



PALYNOLOGICAL STUDY IN SOME MEDICINAL SPECIES OF *LAMIACEAE* (LABIATAE) FAMILY IN IRAQ

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Abstract

In this study the pollen morphology of 9 species belonging to 8 genera of *Lamiaceae* family in Iraq: *Lycopus europaeus* L., *Marrubium vulgare* L., *Melissa officinalis* L., *Mentha longifolia* (L.) Huds., *Ocimum basillicium* L., *Stachys lavandualifolia*, *Stachys sylvatica* L., *Thymus kotschyana* and *Teucrium polium*. have been investigated by aid of light microscopy (LM). In order to perform the pollen micro-morphology of *Lamiaceae* genera, and to find its significance in taxonomy of the group, qualitative and quantitative variables related to the shape, size, ornamentations and colpate were studied. The shape is mostly Prolate - spheroidal, Oblate-spheroidal, Subprolate and Oblate, apertures are mostly tricolpate and hexacolpate. Significant differences in grain size also found in all species, the small grains in *Lycopus europaeus*, whereas the small-medium grains are *Mentha longifolia*, *Stachys lavandualifolia*, *Stachys sylvatica* and *Thymus kotschyana* species, medium grains in *Marrubium vulgare*, *Melissa officinalis* and *Teucrium polium* and Medium-Large grains in *Ocimum basillicium*. Ornamentation varies from Verrucate, Reticulate and Perforate.

Keywords: *Lamiaceae*, Palynological, Mint family, Iraq

Introduction

The *Lamiaceae* (Labiatae) or Mint family is a large, cosmopolite family of 240 genera and about 7000 species of herbs, shrubs and trees, the family *Lamiaceae* is important due to medicinal as well as ornamental properties) Harley, 2003, Bazarragchaa *et al.* 2012, Azzazy, 2016), Mint family is represented by 32 genera and about 140 species in Iraq (Al-Musawi, 1987).

Lamiaceae family was divided into 7 subfamilies *Ajugoideae*, *Scutellarioideae*, *Nepetoideae*, *Viticoideae*, *Symphorematoidea*, *Postantheroidea* and *Lamioideae* (Wagstaff *et al.* 1998; Raymond *et al.* 2004; Walker and Sytsma, 2007; Bramley *et al.* 2009; Zhong *et al.* 2010, Ryding 2010 and Yuan *et al.* 2010), Genus *Ocimum*, *Thymus*, *Lycopus*, *Melissa* and *Mentha* belongs to the subfamily *Nepetoideae*, Genus *Teucrium* belongs to the subfamily *Ajugoideae*, Genus *Stachy* and *Marrubium* belongs to the subfamily *Lamioideae*

Pollen morphology of the family *Lamiaceae* has been examined by a number of workers such as Fritzsche *et al.* (1832); Erdtman (1945); Asu-Asab and Cantino

(1992); Pozhidaev (1992); Moon and Hong (2003); Akgul *et al.* (2008); Celenk *et al.* (2008); Bazarragchaa1 *et al.* (2012); Salmaki *et al.* (2012); Azzazy, (2016); Marzouk *et al.* (2017). Either in Iraq it has studied the pollen of some genus such as *Sideritis* L. (Al-Musawi *et al.*, 2009), *Lycopus* L. (AL-Saadi and AL-Mayah, 2012) and *Satureja* L. (Nasrullah, 2016).

In this study, to aim investigated for palynological information of 9 medicinal species belonging to 8 genera of Labiatae in Iraq which would be helpful to establish classification and phylogenetic relationship with Labiatae.

Materials and Methods

In the present study, nine taxa of the *Lamiaceae* have been investigated. The study was based on fresh samples collected during field trips between the years (2017-2018). We also depended on the Herbarium of Misan University (MISUH), Herbarium of Basra University Collage of Science (BSRA). the list of specimens is shown in (Table 1).

The pollen grains were prepared for Light Microscope, using acetolysis method of (Erdtman, 1952)

and the pollen grains were mounted in glycerin-jelly mixture and the general exomorphological features of the pollen were examined using Olympus microscope model SMZ 600 using image analysis software Dc-2. The measurements are based on 15-20 readings from each specimen. Pollen diameter, polar axis (P) and equatorial diameter (E), aperture size, colpate diameter, number colpate and exine thickness were measured (Tables 2).

