



CHARACTERIZATION AND IDENTIFICATION OF *Origanum Spp.* FROM LEBANON USING MORPHOLOGICAL DESCRIPTORS

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Abstract- *Origanum* species are growing wild in the Lebanese mountains. It can be found everywhere in rocky places from the North to the South and at different altitudes. In order to contribute to the characterization of economically important *Origanum* spp., seventy samples of oregano-like plants were collected from 37 different locations of Lebanon. To simplify the identification and characterization of the accession entries, a chart containing 40 descriptors has been adopted, modified and further developed. Application and analysis of these descriptors revealed two *Origanum* spp., namely, *O. syriacum* L., and *O. ehrenbergii* Boissier and other species from different genera, namely, *Thymus hirsutus* M. B., *Thymbra spicata* L., *Saturia hortensis* L. and *Saturia thymbra* L.

Key words- Characterization, identification, morphological descriptors, *O. syriacum* L., *O. ehrenbergii* Boissier, *Thymus hirsutus* M.B., *Thymbra spicata* L., *Saturia hortensis* L., *Saturia thymbra* L.

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Introduction

Many species belonging to several botanical families are used worldwide under denomination of "oregano" [1-2]. Oregano is the common name for a general aroma and flavour used as a spice. The majority of aromatic oregano belongs to Lamiaceae and Verbenaceae families, while the large distinction is made between European (*Origanum* spp.) and Mexican (*Lippia* spp.) oregano. They have two main commercial uses: as a fresh or dried herb added to food or beverages and to obtain different extracts, mainly essential oils which are employed in food, cosmetic, aromatherapy and pharmaceutical industries due to its carvacrol and thymol content [3].

The taxonomy of *Origanum* is rather complicated and currently under much debate [4]. *Origanum* sp. is characterised by a large (and still little investigated) morphological and chemical diversity resulting in 10 distinct sections consisting of 49 taxa and 42 spe-

cies. During the last 150 years more than 300 names were given but only 70 are presently recognised as *Origanum* species, subspecies and hybrids. Plenthora of different names reflects the extent of morphological variation this genus exhibits in nature. Majority of the taxa are located in the Mediterranean region with 9 species being distributed in Greece and 21 species in Turkey. Sixty per cent of all *Origanum* taxa are recorded to grow in Turkey making this country as the gene centre of *Origanum*. In addition, 17 hybrids between different species have been described some of which are known only from artificial crosses. Very complex in their taxonomy, *Origanum* biotypes vary in their essential oil content or essential oil composition. Oregano species are mainly characterized by the presence of two chemotypes, carvacrol and thymol. Another intermediate type would contain high content of two monoterpene hydrocarbons, *g*-terpinene or *p*-cymene, whereas some species were found with high values of linalool, phenols,

alcohols, ethers, aldehydes and ketones [5-8].

Bentham [9] recognised three genera: *Amaracus*, *Majorana* and *Origanum* but later [10] returned to Linnaean concept and described within *Origanum* genus four sections: *Amaracus*, *Majorana*, *Origanum* and *Anatolicon*. Briquet [11], on the other hand, have accepted the earlier three genera concept of Bentham [9] and described some more sections in the genus *Marjorana*. One genus and three genera concept of *Origanum* taxonomy was widely used until Letswaart [12].

According to the current status and classification of the genera within Labiatae family, there are 8 subfamilies: Ajugoideae, Chlorthoideae, Lamioideae, Nepetoideae, Pogostemonoideae, Scutellarioideae, Teucroideae and Viticoideae [13]. The Nepetoideae, the biggest subfamily is further divided into 4 tribes: Escholtziaceae, Lavanduleae, Mentheae and Ocimeae.

According to classification of Letswaart [12], *Amaracus*, *Majorana* and *Origanum* should be considered as one genus. Within the genus *Origanum* he recognised three groups, 10 sections, 38 species, eight subspecies and 17 hybrids. Since Letswaart's publication, five more species [14-17] and one hybrid [18] have been recognised. This classification was based on many morphological characters (e.g. length of stems, arrangement, number and length of branches, shape of leaves, length of petioles, etc).

