl geranate	1323	0.3	0.3	N/A	0.3	0.3	0.4
45	Citronellyl acetate	1354	3.0	2.7	N/A	2.4	4.4	2.9
46	Neryl acetate	1365	N/A	4.0	N/A	N/A	N/A	N/A
47	β-elemene	1375	1.4	1.5	2.8	0.2	5.8	4.0
48	α-copaene	1376	9.9	8.0	8.7	2.5	9.3	8.2
49	β-copaene	1378	N/A	N/A	0.2	N/A	N/A	N/A
50	Geranyl acetate	1383	N/A	N/A	N/A	N/A	N/A	1.5
51	6-methyl octahydrocoumarin	1388	N/A	N/A	4.8	N/A	N/A	N/A
52	1-octadecyne	N/A	2.4	N/A	N/A	N/A	1.6	N/A
53	8-hydroxyneomenthol	1423	N/A	1.8	N/A	0.3	3.8	3.1
54	Trans-α-bergamotene	1436	N/A	N/A	1.5	N/A	N/A	N/A

Table 1 (Contd)

S/N	Compounds	KI	% Composition					
			Fresh	DSA1	DSA2	DSA3	DSA4	DSA5
55	Humulene	1440	0.5	0.5	0.9	1.3	1.8	0.8
56	Citronellyl propionate	1444	N/A	N/A	2.7	N/A	N/A	N/A
57	β-Caryophyllene	1454	1.3	1.3	2.3	3.2	4.5	2.4
58	Cis-β-farnesene	1458	0.4	0.8	1.9	2.7	2.7	1.3
59	Alloaromadendrene	1461	N/A	N/A	N/A	N/A	0.2	N/A
60	β-selinene	1485	N/A	N/A	N/A	3.6	N/A	N/A
61	Eremophilene	1486	1.2	1.3	0.3	0.3	0.5	N/A
62	β-guaiene	1490	0.3	N/A	2.3	3.0	4.1	N/A
63	Valencene	1491	N/A	N/A	0.2	0.6	0.3	N/A
64	α-farnesene	1508	N/A	1.1	N/A	1.1	N/A	N/A
65	β-bisabolene	1509	N/A	N/A	N/A	0.2	0.2	N/A
66	δ-cadinene	1524	N/A	N/A	0.4	0.4	0.4	N/A
67	Spathulenol	1576	N/A	N/A	N/A	0.2	0.2	N/A
68	Caryophyllene oxide	1581	0.4	0.4	N/A	N/A	1.1	0.5
69	Caparratriene	N/A	N/A	N/A	N/A	0.7	N/A	N/A
70	Eudesmol	1652	N/A	N/A	0.4	0.2	1.0	N/A
71	Trans-farnesol	1722	N/A	N/A	0.6	N/A	N/A	N/A
72	α-sinensal	1752	2.7	1.1	N/A	N/A	1.5	1.7

Table 1 (Contd)

S/N	Compounds	KI	% Composition					
			Fresh	DSA1	DSA2	DSA3	DSA4	DSA5
73	Supraene	N/A	N/A	N/A	N/A	0.9	N/A	N/A
74	Epicamphor	N/A	N/A	N/A	N/A	0.3	N/A	N/A
75	Neopentylidenecyclohexane	N/A	N/A	N/A	0.4	N/A	N/A	N/A
76	Ladene oxide (II)	1890	N/A	N/A	0.7	N/A	N/A	N/A
77	Dehydroneoisolongifolene	N/A	0.4	N/A	N/A	N/A	0.2	N/A
78	α-springene	2019	0.5	N/A	N/A	0.6	0.6	N/A
79	Phytol	1949	2.5	1.5	2.5	N/A	3.3	3.3
80	Phytol acetate	2223	N/A	N/A	N/A	3.0	N/A	N/A
	Total (%)		**94.7**	**98.5**	**91.5**	**86.6**	**85.4**	**97.6**
	Number of compounds		**37**	**35**	**43**	**45**	**44**	**34**

Notes:

N/A means no data, KI means Kovats Index.

Table 2. Percentage mortality at 6h interval over 42 h exposure period.

Treatments	% Mean mortality						
	6 h	**12 h**	**18 h**	**24 h**	**30 h**	**36 h**	**42 h**
Fresh	0c	5e	25c	30c	45c	45c	50c
DSA1	0c	20d	25c	30c	35c	40c	45c
DSA2	25b	45c	60b	75b	80b	80b	80b
DSA3	10c	40c	70b	70b	75b	90ab	90ab
DSA4	30b	60b	100a	100a	100a	100a	100a
DSA5	60a	85a	100a	100a	100a	100a	100a
Control	0c	0e	0d	0d	0d	0d	0d

PS: Values in the same column followed by the same letter(s) are not significantly different at P=0.05.

