



Phytochemical Analysis of Cultivated and Wild *Salvia Palaestina* using GC-MS: A comparative study

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ABSTRACT

The leaves of cultivated populations of *Salvia palaestina* (Lamiaceae) were collected from seven different governorates in Palestine to compare their phytochemical profiles to wild populations. Twenty volatile and semivolatile components were separated and identified by GC-MS. The major components in all the cultivated *S. palaestina* leaves were eucalyptol and camphor excluding one sample that was collected from Jericho. This sample revealed camphor as the predominant component (30.65%) while in the rest of the cultivated samples, camphor did not exceed 9.2% level. Moreover, thujone derivatives in Jericho's sample were abundant at high concentrations (28.9%) in comparison to other populations which did not exceed more than 2%. The wild *S. palaestina* leaves, showed eucalyptol as a major component in all samples from different locations with higher concentration than the cultivated leaves, while the later had a higher amount of camphor.

Key words: *Salvia palaestina*, Phytochemicals, GC-MS, Eucalyptol, Thujone.

INTRODUCTION

Due to its diverse topographical features, Palestine is very rich in biodiversity. More than 2953 plant species are documented of which about 700 are recognized for their medicinal use [1, 2]. Herbal medicines is considered as an integral part of Palestinian culture and still plays a significant role in their everyday public healthcare. Usually, the medicinal benefits and procedure of preparations of these herbs were verbally inherited from one generation to another but rarely reported or investigated [3].

One of the most acknowledged curable herbal plant to Palestinians is *Salvia palaestina* (Lamiaceae), called *Meramia* in Arabic. This plant is also common to Egypt, Syria, Lebanon, Southern Turkey, Iran, Northern Iraq and Jordan [4]. Typically, infusion of the leaves with tea is intensively used to add the distinctive pleasant aroma. However, decoction in boiling water is preferred for prevention and remedial purposes. *S. palaestina* leaves has glands that are rich in secondary metabolites of essential oils which are released when rubbed or heated.

The wild *S. palaestina* essential oils phytochemical profiling from different locations in Palestine were investigated by us along with *in-vitro* antioxidant and antimicrobial effects [5, 6]. Our current investigation aims to explore the difference between the wild and cultivated phytochemical profiles using GC-MS. Therefore, we screened the secondary metabolites of the cultivated populations of *S. palaestina* from the same locations and dates as that of the wild leaves and compared their components abundance to wild populations.

MATERIALS AND METHOD

Collection of plant materials: Wild and cultivated *S. palaestina* leaves were collected from seven different governorates (9 locations) in Palestine between April-May 2013 (Table 1). The leaves were air dried in the absence of light at room temperature for about one week until constant weight was achieved. Dried samples were stored in sealed paper bags and protected from light.

Reagents: GC grade n-hexane solvent and anhydrous sodium sulfate salt were purchased from Sigma-Aldrich Inc. (USA). Kovats retention index

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(KI) reagent that consists of alkane standard mixture between C₁₀-C₄₀ (even numbered) were purchased from Fluka, Switzerland.

Instrumentation: Essential oils were analyzed using Perkin Elmer, Clarus Gas Chromatography connected to Clarus 600 C mass spectrometer (USA). The GC-MS was operated in the electron impact ionization mode (EI) at 70 eV. Perkin Elmer autosampler was used with 2ml vials. The GC is equipped with a fused silica capillary column; DB-5 MS consisted of (5% diphenyl polysiloxane, 95% dimethyl polysiloxane) 28 m x 0.25 mm, coating film thickness is 0.25 µm (Restck, USA).

Scanning electron microscope (SEM) was employed to analyze the morphology of fresh *S. palaestina* wild and cultivated leaves. The experiments were performed on high resolution scanning electron microscope (HR SEM) Sirion (FEI Company) using Shottky-type field emission source and secondary electron (SE) detector. The images were scanned at voltage of 5kV. The simple distillation system (Clevenger apparatus), the analytical balance (Sartorius, accuracy ±0.0001g, Germany) and rotary evaporator (Steroglass-strike202, Italy) were utilized throughout the experiments.