Difficulties, controversies and confusions in *Origanum* taxonomy prompted many scientists all over the world to study the chemistry, oil compositions and its biological activities of *Origanum* species. At the time of Letswaart's revision chemical data was too sparse to be useful in the taxonomic classification. Although there are a large number of publications reporting chemistry of *Origanum* genus today, the present data on oil compositions of *Origanum* taxa are not sufficient enough to draw the conclusions for the use of essential oil constituents in the chemotaxonomy in the genus *Origanum*.

According to Post and Dinsmore [19], Letswaart [12] and Skoula and Harborne [20] in Lebanon there have been *Origanum* spp. recorded as follows:

1. Group A, Section *Anatolicon* Bentham: *O. libanoticum* Boiss,
2. Group B, Section *Marjorana* Bentham: *O. syriacum* L. (*O. maru* L.), *O. syriacum* L. var *bevanii* Letswaart,
3. Group C, Section *Origanum*: *O. vulgare* L., *O. vulgare* var *viride* (Willd.) Boiss, *O. vulgare* var *prismaticum* Bentham., *O. vulgare* var *laxiflorum* Post, *O. vulgare* var *longespdatum* Post; Section *Prolaticorolla*: *O. ehrenbergii* Boiss, *O. ehrenbergii* var *pariflorum* Bornm,
4. Hybrids: *O. X barbarae* Bornm (*O. ehrenbergii* Boiss. x *O. syriacum* L. var *bevanii* Letswaart), *O. X adonidis* Mouterde (*O. libanoticum* Boiss x *O. syriacum* L. var *bevanii* Letswaart).

Origanum spp. are growing wild in the Lebanese mountains. It can be found everywhere in rocky places from the North to the South and at different altitudes. Recently and due to its importance in the economic use, *Origanum syriacum* has become a cultivated crop to cover the need of the local market. However, large amounts of the consumed *Origanum* product are still imported. This plant with "Thymus - like" smell represents the fastest, easiest, and most common and popular breakfast ("za'atar") of the Lebanese people. It is a very important plant as far as its economic use is concerned. "Za'atar" is found in every shop, in every supermarket and in every home. Tons of plants that are being cultivated or collect-

ed from the wild are consumed every year. The economic potential of *Origanum syriacum* of becoming a new crop in Lebanon is feasible in three of four different economic scenarios: za'atar production, herbal tea, spice and essential oil production. In order to develop the diversity of cultivation areas together with the different methodologies and technologies of primary production, dehydration and oil extraction, it is imperative to generate a wide variety of products with different qualities of the raw material and essential oils content and compositions. To have such a contrasting quality tool, there is a need for work completion on botanical identity correlated with physical and chemical characterisation of essential oils from *Origanum spp.* grown in Lebanon.

For these reasons, the main objectives of the current work were carried out as follow: (1) collection of different samples of origano from different regions of Lebanon, (2) development and establishment of the characterization descriptors for the genus *Origanum*, (3) application of the descriptors to the collected oregano phenotypes and (4) identification of *Origanum* spp.

Experimental

Sample Collection

Different samples of economically important oregano plants (including soil) were collected from different regions, areas, sites and altitudes of Lebanon (Tab. 1) during March-May 2009 and 2011. However, *Origanum libanoticum* plants were not sampled in this survey. All collected samples were divided into two parts. One part was potted using the same soil brought from the site of sampling and cultured in the laboratory at the Department of Plant Protection, Faculty of Agricultural and Veterinary Sciences, Dekwaneh, Beirut. Second part was cultured in a field at Gazeer Research Station, Faculty of Agricultural and Veterinary Sciences.

