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© 2008 S. Abu-Lafi[1], I. Odeh[1], H. Dewik[1], M. Qqabajah[1], A. Iimam[2], V.M. Dembitsky[3] and L. O. Hanuš[3*]

DIVERSITY OF THE TERPENOIDS AND PHENOLIC COMPOUNDS IN *MAJORANA SYRIACA* (LABIATAE) LEAVES GROWING WILD IN PALESTINE

Абу-Лафи С., Одех И., Девик Х., Кквабаджах М., Имам А., В.М. Дембицкий, Хануш Л.О.

РАЗНООБРАЗИЕ ТЕРПИНОИДНЫХ И ФЕНОЛИТИЧЕСКИХ ВЕЩЕСТВ В ЛИСТЬЯХ *MAJORANA SYRIACA* (LABIATAE), ДИКО ПРОИЗРАСТАЮЩЕГО В ПАЛЕСТИНЕ.

Essential oils, which including terpenoids, phenolic compounds, and other volatiles of *Majorana syriaca* leaves growing wild in Palestine were analyzed using static headspace gas chromatography mass spectrometry technique (HS-GCMS) to check for their chemical variability. The samples were collected from thirty-four individual plants naturally growing in different locations from Palestine. In such a small-restricted area, a wide range of variation in oil characteristics was observed indicating that the Palestinian region is an important center of diversity. The essential oil yield, based on air-dried weight, ranged from 10.5-mg g^{-1} to 54-mg g^{-1}. The major constituents identified throughout all the harvesting periods were varied greatly among the samples examined. HS revealed major volatiles and semi-volatiles of α-phellandrene (1.62-8.13%), α-pinene (1.22-4.61%), β-myrecene (0.5-11%), m-cymene (1.86-8.61%), p-cymene (8.44-48.6%), γ-terpinene (11.96-30.8%), thymol (0.26-11.6%), and carvacrol (0.65-21.7%). As for the phenolic compounds, the results revealed that the wild growing *Majorana syriaca* could be characterized by the dominant presence of carvacrol. Conversely, water irrigation has showed a prominent effect on the thymol isomer production. Therefore, we suggest using isomeric distribution ratios of these isomers as a marker to distinguish wild from cultivated *Majorana syriaca*.

INTRODUCTION

Plant species belonging to the genus *Origanum* are very well represented in the Mediterranean area with more than 2/3 of species being restricted to the Eastern regions, including Palestine [1, 2]. Species of *Origanum* consider among the most important aromatic plants worldwide. Many representatives of the genus are widely used as culinary herbs, as garden plants and for essential oil production [3].

* [1]Chemistry and Chemical Technology Department, P.O. Box 20002, Al-Quds University, Abu-Deis, Palestine,
[2]Techno-Line Advanced Instrumentation, P.O. Box 27292, East Jerusalem, Palestine and
[3]Department of Medicinal Chemistry & Natural Products, School of Pharmacy, P.O. Box 12065, Hebrew University, Jerusalem 91120, Israel

Hippocrates, in the 5th century (B.C.), used essential oils of some plants of the genus *Origanum* for curing various diseases such as stomach pain and respiratory diseases. More recently, in the 15th century, Paracelsus used the same essential oils to treat diarrhea, psoriasis, vomiting, jaundice, and fungal diseases [4]. Mediterranean people used it as a meat preservative. At present, the essential oils isolated from the family Labiatae are used internally for joint pain, aching muscles, chronic cough, infections, asthma and bronchital congestion, and topically as an antifungal, antiparasitic, antiviral and antibacterial [5, 6]. The fact that Palestinians prefer wild over cultivated thyme as an effective healer for many diseases instigated this work.

Majorana syriaca (= *Origanum syriacum* var. *syriacum*) that belongs to the mint family Labiatae, is considered among the most popular herbs in the Palestinian territories (Al Sheikh et al., 2000. The plant is cultivated widely and grows naturally between the rocks of the mountains of Palestine [7, 8]. The wild herb possesses distinctive aroma with a warm pungent taste. The green leaves of the herb are rich in essential oil, which is responsible for its characteristic flavor and fragrance [9, 10]. Flowering of *M. syriaca* occurs from May to October [7]. Leaves are normally collected from wild populations once a year, before flowering. In Palestine, scarce information is available dealing with local wild thyme variability [11].