Results

Quantitative and qualitative characters are presented (Tables 2) and (terminology according to Erdtman 1952 and Punt *et al.* 1994).

Quantitative Characters

Pollen grain size

Size of pollen grain was found varying from 20 to 56 μm . In it *Lycopus europaeus* was found small, in *Mentha longifolia*, *Stachys lavandulifolia*, *Stachys sylvatica* and *Thymus kotschyau* were small-medium, in *Marrubium vulgare*, *Melissa officinalis* and *Teucrium polium* were medium, whereas in *Ocimum basillicium* it was medium-large in size (table 2).

Number and size of apertures

The apertures of pollen grains of *Lamiaceae* were observed as tricolpate and hexacolpate, Pollen grains in

Lycopus europaeus, *Melissa officinalis*, *Mentha longifolia*, *Ocimum basillicium* and *Thymus kotschyau* are hexacolpate, whereas, others species are tricolpate (Table 2) (fig. 1).

The maximum length for colp was (30.75) μm found in *Mentha longifolia*, and the minimum length was (13.55) μm in *Stachys lavandulifolia*. While the maximum width for colp was (3.88) μm in *Thymus kotschyau*, and the minimum width was (1.66) μm in *Marrubium vulgare*.

Wall thickness

The results showed that exine is thicker than entine. the minimum thickness in exine was found in *Ocimum basillicium* (3.01) μm , and the maximum found in *Stachys sylvatica* (1.00) μm . While the minimum thickness for entine is in *Ocimum basillicium* (0.99) μm and the maximum is in *Lycopus europaeus* (0.33) μm (table 2) (fig 1)

Qualitative Characters

Shape

Our results show that pollen grains in the species under study were variable in shape; they were prolate - spheroidal in *Lycopus europaeus* and *Stachys sylvatica*, oblate-spheroidal in *Marrubium vulgare*, *Mentha longifolia* and *Stachys lavandulifolia*.

Table 1: The list of the species used for pollen micro-morphological study and their localities

N.	Taxa	Localities	Herbarium No.
1	<i>Lycopus europaeus</i> L.	MSU : Qaliansan, 2/8/2007 Basra	0160 0155
2	<i>Marrubium vulgare</i> L.	MSU : Sulaimaniya- Khormana towards Dola Bamo, Qaragagh MRO: Rania, 26/5/1990	0188 0183
3	<i>Melissa officinalis</i> L.	Baqula, on road side, 10/6/1969 Between chuarta, 5/8/2009	36999
4	<i>Mentha longifolia</i> (L.) Huds.	Qandeal read to mergaon 4/8/2007 MSU: Pengawen, Dokan MRO : Haji Umran MAM: Amadiya Amara 23/8/2018	0130 0139 0144 0132 0100
5	<i>Ocimum basillicium</i> L.	Misan, Almajar alkaber, 23/8/2018 Basra, 25/9/2018	0115 0120
6	<i>Stachys lavandulifolia</i>	MSU : Azmar Mountain MRO : Rania	0177 0170
7	<i>Stachys sylvatica</i> L.	MRO : Rania	102
8	<i>Thymus kotschyau</i>	Darband 2007 MSU: 3 km N. Dokan MAM: Amadiya	0122 0120 01288
9	<i>Teucrium polium</i> L.	Jabal keara, 19/7/1988 Aqra, 13/5/1988 MAM: Amadiya Snam M. 22/6/2017	46, 47 33 0133 0132

Table 2: General pollen characters of species *Lamiaceae* (Labiatae) family in Iraq (values in μm).