Herbarium Collection

Upon establishment of pot culture in the laboratory, Herbarium specimens were taken from each plant sample and deposited at the Department of Crop Protection, Faculty of Agricultural and Veterinary Sciences, Lebanese University, Dekwaneh, Beirut for further reference.

Establishment of Characterization Descriptors for the Genus *Origanum* L.

To simplify the identification and characterisation of the accession entries (collected samples), a chart containing 40 descriptors has been adopted [3], elaborated and further developed to allow easy and quick individualisation of present and future accession entries added to the collection of *Origanum* spp. A numerical value was assigned to each status of each descriptor (Tab. 2). The descriptor value corresponding to each sample was then determined by observing and measuring 10 stems.

Taxonomical Determination

Taxonomical determination of the material was performed by observation and measurements of the vegetative and floral features and by using specific bibliography [12,19].

Results

Collection of Oregano

Seventy samples of oregano plants were collected over the period

of two years (2009-2011) from 37 different areas/locations of Lebanon. Designated sample numbers and locations of sampling are presented in Table 1.

Table 1- The region, location, altitude and sample number of collected oregano plants

Region	Place	Altitude, m.s.l.	Sample No
South Lebanon	Kfarhamam	800	2 a, b, c, d
	Kfarhattah	400	3 b, c, d, e
	Bazouriyeh	170	4 a, b, c
	Hasbaya	750	5 a, b, c
	Sarafand	50	6
	Cheik Taba Akkar	200	19, 19'
	El Khiam	695	22 a, b, c, 26
	Chehour	320	25
	Jezzine	950	27
	El Doueir	370	28
	Rouaisset Neaman	200	33
	Marjeyoun	750	35 a,b,c
	Chekka	20	11 a, b
	Koura	25	11 c
North Lebanon	Bcherri	1400	13
	Nafiseh Akkar	150	14, 14'
	Hadoun Batroun	1030	15, 15'
	Rahbeh Akkar	750	16' a, b, c
	Ouainat Akkar	350	17 a, b, cultivated
	Mechref	280	1 a, b, c
	Kfarthebian Keserouan	1250	7, 7'
	Blat Mestita Jbeil	200	8
	Mouhrine Jbeil	15	9, 9'
	Bkirki Keserouan	240	10
Mount Lebanon	Halat Nahr Ibrahim Jbeil	50	12 a, b, 12' a, b
	Bsaba Baabda	330	18
	Bhannes Dahr El Souan	750	20
	Kfour Kisrouan	800	21 a, b, c
	Chaouie Al Maten	530	23, 23'
	Bsaba (Baabda)	330	24 a, b, 24' a, b
	Barouk	1110	34
	Wardaneh Iklim	100	30
	Brital	1200	31
	Nabbi Cheit	1289	32
Bekaa	Kherbet Kanafar (plain)	1090	36
	Kherbet Kanafar	1100	37

' – *Thymus* – like

Herbarium Collection

Most of the samples collected from the wild have successfully established as the pot cultures in the laboratory and in the field of Gazeer Research Station.

Establishment of Characterization Descriptors for the Genus *Origanum* L.

Oregano samples were studied and characterized by using developed chart of morphological descriptors specifically designed for the *Origanum* spp. The chart comprises of 40 vegetative and floral descriptors (Tab. 2). These descriptors significantly substantiated speed of sample processing and proved to be important and useful for typifying an oregano sample. The values observed and measured are presented in Table 2. Some oregano samples resembled each other; some differed greatly from one another. When analysed, it was obvious that collection of *Origanum* plants was falling into 9 separate groups (Tab. 2).

Table 2- Morphological descriptors for Lebanese *Origanum* spp.