The aim of the present study is to investigate the extent of the qualitative and quantitative terpenoids and phenolic compounds variability within and between wild populations in the composition of the volatiles of *M. syriaca*.

EXPERIMENTAL

Material and Methods

Plant material

The wild leaves materials were collected from six different environmental sites in the West Bank territories. The chosen sites were distributed geographically as follows: the northern area: Nablus, Jenin and Tulkarim, the southern area: Hebron (Tarqumia, Halhool, Dora and Bet O"la), the central area: Bethlehem, and Ramalla.

The collection was carried out for two years, starting February 2003 and ending June 2004. During this period, thirty four samples were collected. Thyme leaves were air dried in the absence of light at room temperature for a fixed period of time (three months) for all of the samples, followed by storing in sealed paper bags; this is the most effective method to preserve the components of the essential oils from being damaged or altered [12]. Leaves were analyzed by HS-GCMS without being grounded. The amounts of the dry leaves used for the analysis were 0.6g and 6g for the HS and steam distillation experiments respectively.

HS-GC-MS analysis

Essential oils were analyzed using Shimadzu GC-17A connected to MS-QP5050A. The GCMS was operated in the electron impact ionization mode (EI)

at 70 eV. Shimadzu auto sampler AOC-20i was used with 2ml vials. An equilibrium headspace Shimadzu HSS-4A auto sampler was used with 27-ml HS vials. The HS vials were sealed with silicon rubber septa and aluminum caps after introduction of the thyme sample while the AOC vials were sealed with 8mm double-faced rubber septa and screw cap with 12 mm hole. The GC is equipped with a fused silica capillary column; DB-5 MS containing (5% diphenyl poly siloxane, 95% dimethyl polysiloxane) 30 m x 0.25 mm i.d., coating thickness is 0.25 µm, Supelco (Sigma-Aldrich Inc., USA).

The carrier gas flow rate was 1.6 ml He/min. Injector and detector temperatures were 230^{o}C and 250^{o}C respectively. Split ratio was 1:30. The column temperature was held at 60°C for 2 minutes, then raised from 60°C to 100°C at 3°C/min and from 100° to 280°C at 30°C/min and held there for 2 min. Solvent cut time was 4 minutes and the starting time of the chromatogram was 5 minutes. Mass range was from 30 to 350 u, and scan interval was 0.5 seconds. Detector voltage was set to 1.50 kV.

Before each HS-GCMS analysis, a blank consisting of an empty vial is carried out to check whether any constituents of the materials of the septum or the vial are being emitted. 0.6 g samples of dried leaves of thyme were placed in HS vials and immediately sealed with silicone rubber septa and aluminum caps. The vials were then transferred to the headspace tray.

The identification of the compounds was based mainly on their retention times in comparison with those from authentic standards. The standards were injected separately in addition to adding them to the thyme matrix (spiking) to enhance the relevant peaks of interest. Identification of some peaks was based on matching of their MS spectra with NIST/EPA/NIH Mass spectral library (NIST 98).

RESULTS AND DISCUSSION

The essential oils of wild *M. syriaca* dry leaves were isolated first by normal steam distillation (SD) and analyzed by GCMS using the EI mode. The samples were collected from thirty-four plants growing wild in six major districts from Palestine. The harvesting time (January to June) over two year's period seems to greatly influence the oil yields.

The results reported that the yields ranged between from 10.5-mg g^{-1} to 54-mg g^{-1}. The differences in the yield of the wild samples are as much as 80.4%. The maximum yield recorded was for the months May-June, and the minimum value recorded was for the months of January.[11] SD-GCMS analysis reveals that there are no significant differences in oil yields as it related to different geographical sites.