N	Taxa	Pollen shape	Aperture	Polar axis	Equatorial diameter	P/E	Size of pollen	Aperture dimensions		Wall thickness		Ornamentation
								Length	width	Exine thickness	Intine thickness	
1	<i>Lycopus europaeus</i>	Prolate spheroidal	6 colpate	(22-23.5) 22.83	(21.5-22) 21.83	1.04	Small	(15-18) 16.83	(2-2.5) 2.2	(1-1.3) 1.05	(0.2-0.4) 0.33	Perforate
2	<i>Marrubium vulgare</i>	Oblate spheroidal	3 colpate	(27-31.5) 30.11	(29-36) 33.25	0.9	Medium	(15-17.5) 16.37	(1.5-2) 1.66	(1-2) 1.06	(0.45-0.5) 0.48	Perforate
3	<i>Melissa officinalis</i>	Subprolate	6 colpate	(39-49) 45.75	(32-35) 34.22	1.33	Medium	(29-31) 29.75	(1.9-2.5) 2.13	(2-2.5) 2.11	(0.5-0.6) 0.51	Reticulate
4	<i>Mentha longifolia</i>	Oblate spheroidal	6 colpate	(24-30) 29.62	(25-32) 31.07	0.95	Small-Medium	(30-32) 30.75	(2-2.5) 2.02	(1-1.2) 1.1	(0.4-0.8) 0.56	Reticulate
5	<i>Ocimum basillicium</i>	oblate	6 colpate	(35-37) 35.9	(54-56) 54.75	0.66	Medium-Large	(27-33) 31.2	(2.5-4.5) 3.7	(2.9-3.25) 3.01	(0.85-1.2) 0.99	Reticulate
6	<i>Stachys lavandulifolia</i>	Oblate spheroidal	3 colpate 23.01	(22-23.75) (25-29)	27.75	0.83	Small-Medium	(11.5-16.5) 13.55	(2.5-4) 3.11	(1-1.2) 1.03	(0.5-0.75) 0.51	Reticulate
7	<i>Stachys sylvatica</i>	Prolate spheroidal	3 colpate	(20.1-26) 23.75	(17-24) 22.22	1.10	Small-Medium	(10.5-17.5) 14.75	(2.5-4.2) 3.22	(1-1.1) 1.00	(0.49-0.75) 0.55	Reticulate
8	<i>Thymus kotschyana</i>	Oblate	6 colpate	(22-26) 24.33	(32-35) 33.66	0.75	Small-Medium	(17.5-19) 18	(3-4.5) 3.88	(1.5-3) 2.01	(0.6-1.70) 0.90	Reticulate
9	<i>Teucrium polium</i>	subprolate	3 colpate	(32.11-37) 35.22	(26-33.5) 27.75	1.26	Medium	(19-23.75) 22.5	(3-4.98) 3.45	(1-1.1) 1.02	(0.55-0.75) 0.60	Verrucate

The values between arches represent the mean and the values out the arches represent the minimum and maximum values.