Steam distillation: The essential oils of the *S. palaestina* leaves were isolated by distillation using a Clevenger type apparatus. About 10 gm of the leaves from each governorate were grounded and mixed with 250 ml distilled water. The sample was subjected to steam distillation for three hours at atmospheric pressure. The water distillate was extracted twice with 100 ml hexane using separator funnel. Then the hexane fractions were combined and dried over anhydrous sodium sulfate. 300 µL of hexane extract was diluted to 1 mL with hexane and 1 µL of the resulted diluted sample was injected to GC-MS.

GC-MS chromatographic condition: Perkin Elmer GC-MS at electron impact mode (EI) was used. The flow rate of the carrier gas was 1 ml He/min. Injector temperature was set at 235°C, the source temperature was at 250°C and the interface temperature was at 260°C. Split ratio of 1:20 was adopted during the entire analysis. The column gradient temperature was held at 50°C for 2 minutes, then raised from 50°C to 180°C at a ramp of 5°C/min and from 180° to 280°C at a ramp rate of 15°C/min and held there for extra 5min. Solvent cut time of 4.5 minutes was used to eliminate the hexane solvent peak. The mass range was from m/z of 50 up to 480 Da, and of scan interval of 0.2 seconds.

Peaks identification: The identification of compounds was based mainly on matching their MS spectra with NIST mass spectral library. Moreover, Kovats Retention (KI) calculation was used to support the identification. KI values were compared with literature NIST values. Excellent agreement was obtained even using different chromatographic conditions. Quantitative analysis of the essential oils was performed once the identities of the compounds are known by the MS.

RESULTS AND DISCUSSION

Due to its diverse variability, *Salvia* species exhibit significant phytochemical and morphological variations. To distinguish such differences among cultivated populations of *S. palaestina*, a scanning electron microscopy (SEM) was utilized and compared to fresh wild leaf. The intensity of trichomes in the lower surface of both cultivated and wild fresh leaves was always higher than the upper surface. Figure 1 shows SEM snapshots of wild and cultivated fresh leaves and their corresponding trichomes. The cultivated leaf was larger, wider and flattened when compared to wild leaves which was smaller; bent inward at the edges to avoid harsh environmental conditions and water losses.

Oil yields of dry cultivated *S. palaestina*: Cultivated *S. palaestina* leaves were collected from seven different Palestinian governorates between April-May 2013. Prior to extracting the oil, the fresh leaves were dried and the water loss was calculated to acquire information about water content in the leaves. The essential oils of dried leaves were then isolated by steam distillation (SD). The harvesting time, locations, water loss and oil yields are summarized in Table 1.

Upon comparing results of table 1 with wild samples that has been reported lately on wild populations from the same locations, average water loss was higher in the cultivated samples versus wild ones [5]. Moreover, the average oil yields, which were calculated based on oil to dried sample weights, were approximately 0.46% and 0.63% for wild and cultivated samples respectively.

GC-MS profile analysis: The essential oils were analyzed by GC-MS in the electron impact mode and identified by comparing their Kovats Indices (KI) and mass spectra with authentic NIST-MS library. Twenty major components were identified. The name, molecular formula, retention time and KI values are summarized in table 2. Figure 2 shows the total ion chromatograms (TIC) of the GC-MS of a cultivated sample collected from Ya'bad/Jenin.

The essential oils of *S. palaestina* contain mainly monoterpenoids, oxygenated monoterpenoids and to a lesser extent sesquiterpens and diterpens (figure 2 and table 2). Among all, the oxygenated monoterpenoid eucalyptol was the predominant component in an average percentage of more than 50%. All the essential oils components were analyzed in triplicate and the developed GC-MS method was found to be sensitive, accurate and reproducible.

Cultivated *S. palaestina* from all locations: Comparison was performed between all cultivated samples from all locations collected between April-May 2013. The comparison revealed that the sample from Jericho was unique due to its high concentration of camphor, thujone, 3-thujanone, L-bronyl acetate and camphene (figure 3). Jericho is the deepest point in the world and its soil is different from other places in Palestine in term of texture, moisture content and acidity which probably affects the composition of *S. palaestina* leaves essential oils. In the histograms, few differences were observed in volatiles from other locations rather than that of Jericho (figure 3).