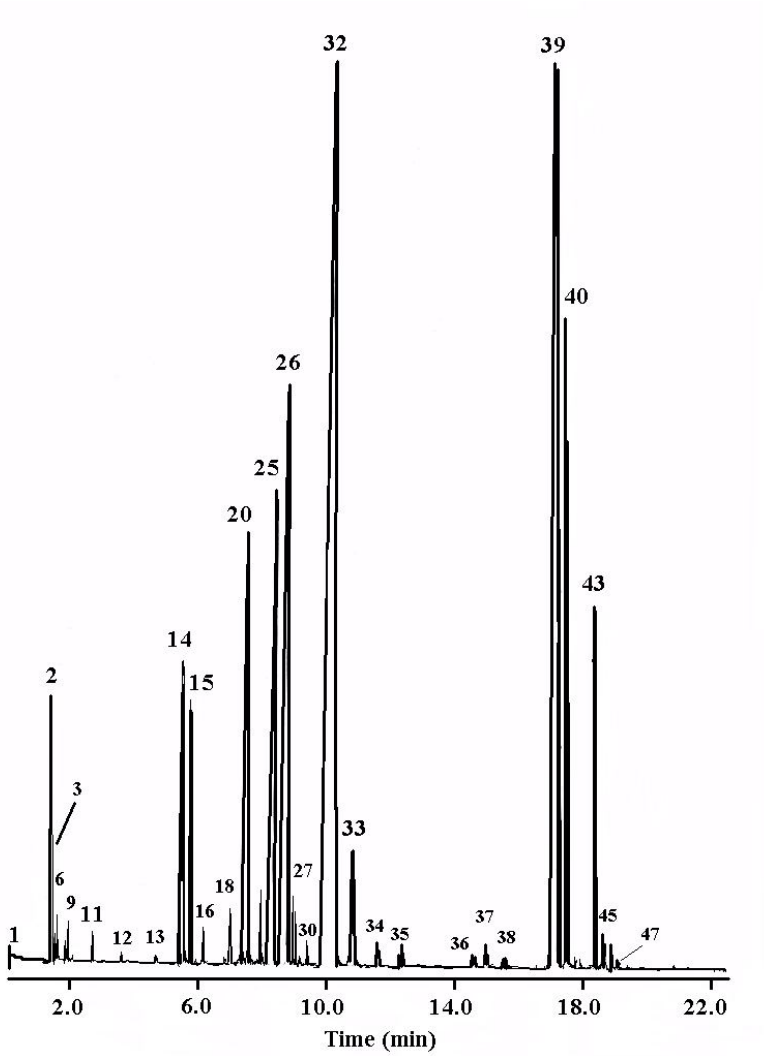

Fig. 1. Typical TIC of the terpenoids from leaves of wild Majorana syriaca using HS-GCMS on nonpolar DB-5 capillary column. Identities of the major terpenoid peaks are as follows: 14, α-Phellandrene, 15, α-Pinene, 20, β-Myrecene, 25, Terpinolen, 26, m-Cymene, 27, p-Cymene, 32, γ-Terpinene, 39, thymol, 40, carvacrol, and 43, iso-caryophellene (See Table 1)

The terpenoids present in *M. syriaca* was characterized by the presence of about 47 compounds using HS-GCMS.[11] Fig. 1 depicted typical chromatogram of terpenoids at the optimized time and temperature conditions. Base line separations with reasonable resolution were obtained for almost all the compounds separated. Terpenes (oxygenated and hydrocarbons) are the main constituents in thyme leaves. Only few hydrocarbons and sesquiterpenes such as caryophyllene, and iso caryophyllene were also present but in smaller quantities in comparison to the oxygenated monoterpenes. The principal constituents of

Table 1

Variability of the major terpenoid components (in %) from M. syriaca growing wild in different districts at different harvesting time