Vegetative parts/Groups	I	II	III	IV	V	VI	VII	VIII	IX
(1) Habitat of growth									
1. Erect		X							X
2. Ascending		X	X					X	
3. Decubent	X				X	X	X		
4. Prostrate									
5. Other (specify)									
(2) Plant height (cm)									
1. Low, less than 20 cm	X								
2. Medium, 20-50 cm			X	X		X	X	X	
3. High, 50-80 cm		X	X			X			
4. Very high, more than 80 cm									
(3) Branching									
1. Lowly branched	X	X	X	X	X	X	X	X	X
2. Highly branched		X							
3. Other (specify)									Thick
(4) Stem colour									
1. Dark green									
2. Pale green				X		X		X	X
3. Pale grey									
4. Grayish brown		X	X				X		
5. Greyish purple	X				X				
6. Dark purple						X			
7. Other (specify)									
(5) Trichomes in stems									
1. Antrose									
2. Retrose	X	X	X	X	X	X		X	
(6) Leaves colour									
1. Pale green									
2. Yellowish green			X					X	
3. Dark green		X		X		X	X		X
4. Blue green									
5. Greenish green	X				X				
6. Pale purple									
7. Variegated									
8. Other (specify)									
(7) Leaves colour (upper & lower surfaces)									
1. Concolourous	X	X	X	X	X	X	X	X	X
2. Discolourous									
(8) Leaf shape									
1. Oblong					X				
2. Oval									
3. Ovate	X	X	X	X	X	X		X	
4. Rounded							X		
5. Parabolic									
6. Cordate									
7. Lanceolate									
8. Other (specify)									Linear
(9) Leaf apex									
1. Acute						X		X	
2. Obtuse	X	X	X	X	X				X
3. Truncate							X		
4. Other (specify)									
(10) Leaf base									
1. Cordate									
2. Subcordate						X		X	
3. Acute									X
4. Rounded	X	X	X	X	X		X		
5. Other (specify)									
(11) Leaf margins									
1. Crenate					X				
2. Serrate						X	X	X	
3. Entire-smooth			X	X					X
4. Ondulate									
5. Different in upper third	X	X							
6. Other (specify)									Revolute, ciliate

Table 2 Continue

Vegetative parts/Groups	I	II	III	IV	V	VI	VII	VIII	IX
(12) Leaf venation (midrib)									
1. Prominent	X	X	X	X			X	X	X
2. At the same level					X				
3. Shortly prominent						X			
4. Other (specify)									
(13) Leaf upper surface									
1. Rugged	X	X	X	X	X		X	X	
2. Reticulate									
3. Smooth						X			X
4. Other (specify)									
(14) Leaf length									
1. Small, less than 1 cm			X						X
2. Medium, 1-1.5 cm	X			X	X	X	X		
3. Big, more than 1.5 cm		X						X	X
(15) Leaf width									
1. Narrow, less than 1 cm	X		X		X				X
2. Medium, 1-1.5 cm				X		X	X		
3. Broad, more than 1.5 cm		X						X	
(16) Leaf surface									
1. Glabrous						X			X
2. Pilose									
3. Pubescent									
4. Hirsute									
5. Incanous									
6. Tomentose	X	X	X	X	X		X	X	
7. Glandular	X	X	X	X	X	X	X	X	X
(17) Trichomes in leaves									
1. Single unicellular	X		X						
2. Single pluricellular									
3. Stellate									
4. Glandular	X	X	X	X	X	X	X	X	X
5. Capitate									
(18) Leaf petiole length									
1. Short, less than 0.5 cm	X	X	X	X	X		X		
2. Medium, 0.5-1.5 cm						X		X	
3. Long, more than 1.5 cm									
4. Sessile									X
(19) Branches axil									
1. Acute	X	X	X		X	X	X	X	X
2. Abtuse									
3. Straight				X					
4. Unspecified									
FLOWERING PARTS									
(20) Inflorescence location									
1. Terminal			X						X
2. Axillary									
3. Terminal and axillary	X	X		X	X	X	X	X	
(21) Inflorescence type									
1. Spike	X	X	X	X	X	X	X	X	
2. Glomerules									
3. Cyme carimbose									
4. Cyme paniculate	X	X	X	X	X			X	
5. Corymb									
6. Thyrsus						X			
7. Other (specify)									Head or spikes
(22) Flower number									
1. Few									
2. Many	X	X	X	X	X	X	X	X	X
(23) Flowers colour									
1. White	X	X	X	X	X	X	X	X	
2. Pink									X
3. Pale purple									
4. Purple									
5. Pale blue									
6. Other (specify)									