Wild vs. cultivated *S. palaestina* main components from all locations: Focused comparison between main components percentages is summarized in the histograms (figures 4). The main components in wild *S. palaestina* were eucalyptol, camphor, caryophyllene, terpineol, β -thujene and β -myrcene, while the main components in cultivated leaves were: eucalyptol, camphor, caryophyllene, α -terpineol, thujone and 3-thujanone. The above major six components represented about 80% of cultivated *S. palaestina* components as in Jericho's sample, while in the wild leaves they represented about 70% of all components which probably indicate that components in wild leaves are more variable than that of the cultivated. Moreover, the concentration of similar components varies between cultivated and wild samples; in general the wild *S. palaestina* has higher concentration of eucalyptol, while the cultivated has a higher concentration of camphor. This symbolize to choose wild *S. palaestina* when the eucalyptol effects are desired, while cultivated *S. palaestina* is the one to be chosen when camphor effect is pursuit.

Figure 4 also revealed that both leaves of Anabta's/Tulkarem sample contain nearly the same amount of eucalyptol as a major component while the wild sample contain a doubled amount of caryophyllene. Conversely, α -terpieol and camphor were slightly pronounced in the cultivated leaves. The cultivated samples from Beita/Nablus showed a lower number of components and concentrations.

Excluding camphor which was higher in cultivated *Salvia* from Ya'bad/Jenin, the components were nearly the same in both samples but the amount of components were higher in the wild sample. Halhul/Hebron sample revealed that the number of identified volatiles was less than other investigated locations. The concentration of identified volatiles was higher in wild leaves except for β -caryophyllene, β -myrcene and epiglobulol. Al-Khader/Beithlaham sample was especially distinguished with its higher levels of eucalyptol (63.06%) in wild leaves and high levels of camphor (8.42%) in cultivated ones. As in previous samples, the main component in Kafr Ni'ma/Ramallah sample was eucalyptol and its level is higher in wild than in cultivated, conversely, the later has higher levels of camphor and caryophyllene. In general, comparison between wild and cultivated *S. palaestina* leaves components from multiple locations revealed that the concentration of eucalyptol was always higher in wild leaves, while in the majority, the concentrations of camphor, caryophellene and β -myrcene were higher in cultivated samples.

Main *S. palaestina* components medical applications and limitations: Eucalyptol, which is the main component in the wild leaves, is mainly used as an active ingredient in mouthwash, lozenges, ointments, inhalants, body powder and cough suppressant preparations. It controls airway mucus hypersecretion and asthma via inhibition of cytokine production in human monocytes [7, 8]. In addition, it stimulates immune system response by enhancing the phagocytic ability of human monocytes [9]. Eucalyptol has noticeable antimicrobial activity with minimal side effects either when applied topically or systemically. Therefore, it is used in many preparations as an active antiseptic and to reduce inflammation and pain. Due to its pleasant smell, it is used as a fragrance to impart a fresh and clean aroma in soaps, lotions, detergents and cosmetics. Recently, several studies revealed that it might have anti-tumour activity since it kills leukaemia cells *in vitro* [10]. Thus, it is advisable to use wild *S. palaestina* leaves in the preparation of pharmaceutical dosage forms that contain eucalyptol as an active or inactive ingredient.

Camphor, which is available in relatively high concentrations in cultivated leaves, has a long history of use as an analgesic, antiseptic, antipruritic, counterirritant and rubefacient. Its success in topical use is mainly related to its mild local anesthetizing effect and to the production of heat sensation followed by a feel of cooling [11, 12]. Nowadays, camphor is mostly used in the form of inhalant preparations for home treatment of

colds and nasal decongestion [13]. It is also used as an active ingredient in topical analgesic and anti-inflammatory preparations to treat sprains and swellings [12, 14, 15].

Systemic use is associated with tachycardia, vasodilation in skin (flushing), slower breathing, reduced appetite, increased secretions and excretions such as perspiration and diuresis [16]. Moreover, it can modulate the activities of hepatic enzymes involved in phase I and phase II drug metabolism and inhibit mitochondrial respiration [17, 18]. Camphor can also be a potential radiosensitizing agent in radiotherapy [19]. Nonetheless, medicinal use of camphor is discouraged by the FDA, except for skin-related uses in relatively small amounts [19-22]. Thus, it is advisable to use cultivated leaves in skin pharmaceutical preparations when camphor is preferred. In order to qualitatively understand the accumulated results one must keep in mind that secondary metabolites biosynthesis and accumulation in plants is strongly influenced by various biotic and abiotic factors [23]. Plants are exposed to various degrees of stress, which might be either natural or human-induced factors. Drought, salinization, water, light, radiation, humidity, atmosphere, pressure, sound waves, soil type and the presence of heavy metals in the soil are all causes of substantial effect on yield, type and quality of bioactive components in the oil [24].