Sample No.	subdistrict	Harvesting time	α-Phellandrene	α-Pinene	β-Myrecene	m-Cymene	p-Cymene	γ-Terpinene	Thymol	Carvacrol
1	2	3	4	5	6	7	8	9	10	11
1	Tarqumia	April 2003	1.63	2.40	7.03	4.31	19.0	21.6	11.6	4.70
2	Tarqumia	May 2003	2.69	2.67	7.91	6.11	16.7	28.3	7.87	5.81
3	Tarqumia	June 2003	2.70	3.47	9.61	7.30	18.6	24.3	6.21	5.55
4	Tarqumia	March 2004	5.84	3.34	8.46	6.46	21.6	21.8	6.00	2.28
5	Halhool	May 2003	3.50	3.32	7.89	5.49	14.4	28.4	5.07	8.40
6	Halhool	February 2004	3.92	3.31	0.50	0.72	42.5	21.01	0.37	1.35
7	Halhool	March 2004	6.79	3.43	9.09	7.44	13.0	29.5	3.36	8.96
8	Halhool	April 2004	5.27	2.75	7.35	5.82	21.8	23.7	2.07	6.59
9	Dora	April 2003	7.05	2.90	9.28	8.61	11.9	29.0	2.31	11.0
10	Dora	June 2003	2.10	3.13	8.27	5.84	17.5	23.7	4.45	10.2
11	Dora	March 2004	4.08	3.18	8.10	4.60	21.8	17.9	0.63	15.8
12	Dora	April 2004	7.08	3.04	10.1	5.23	12.1	17.5	0.74	21.7
13	Beit Ola	April 2003	4.00	3.63	8.49	6.47	16.3	26.6	4.70	8.01
14	Beit Ola	May 2003	3.55	3.27	8.94	6.50	16.8	24.4	5.51	9.96
15	Beit Ola	February 2004	3.29	2.12	3.43	3.48	40.0	18.2	3.04	1.53
16	Menea	February 2004	5.23	3.65	7.58	3.36	25.8	15.2	0.44	9.01
17	Menea	March 2004	5.46	2.48	5.64	6.40	24.0	25.4	1.24	5.72
18	Menea	April 2004	8.09	3.39	9.63	7.10	14.0	25.8	1.47	10.1
19	Menea	May 2004	6.01	2.58	8.82	5.93	9.79	24.8	4.54	14.9
20	Kafermalek	May 2003	1.93	3.41	9.10	6.76	18.8	21.7	4.47	8.39
21	Kafermalek	June 2003	2.90	3.67	8.29	5.50	21.2	17.2	3.36	10.7
22	Kafermalek	March 2004	4.59	2.26	6.65	6.34	17.4	30.8	6.20	6.20
23	Beata	April 2003	2.55	3.82	8.88	5.88	15.0	24.2	2.59	13.4

the continue of table 1

1	2	3	4	5	6	7	8	9	10	11
24	Beata	May 2003	2.41	2.93	8.33	5.15	15.4	24.3	1.39	11.3
25	Beata	June 2003	2.87	4.61	11.0	7.50	17.9	19.9	1.04	12.4
26	Beata	February2004	5.04	3.28	5.95	4.76	21.3	15.3	1.78	7.41
27	Beata	March 2004	4.79	3.03	7.26	6.10	16.6	25.4	0.26	8.32
28	Yaabad	April 2003	1.62	1.81	2.86	1.86	8.44	11.9	5.16	0.65
29	Yaabad	May 2003	3.04	3.80	9.48	6.62	14.6	22.7	2.79	10.1
30	Yaabad	January 2004	1.77	1.22	1.85	2.89	48.6	15.8	3.38	1.26
31	Rameen	April 2003	3.91	3.13	7.80	6.88	17.9	27.3	9.84	2.69
32	Rameen	May 2003	3.61	3.25	9.46	6.72	16.4	26.0	5.98	7.38
33	Rameen	June 2003	2.93	3.23	8.79	5.79	15.4	25.3	0.97	11.8
34	Rameen	March 2004	8.13	3.69	9.70	8.26	11.1	27.1	5.74	6.56

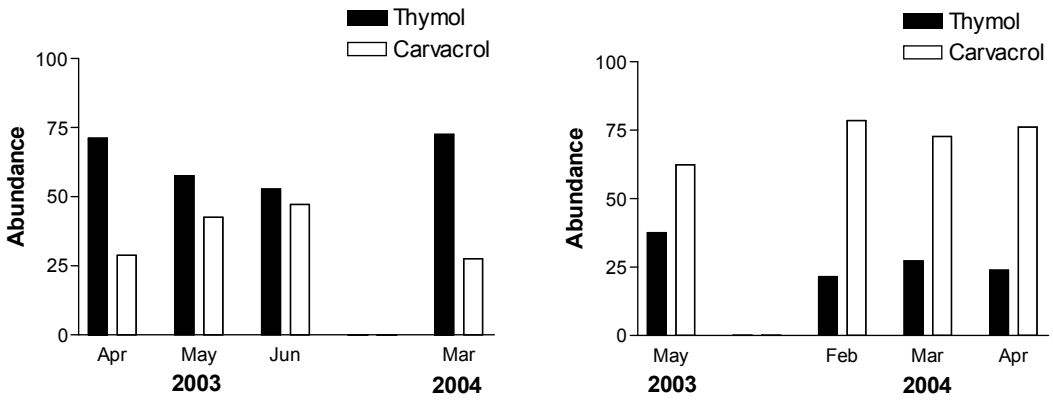

Fig. 2. Left part, Histograms of the isomeric distribution of thymol and carvacrol from wild sampe from Tarqumia (Hebron) at different harvesting time by HS-GCMS; Right part, Histograms of the isomeric distribution of thymol and carvacrol from Halhool (Hebron) at different harvesting time by HS-GCMS