Table 2 Continue

Vegetative parts/Groups	I	II	III	IV	V	VI	VII	VIII	IX
(24) Flower corolla shape									
1. Tubular						X			
2. Bilabiate	X	X	X	X	X	X	X	X	X
3. Filiform									
4. Other (specify)									
(25) Corolla tube									
1. Short, less than 3 mm									X
2. Medium, from 3 to 5 mm	X	X	X	X	X			X	X
3. Long, more than 5 mm							7.5		X
(26) Corolla, bilabiate upper lip									
1. Short, less than 1 mm	X	X	X	X	X			X	
2. Medium, from 1 to 2 mm									X
3. Long more than 2 mm									X
(27) Corolla, bilabiate lower lip									
1. Short, less than 2 mm	X	X	X	X	X		X	X	
2. Medium, from 2 to 3 mm									X
3. Long more than 3 mm									X
(28) Calyx									
1. Regular, 5 dentate, actinomorphic								X	
2. Irregular, 5 dentate, bilabiate, zygomorphic	X	X	X	X	X			X	X
3. Irregular, unilipped									X
4. Reduced to a single sepal	X	X	X	X	X			X	X
(29) Calyx shape									
1. Tubular								X	
2. Campanulate									
3. Bilabiate									X
4. Bracteoid	X	X	X	X	X			X	X
5. Infundibuliform									
6. Other (specify)									
(30) Calyx length									
1. Short, less than 2 mm	X	X	X	X	X				
2. Medium, from 2 to 4 mm								X	X
3. Long, from 4 to 6 mm								X	X
(31) Calyx surface									
1. Glabrous									
2. Hirsute									X
3. Glandular dotted	X								
4. Sparce									
5. Dense									
6. Other (specify)									Reddish
(32) Floral bracts-shape									
1. Lanceolate			X						
2. Ovate	X	X	X					X	X
3. Obovate							X	X	
4. Cordate									
5. Obcordate									X
6. Spatulate									
7. Other (specify)									Subulate
(33) Floral bracts-size									
1. Short, less than 1 mm									
2. Medium, from 1 to 4 mm	X	X	X	X	X			X	X
3. Big, greater than 4 mm									
(34) Floral bracts-color									
1. Green	X	X	X	X	X		X	X	X
2. Brown									
3. Purple									
4. Leaden									
5. Green with purple edges									
6. Other (specify)									
(35) Floral bracts-surface									
1. Glabrous								X	
2. Little pubescent									
3. Pubescent	lower	X			lower			X	
4. Canescent	lower	X		lower			X		
5. Other (specify)									Ciliate or glabrous

Table 2 Continue

Vegetative parts/Groups	I	II	III	IV	V	VI	VII	VIII	IX
(36) Stamina number									
1. None									
2. Two									
4. Four	X		X	X	X	X	X	X	X
(37) Stamina-length									
1. All equal									
2. Didynamous	X		X	X	X	X	X	X	X
(38) Stamina-anther									
1. White	Purple fringes	X							
2. Yellow						X			
3. Purple				X	X				X
4. Brown			X				X	X	
5. Other									
(39) Gynoecium-length									
1. Short, less than 5 mm								X	
2. Medium, from 5 to 7 mm			X	X	X				
3. Long, from 7 to 9 mm	>9					X	X		X
(40) Gynoeciem-ovules developed									
1. 1									
2. 2									
3. 3									
4. 4	X		X	X	X	X	X	X	X