Due to the aforementioned factors, differences between *S. palaestina* components from one governorate to another are justified. In the case of the cultivated sample from Jericho, significant differences were observed probably due to the fact that Jericho is the deepest place in the world which affects the atmospheric temperature and pressure. An important recent study compared Jericho's soil to Tulkarem's soil. The study revealed that Jericho's soil texture comprises 60.5% sand, 23.5% gravel, 11.2% slit and 4.8% clay, while Tulkarem's soil comprises 9.56% sand, 0% gravel, 66.92% slit and 23.52% clay. Moreover, the total dissolved substances (TDS) in Jericho's soil was 65 mg/L while in Tulkarem it was 185 mg/L and the moisture content was about 3.73% in Jericho soil, while in Tulkarem it was 19.57% [25]. Even the acidity values were different between both, it was more acidic in Tulkarem sample. Thus, it's not surprising that *S. palaestina* from Jericho is unique. *S. palaestina* from Jericho is rich in thujone and its isomers, which all have some structural similarities to tetrahydrocannabinol, the main psychoactive substance found in marijuana. It was hypothesized, few decades ago, that it might act in the same way on the brain receptors [26]. Later on, thujone was considered as a gamma-amino butyric acid

(GABA) receptor inhibitor [27]. By inhibiting GABA receptor activation, neurons may fire more easily also it effect 5-HT₃ receptors [28]. Moreover, thujone and its derivatives are considered toxic substances due to its adverse reactions on brain, liver and kidney cells. It might cause convulsions (muscle spasms), rapid heart rate, restlessness, anxiety, sleeplessness, vomiting, kidney damage, vertigo, epileptic seizures, and psychedelic effects if ingested in high doses. It's contraindicated during pregnancy because it might cause abortion [29].

Since *S. palaestina* from Jericho is completely distinguished with its high levels of thujone which if ingested might many undesirable effects as aforementioned, its usage should be controlled and restricted as in other countries such as in European Union, Canada and United states.

CONCLUSION

Comparison between wild and cultivated leaves from the same location and dates revealed that the wild samples were characterized by eucalyptol unequivocal dominance while the cultivated was distinguished with higher levels of camphor. Therefore, when camphor pharmacological activity is required (topical applications), the usage of cultivated leaves is recommended, while the wild leaves will be appropriate when eucalyptol pharmacological activity is required. In other words, it is advisable to use wild *S. palaestina* leaves in the preparation of pharmaceutical dosage forms that contain eucalyptol as an active or inactive ingredient and to use cultivated leaves in skin preparations to benefit from camphor. Jericho's cultivated sample is unique, in which camphor volatile compound was the main constituent. Its amount was three times more than other samples from different locations. Therefore, it might be suitable to benefit from its high content in topical applications. On the other hand, Jericho's sample contains high amount of thujone derivatives, which is harmful if ingested. Thus, special restrictions for the usage of Jericho's *S. palaestina* are recommended.

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Table 1: Cultivated *Salvia palaestina* leaves dried/fresh ratio, water loss % and oil yield %*

Location	Dried/Fresh Weight Ratio	Average water loss w/w% \pm SD (n=3)	Average oil yield w/w% \pm SD (n=3)
Anabta/Tulkarem	31.0/100	69.0 \pm 1.007	ND**
Beita/Nablus	23.1/100	76.9 \pm 0.971	0.275 \pm 0.067
Kafr Ni'ma/Ramallah	45.7/100	54.3 \pm 0.814	0.64 \pm 0.054
Halhul/Hebron	25.4/100	74.6 \pm 1.026	0.70 \pm 0.104
Al-Khader/Bethlehem	29.5/100	70.5 \pm 1.179	1.58 \pm 0.199
Ya'bad/Jenin	26.9/100	73.1 \pm 1.124	0.735 \pm 0.136
Jericho	31.2/100	68.8 \pm 0.954	0.434 \pm 0.081