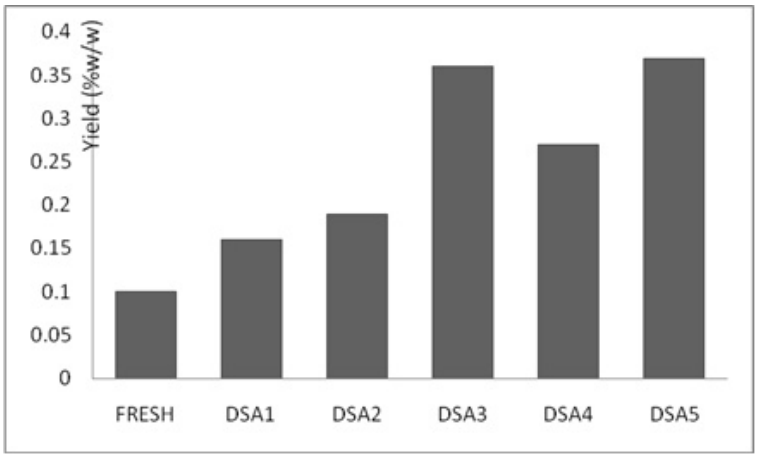
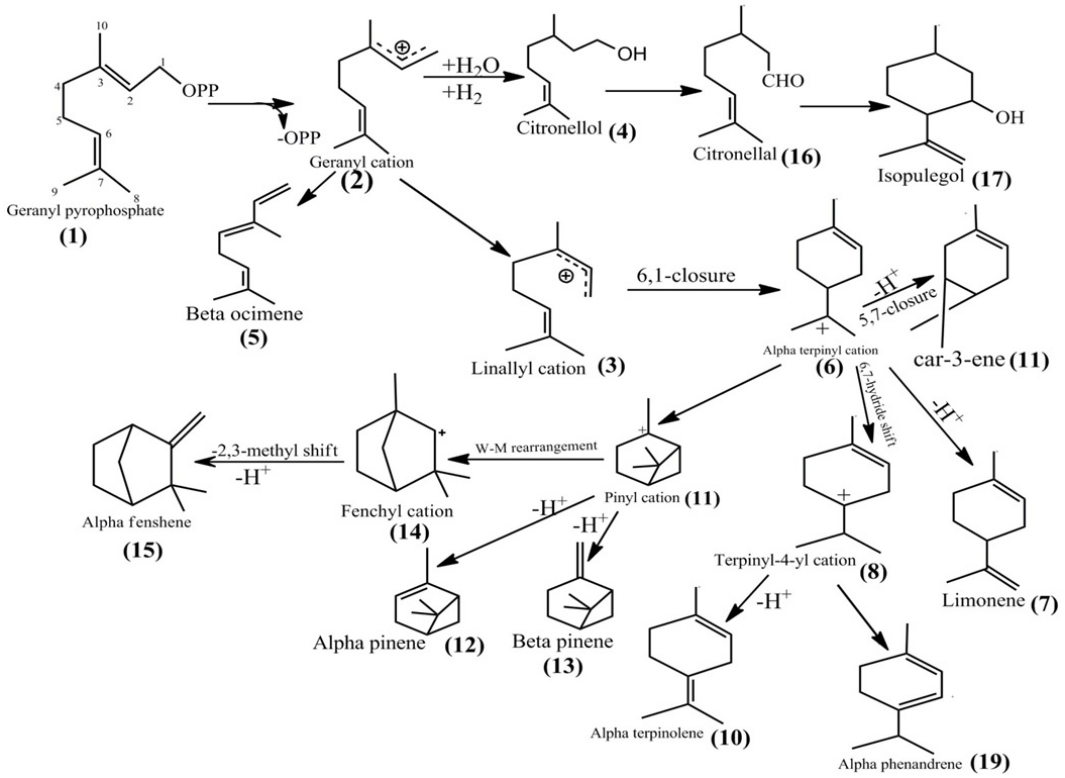
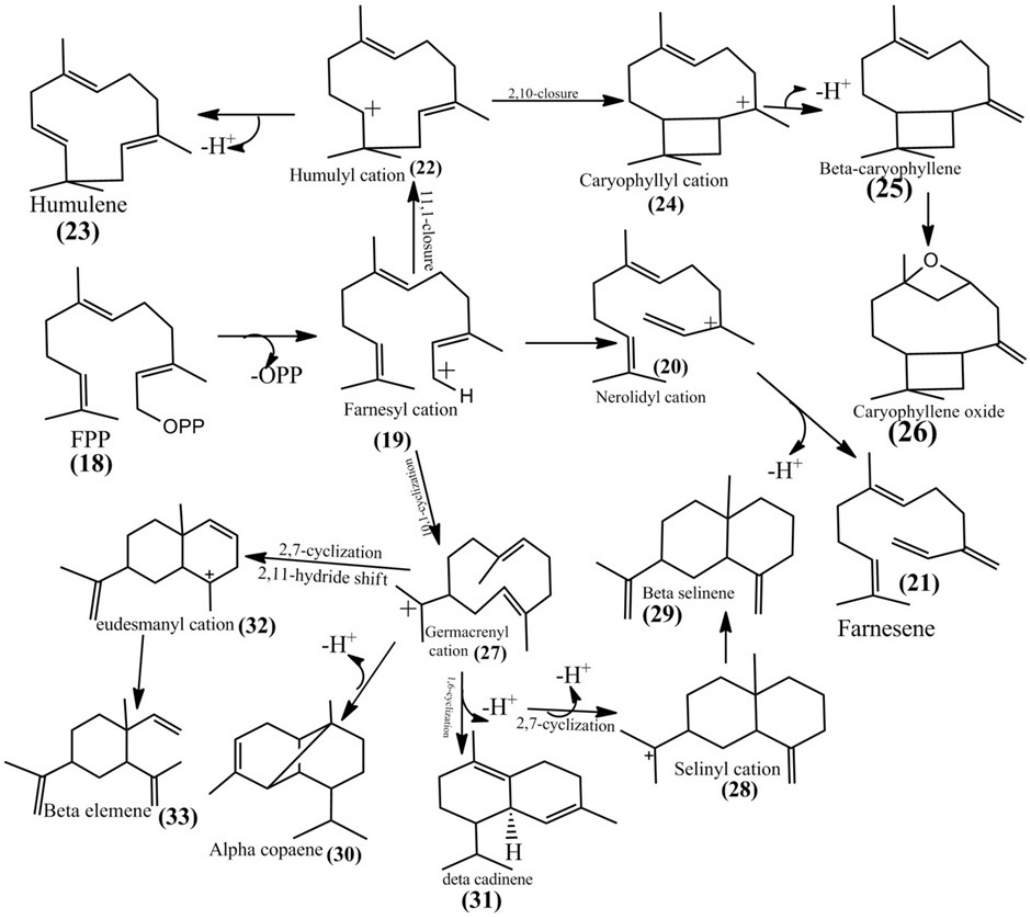