Each group was described in details and the most noticeable features were drawn for further records. First eight groups of plants differed from one another in 27 out of the 40 descriptors analysed (Tab.2). For example, group I consisting of 4 oregano plants were characterised as plants with decumbent plant growth (lying or reclining on the ground, but erect at the terminal). Plant height was medium (20-50 cm) and lowly branched. Colours of the stems were greyish purple. Trichomes were retrose (directed or turned backwards or downwards). Leaves described as greyish-green, concolourous (the same colour on the two surfaces), ovate. Leaf apexes were obtuse, whereas bases were rounded. Leaf margins were different in the upper third. Leaf venation was prominent. Leaf upper surface was rugose (wrinkled). In length leaves were medium 1.2 cm long and narrow (0.9 cm). Leaf surface were tomentose (closely covered with down or malted hairs). Leaf trichomes were observed as unicellular or brown glandular. Leaf had short (0.2 cm) petioles. Branches axis showed acute disposition. Inflorescences were located terminally and axillary spikes were located terminally with many white flowers. Bracts were sessile, 8 veined, glandular dotted with glabrous upper and woolly lower surfaces. Calyx resembled bracts in shape and measured 2x2 mm. White corolla consisted of small flowers measuring 7 mm. Filaments of upper stamens measured 4 mm, whereas lower filaments were 5 mm long. 2-celled anthers appeared white with purplish fringes. Group VIII, on the other hand, included 15 samples of oregano plants and was described as group of plants with erect and stiff growth. Colours of the stems were characterised as pale green. Leaves colour was yellowish-green. Leaf apexes were acute, whereas bases were subcordate with serrate leaf margins and prominent venation. Leaf upper surface was rugged (rocky uneven surface). In length and in breadth leaves were reaching more than 4 cm and 2.5 cm. Leaf petioles were medium. Spikes with many white flowers measured 0.6 cm long and 0.4 cm wide. Bracts measured up to 3.5 mm with obcordate apices. Size of calyx and corolla were similar to the flower size of flowers in group 1. However, upper filaments were much shorter reaching only 0.5

mm and lower filament reaching 1.5 mm. Anthers were 2-celled and brown. Group IX was considerably different from the other eight groups.