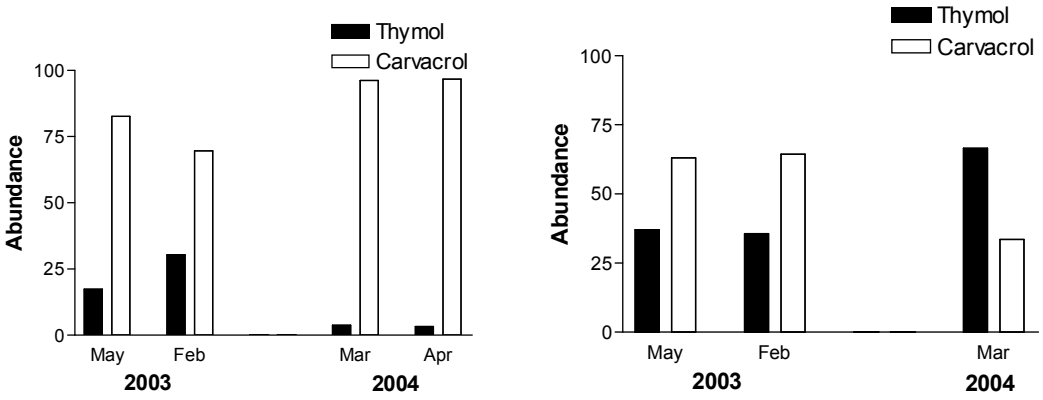

Fig. 3. Left part, Histograms of the isomeric distribution of thymol and carvacrol from wild sampe from Dora Hebron) at different harvesting time by HS-GCMS; Right part, Histograms of the isomeric distribution of thymol and carvacrol from Beit Ola (Hebrn) at different harvesting time by HS-GCMS

oxygenated monoterpenes are the phenolic compounds, thymol and its geometrical isomer, the carvacrol.

Previous work has been carried out on leaves of plants collected from different places all over the world. For example, the variability in the composition of the monoterpenoids of *Origanum microphyllum,* a species endemic to Crete, was reported recently [13]. Headspace analysis showed that plants were rich in *cis*-sabinene-hydrate (varied from 3 to 68%), sabinene (3–45%) and *trans*-sabinene-hydrate (0.5–34%).

Cosentino et. al. [14] has conducted investigation on *Thymus capitatus* oil. In his work, total of nineteen components have been identified, including α-terrpineol, linalool alcohols, the two isomeric phenols carvacrol and thymol, as well as, α-terpinene and p- and α-pinene. The concentration of these components varied from 2.3 to 20.6% for carvacrol and 29.3 to 50.3% for thymol.

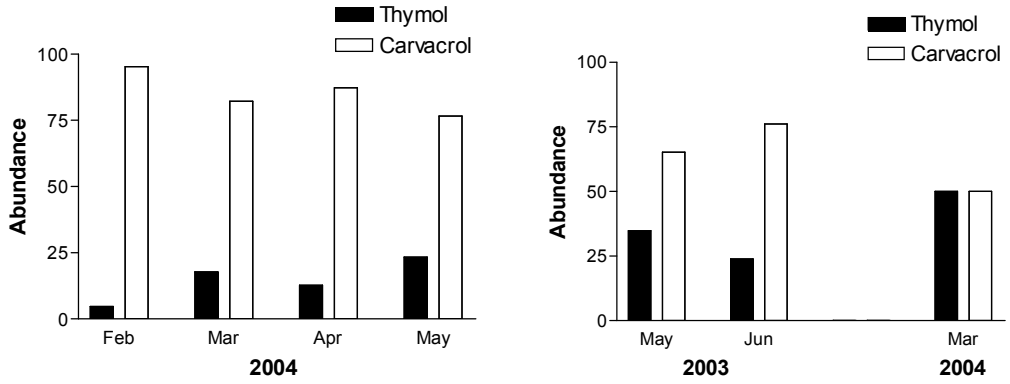

Fig. 4. Left part, Histograms of the isomeric distribution of thymol and carvacrol from wild sampe from Menea (Bethlehem) at different harvesting time by HS-GCMS; Right part, Histograms of the isomeric distribution of thymol and carvacrol from Kafermalek (Ramallah) at different harvesting time by HS-GCMS