* The oil yield was calculated based on oil to dried sample weight

** ND: Not determined

Table 2: GC-MS components of the secondary metabolites of cultivated *S. palaestina* leaves

#	Component	M Formula	RT (mins)	KI
1	α -Thujene	C ₁₀ H ₁₆	6.21	
2	Camphene	C ₁₀ H ₁₆	6.64	
3	β -Thujene	C ₁₀ H ₁₆	7.39	
4	β -Myrcene	C ₁₀ H ₁₆	7.74	
5	Eucalyptol	C ₁₀ H ₁₈ O	8.95	1042
6	γ -Terpinene	C ₁₀ H ₁₆	9.71	1072
7	<i>trans</i> -4-Thujanol	C ₁₀ H ₁₈ O	10.05	1084
8	3-Thujanone	C ₁₀ H ₁₆ O	11.09	1120
9	α -Thujone	C ₁₀ H ₁₆ O	11.42	1131
10	(\pm)-Camphor	C ₁₀ H ₁₆ O	12.25	1156
11	3-Pinanone	C ₁₀ H ₁₆ O	12.64	1168
12	Ocimenol	C ₁₀ H ₁₈ O	12.93	1176
13	L-Terpinen-4-ol	C ₁₀ H ₁₈ O	13.24	1184
14	α -Terpineol	C ₁₀ H ₁₈ O	13.64	1196
15	L-Bornyl acetate	C ₁₂ H ₂₀ O ₂	16.13	1293
16	Terpinyl acetate	C ₁₂ H ₂₀ O ₂	17.79	1353
17	β -Caryophyllene	C ₁₅ H ₂₄	19.71	1419
18	α -Caryophyllene	C ₁₅ H ₂₄	20.60	1458
19	Epiglobulol	C ₁₅ H ₂₆ O	23.92	1591
20	13-Epi-manool	C ₂₀ H ₃₄ O	31.49	2066