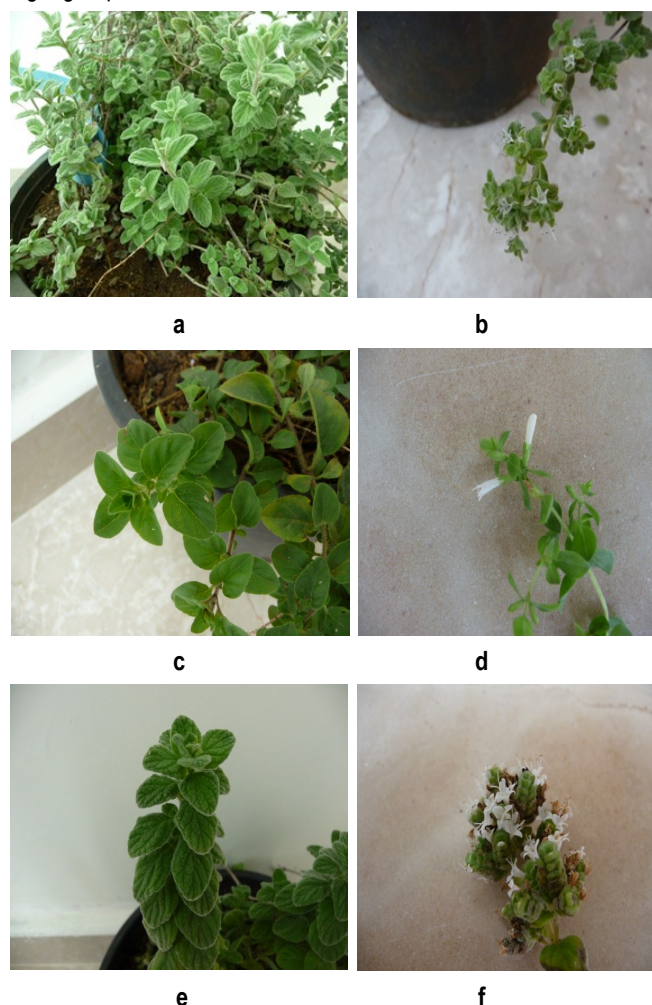


Fig. 1- Samples of oregano plants falling into: a (stems and leaves) and b (inflorescence and flowers); Group VI, c (stems and leaves) and d (inflorescence and flowers); Group VIII, e (stems and leaves) and f (inflorescence and flowers)

Taxonomical Determination

Application of bibliography descriptions [12,19] revealed that groups I, II, III, IV, V, VII and VIII were belonging to Group B, Section *Marjorana* Bentham and identified as *O. syriacum* L. Groups VI was recognised as Group C, Section *Prolaticorolla* and identified as *Origanum ehrenbergii* var *parviflorum* Bornm. Samples fallen into group IX were identified as *Thymus hirsutus* M. B., *Thymbra spicata* L., *Saturia hortensis* L. and *Saturia thymbra* L.

Discussion

The genus *Origanum* is very rich in spices. Taking into account the high degree of diversity and plasticity existing within the genus and the difficulty to differentiate them from each other, some authors have proposed morphological descriptors [21] to discriminate among clones and varieties [3]. For this reason we adopted a list of descriptors (including vegetative and flowering parts) pro-

posed by Farias and colleagues [3], have further modified and finally applied them as a suitable method for quick and easy differentiation. On application of this list to the analysis of 70 samples in present study, 27 differential descriptors were achieved and used in the identification of species.

Although oregano samples in our study were separated into nine different groups, 7 of them were identified as *O. syriacum* L. This discrepancy in morphology is probably due to the usually polymorphic characteristics of *Origanum* spp. Biochemical studies on these groups have also revealed differences not only in esterase and peroxidase activities but also in their electrophoretic analysis [22]. Their phenotype diversity could also be explained by seasonal and altitudinal effects that would alter their morphological, structural and physiological characteristics [23]. Oregano plants grown at high altitude (1760 m) (*O. vulgare* ssp. *vulgare*) were found to be shorter than those grown at low altitude (200 m) (*O. vulgare* ssp. *hirtum*). This plant shortening at high altitude is proposed to associate with the short duration of the growing period and also with reduced temperatures, as well as nutrient and water limitations [24-25]. The reduced height of upland plants further reflects an adaptive strategy to avoid the damaging mechanical effect of strong winds at high elevation. In addition, a prominent observation on oregano plants is that fully expanded leaves are larger and thicker at mid- and high altitudes [23]. In this regard, one could also think of the seasonally dimorphic plants, in which winter leaves are significantly larger than summer leaves [26]. Indeed, majority of oregano plants of group VIII in our studies which were characterized by large and thick leaves were also collected from elevated area (800-1150 m.s.l.). Groups I, II, III, IV, V, VII and VIII should also be analyzed for varietal discrimination.

In conclusion, although 4 different spp., 7 different varieties and 2 hybrids of *Origanum* genus was previously recorded in Lebanon [19, 12, 20], our survey has identified only two different species, namely *O. syriacum* L. and *O. ehrenbergii* var *parviflorum* Bornm. Collected samples also consisted of *Thymus*-like genera, namely *Thymus hirsutus* M. B., *Thymbra spicata* L., *Saturia hortensis* L. and *Saturia thymbra* L.

Why fewer spp. were reported by our survey could be explained by reduction of biodiversity of native plants that is currently taking place in Lebanon [27]. Unregulated harvesting of wild *Origanum* populations leads to over-harvesting, reduction of biodiversity and destruction of ecosystem unnoticeably. These losses could lead to the extinction of the species. Therefore, conservation, documentation and domestication of wild oregano are the urgent actions needed to halt and reverse the process.

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