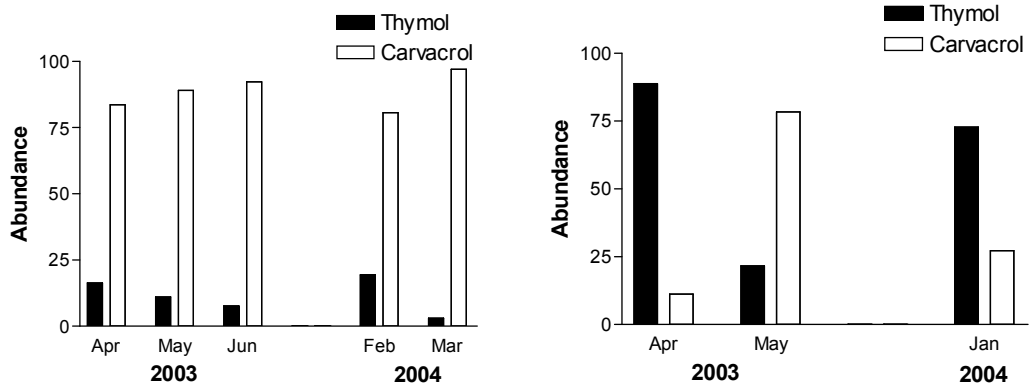

Fig. 5. Left part, Histograms of the isomeric distribution of thymol and carvacrol from wild sampe from Beata (Nablus) at different harvesting time by HS-GCMS; Right part, Histograms of the isomeric distribution of thymol and carvacrol from Yaabad (Jenin) at different harvesting time by HS-GCMS

Senatore [15] has studied of the essential oil of *Thymus pulegioides*. Essential oils were characterized by a high content of γ-terpinene, p-cymene, thymol and

carvacrol, which varied from 57.3% to 62.5% of the total oil content. The essential oil yields varied between 0.38 and 1.11% of the weight of the fresh leaf material in

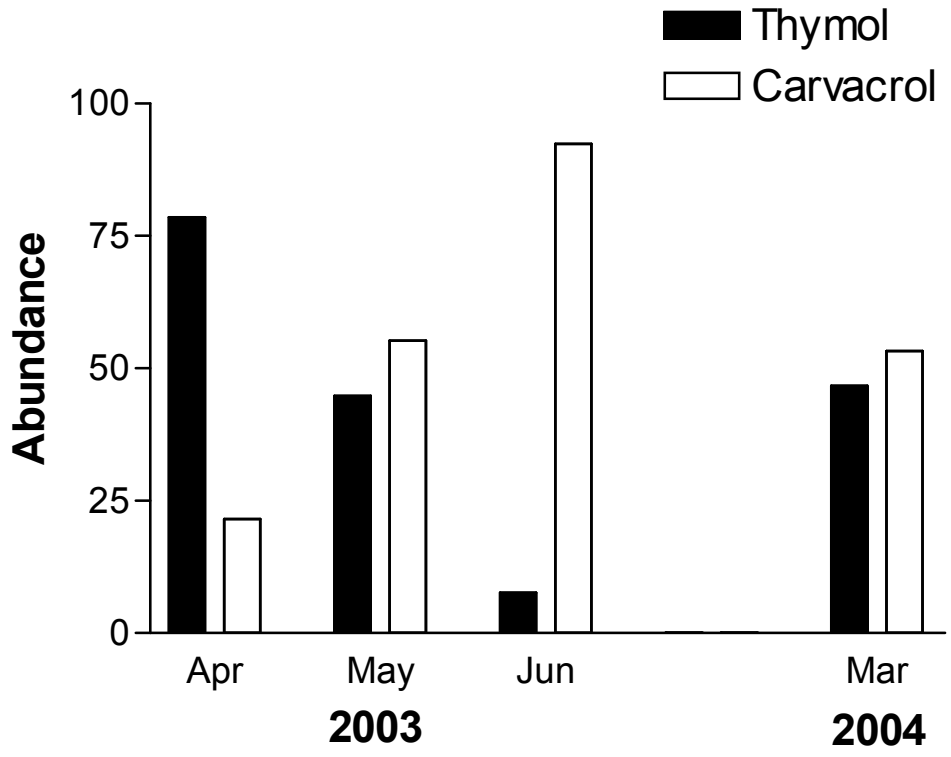

Fig. 6. Histograms of the isomeric distribution of thymol and carvacrol from wild sampe from Rameen (Tulkarim) at different harvesting time by HS-GCMS