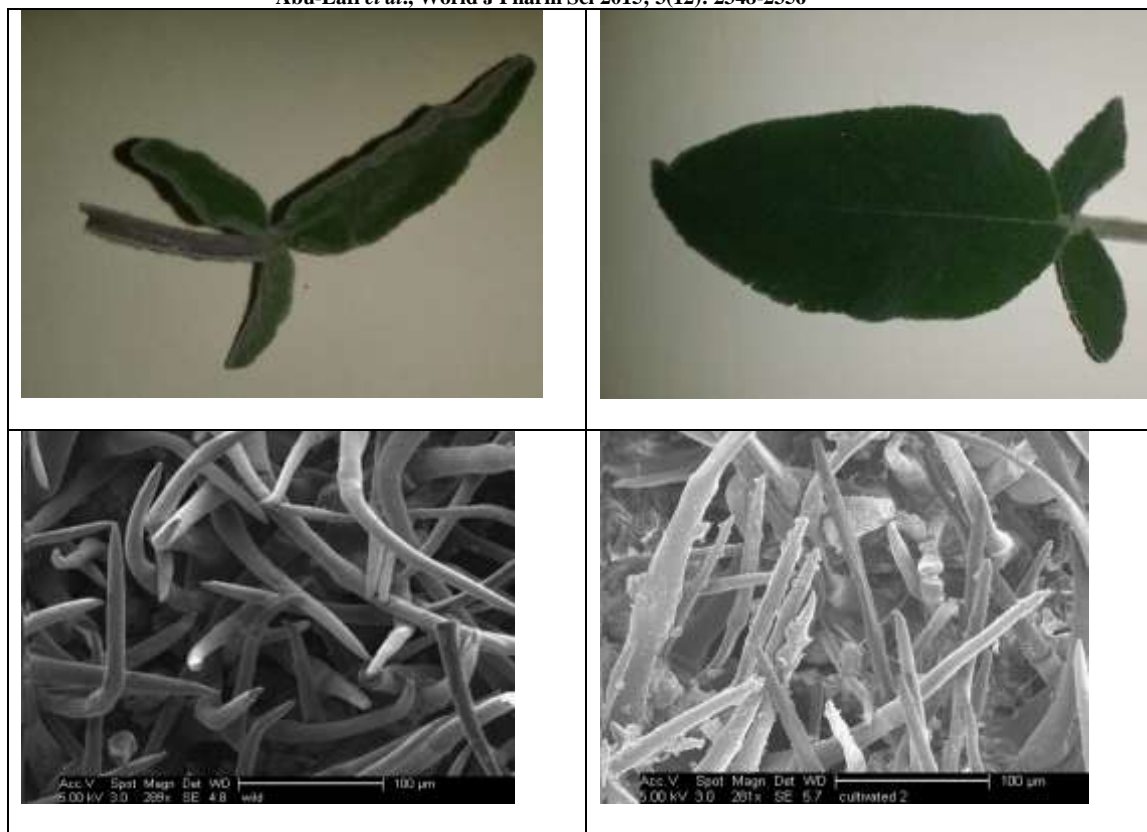


Fig. 1: SEM of wild (left) vs. cultivated (right) of *S. palaestina* fresh leaves

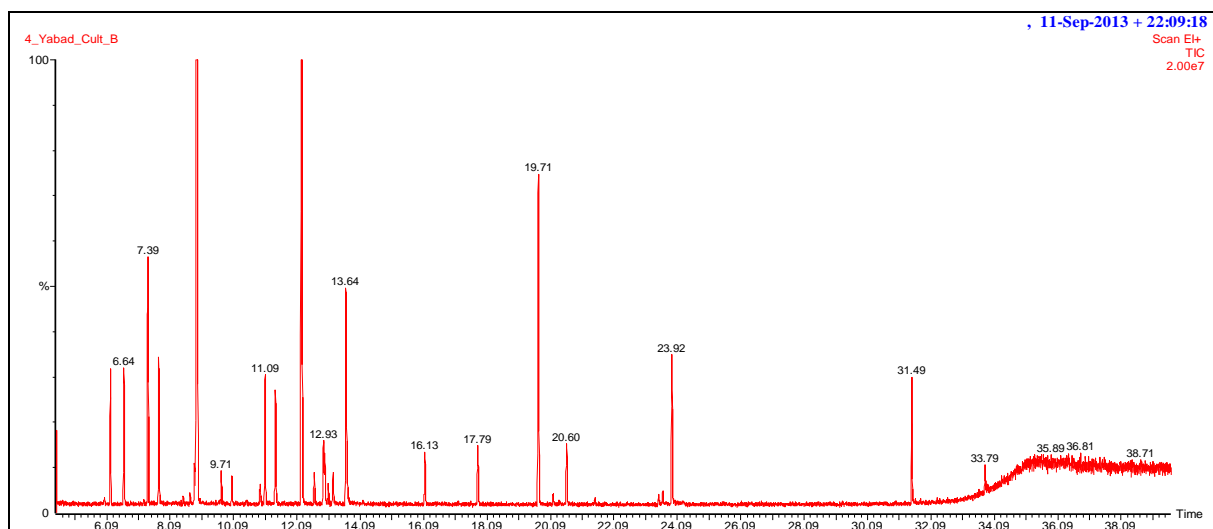


Fig. 2: The TIC of zoomed GC-MS of cultivated *S. palaestina* collected from Ya'bad/Jenin