Figure 1. Yield of *Citrus sinensis* oils.

FRESH: Fresh leaves
DSA1: Leaves dried for one day
DSA2: Leaves dried for two days
DSA3: Leaves dried for three days
DSA4: Leaves dried for four days
DSA5: Leaves dried for five days



Reaction scheme 1. Biosynthesis of major monoterpenoids.



Reaction scheme 2. Biosynthesis of major sesquiterpenoids.

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Türkçe öz ve anahtar kelimeler

KURUTMA İŞLEMİNİN TATLI PORTAKAL (*Citrus sinensis*) YAPRAK ESANSİYEL YAĞININ VERİM, KİMYASAL BİLEŞİM VE İNSEKTİSİT AKTİVİTESİ ÜZERİNE ETKİSİ

Öz: *Citrus sinensis*'in toz edilmiş ve taze, kurutulmuş yaprakları (500 g) kuru sezonda 1-5 gün boyunca kurutulmuş ve ayrı ayrı 3 saat boyunca hidrodistilasyona maruz bırakılmıştır. Örneklerden elde edilen yağ verimleri %0,10-0,37 (w/w) arasında değişmektedir. Yağların gaz kromatografisi – kütle spektrometrisi ile karakterizasyonu sonucunda büyük oranda monoterpenoidlerden oluştuğu (%56,7 – 90,2) anlaşılmaktadır. Car-3-en, taze ve dört gün boyunca kurutulan yaprakların yağ bileşiminde en bol bulunan madde olarak tespit edilmiştir. İlginç şekilde, diğer kurutulmuş yapraklardan elde edilen yağlarda α-fenken, α-terpinolen ve β-pinen ana bileşen olarak gözlenmiştir. Yağların *Callosobruchus maculatus* üzerindeki insektisit aktivitesi de temas yollu zehirlilik biyo-incelemesi ile bulunmuştur. Kuruma seviyesinden bağımsız olarak, yağların *C. maculatus* için zehirli olduğu gözlenmiştir. Beş gün boyunca kurutulmuş yapraklardan elde edilen yağların diğer yağlara göre söz konusu böceğe karşı daha fazla aktif olduğu bulunmuştur.

Anahtar kelimeler: Kurutma, esansiyel yağ, *Citrus sinensis*, terpen sentaz, *Callosobruchus maculatus*.