the season that lasts from mid-April to mid-September. The composition of the essential oils obtained from ten populations of *Thymus caespititiu* were collected in seven different sites on the Island S. Jorge (Azores). The analyzed oils were dominated by their monoterpene fraction (69-91%). The sesquitepene fraction was rather small (4-17%) and consisted mainly of oxygenated compounds, that corresponded with their major components: α-terpineol (43-68%), carvacrol (32-52%), thymol (44-58%), sabinene (41%) [16]. Another work on the variability of essential oils of *Thymus caespititus* from Portugal was also published [17]. Samples were collected at seven different localities from northwest Portugal and fresh plant from the Azores gave an average essential oil yield of 1.1% and 0.2% (w/w) respectively, and more than seventy compounds of the total oil contents were identified. The main components in all samples from northwest Portugal were α-terpineol (30.6-40.5%), p-cymene (6.0-9.1%) and T-cadinol (6.2-8.7%), whereas the major components in the oil sample from the Azores were carvacrol (36.3%), thymol (16.1%). These chemical differences could be related in part to the different soil and climatic conditions that characterize both geographical areas. Twenty one volatiles were characterized on in Moroccan thyme: tricyclene, α-pinene, camphene, β-pinene, myrecene, α-terpinene, limonene, 1,2-cineol, γ-terpinene, p-

cymene, trans-4-thujanol, linalool, camphor, bornyl acetate, carvacrol methyl ether, 1-terpinen-4-ol, β-caryophylene, α-terpineol, borneol, thymol, and carvacrol [18].

As near to our region (Saint Kathrine zone, Sinai.), there was an investigation conducted on *Organum syriacum* [19]. This study showed that *O. syriacum* is a very rich source of carvacrol. The other phenolic isomer, thymol is only present in trace amounts. The other compounds present in rather appreciable amounts are the monoterpene hydrocarbons p-cymene (3.9%), γ-terpinene (3.8%), myrcene (1.4%) and α-thujene (1.2%). The oil does not contain sesquiterpene hydrocarbons except for a minute amount of β-caryophyllene and α-humulene.

Our investigation using HS-GC-MS analysis of wild *M. syriaca* showed that the leaves are rich in monoterpene hydrocarbons and phenolic compounds. The results are summarized in (Table 1). The major constituents identified throughout all the harvesting periods were varied greatly among the thymes examined and were as following: α-phellandrene (1.62-8.13%), α-pinene (1.22-4.61%), β-myrecene (0.5-11%), m-cymene (1.86-8.61%), p-cymene (8.44-48.6%), γ-terpinene (11.96-30.8%), thymol (0.26-11.6%), and carvacrol (0.65-21.7%). In all samples, the most abundant monoterpenes were γ-terpinene and p-cymene, the biogenatic precursors (via enzymic hydroxylation) of the phenolic terpenes thymol and carvacrol [15]. The percentages of γ-terpinene were increased during February, March and April while decreased in May. The percentages of p-cymene were increased during February, March and April while decreased in May.

Wild Palestinian thyme contains thymoquinone in samples that were harvested during January and February. Sample harvested from Jenin in January 2004, revealed 1.96%. In March, April, May and June months, samples have been showing traces of thymoquinone.

As for the phenolic compounds, we have noticed that the wild thyme is characterized by the dominant presence of carvacrol irrespective of harvesting time or location. Out of the large number of wild thyme samples that were analyzed, almost always the highest carvacrol percentages were found in the wild samples (Fig 2-6). This explains why wild thyme, which is rich in carvacrol has a distinct warm pungent taste, a distinctive property of carvacrol [7]. The consistency of the this observation suggests that this HS-GCMS method could be used as a fast indicator to distinguish wild from cultivated thyme which is considered as important quality parameter for consumers to make their purchasing decisions.

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