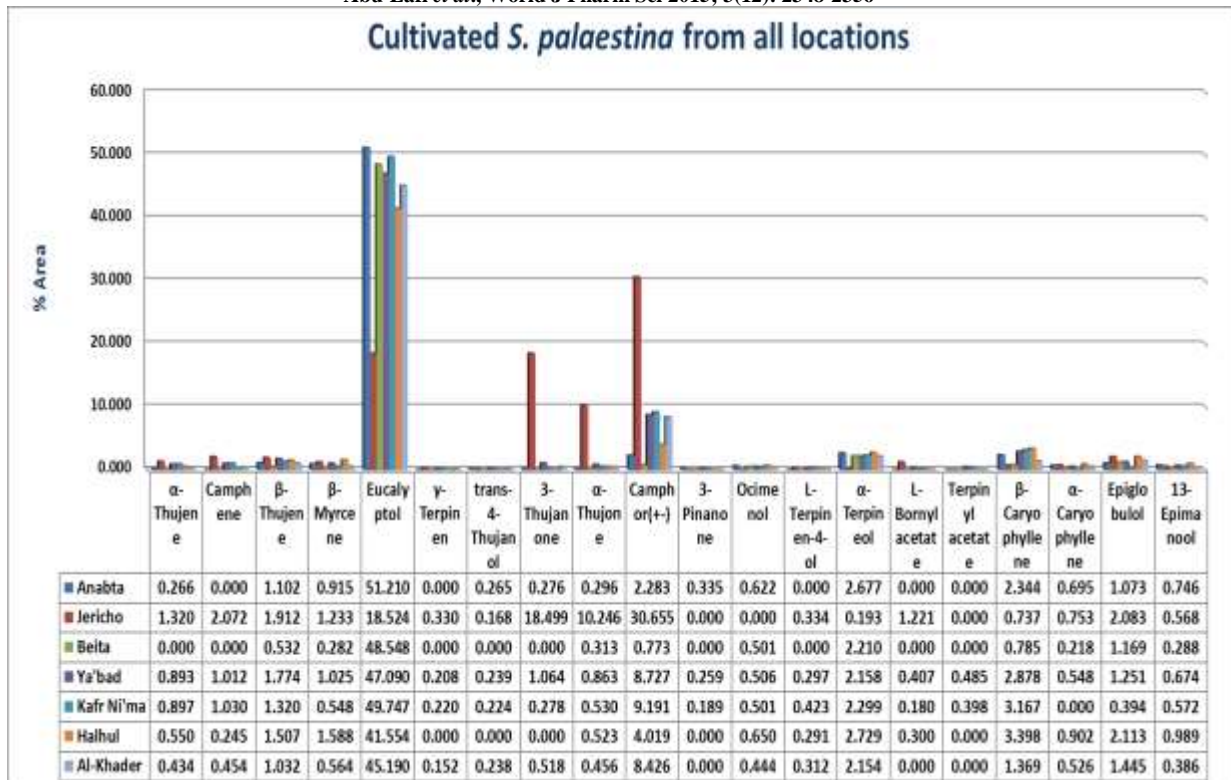
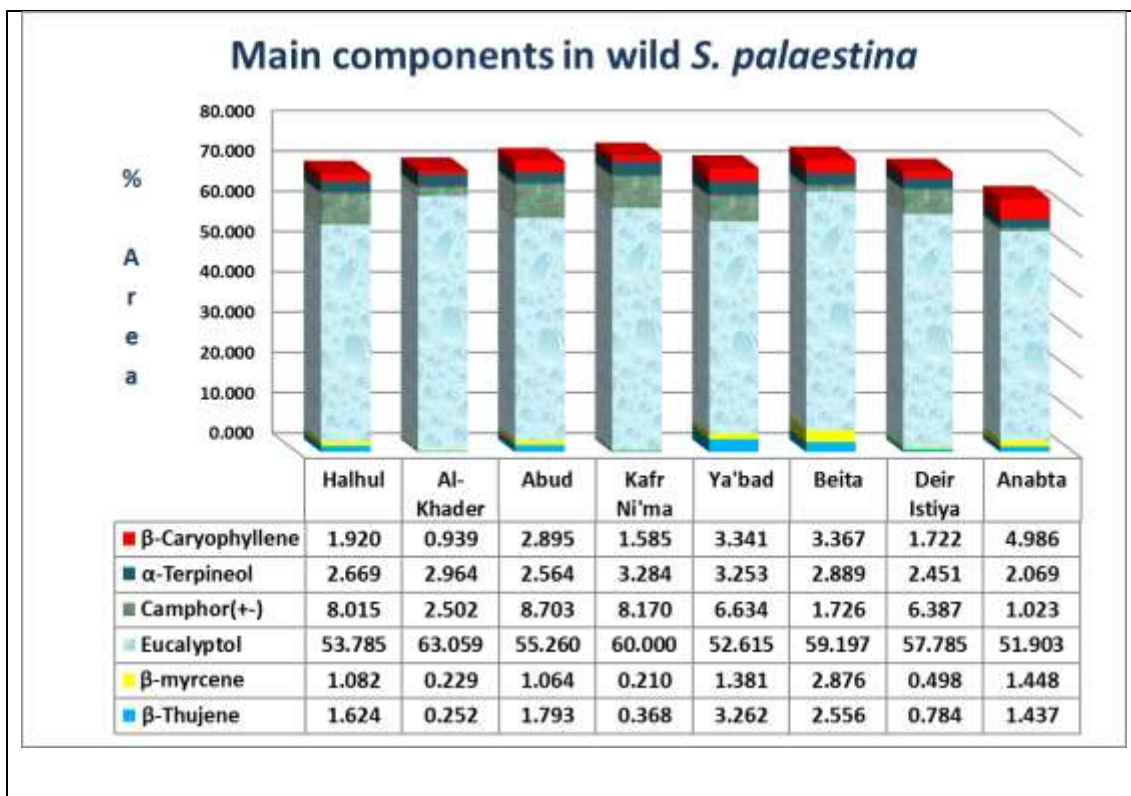


Fig. 3: Main essential oil components in cultivated *S. Palaestina* from all locations



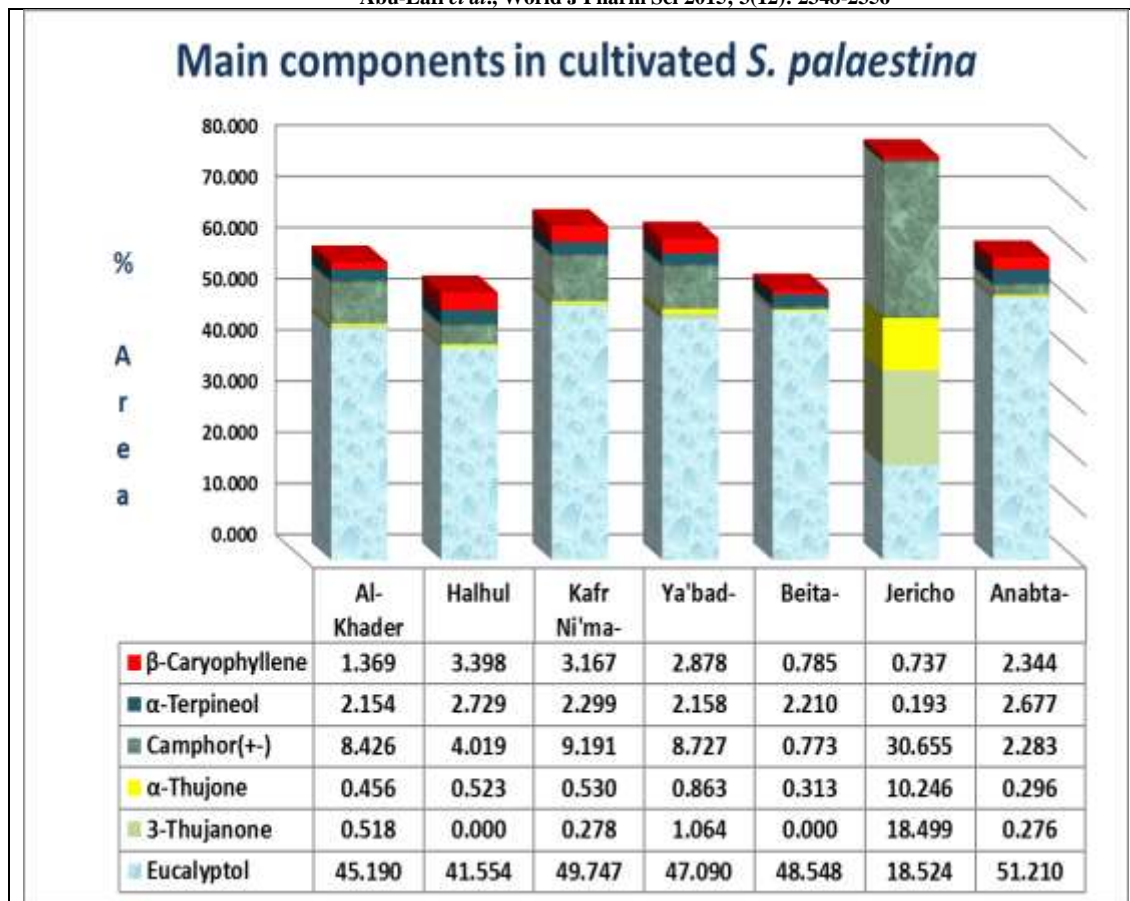


Fig. 4: Main essential oil components in wild and cultivated *S. Palaestina*

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