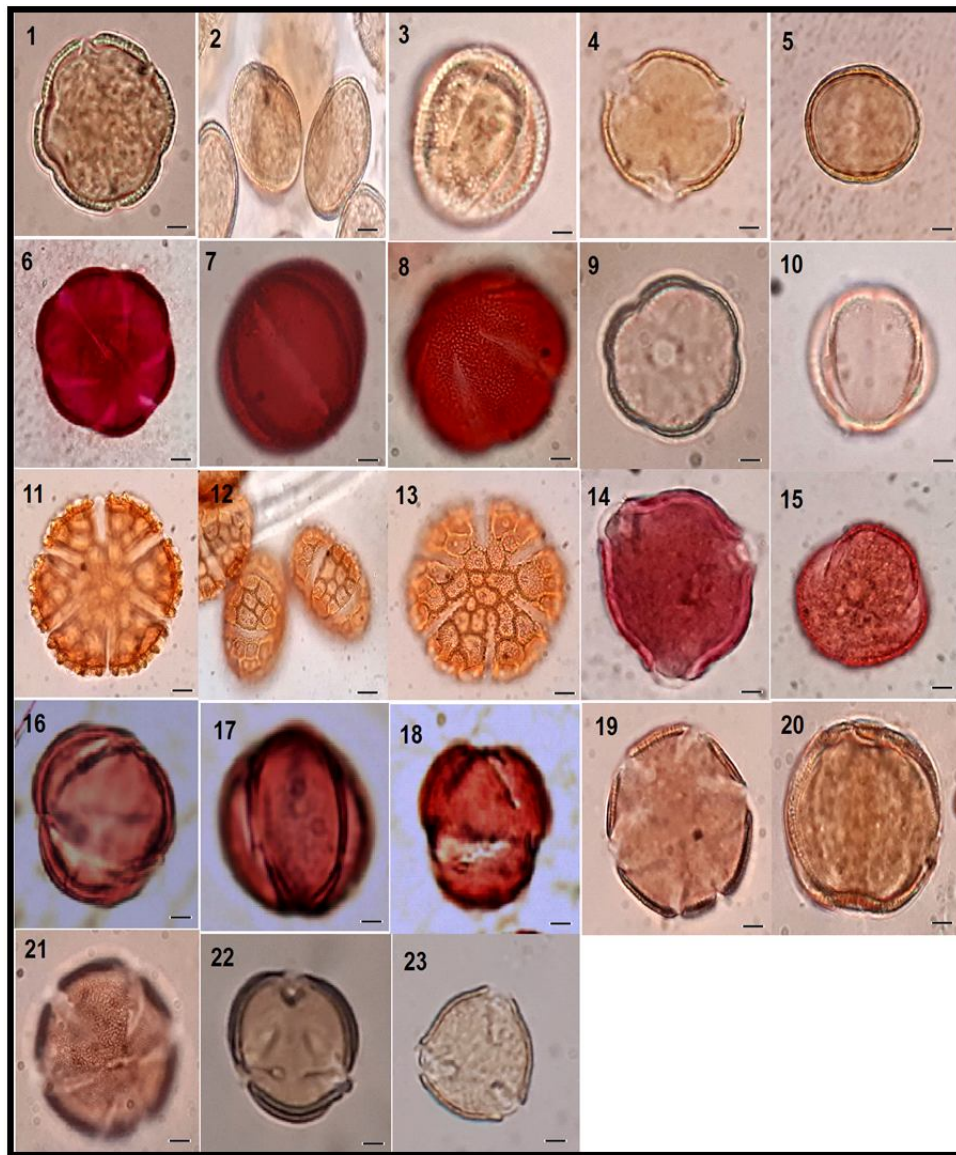


Fig. 1: LM photos of pollen grains of Species: 1-3: *Lycopus europaeus* L.; 4-5: *Marrubium vulgare* L.; 6-8: *Melissa officinalis* L., 9-10: *Mentha longifolia* (L.) Huds.; 11-13: *Ocimum basillicium* L.; 14-15: *Stachys lavandualifolia*, 16-18: *Stachys sylvatica* L.; 19-21: *Thymus kotschyanu*; 22-23: *Teucrium polium* L.(Scales =10 μm)

subprolate in *Melissa officinalis* and *Teucrium polium*, oblate in *Thymus kotschyanu* (table 2) (fig. 1)

Ornamentations

In this study we noted that the ornamentation on the external surface of pollen was; verrucate in *Teucrium polium*, perforate in *Lycopus europaeus* and *Marrubium vulgare*, and reticulate In other species (table 2) (fig. 1).

Key to the pollen Species

- | | |
|--|---|
| 1- 3-coplate apertures.....2 | 2- Perforate or Reticulate ornamentation3 |
| 1- 6- coplate apertures.....5 | 3- Prolate spheroidal shape <i>Stachys sylvatica</i> |
| 2- Verrucate ornamentation..... <i>Teucrium polium</i> | 3- Oblate spheroidal shape4 |
| | 4-Polar axis < 25 μm..... <i>Stachys lavandualifolia</i> |
| | 4-Polar axis > 25 μm..... <i>Marrubium vulgare</i> |
| | 5- Perforate ornamentation <i>Lycopus europaeus</i> |
| | 5- Reticulate ornamentation6 |
| | 6- Subprolate shape <i>Melissa officinalis</i> |
| | 6- Oblate spheroidal or Oblate shape7 |
| | 7- Medium-Large size of pollen <i>Ocimum basillicium</i> |
| | 7-Small-medium size of pollen8 |

- 8- Length Aperture dimensions < 20 μm
*Thymus kotschyanu*
- 8- Length Aperture dimensions > 20 μm
 *Mentha longifolia*

Discussion

The study of the pollen of the species belong to the *Lamiaceae* (Labiatae) or Mint family in Iraq showed that it is monads type, which is characterized by several forms; Oblate, Subprolate, Oblate-spheroidal and Prolate – spheroidal. The pollen of studied species divided into two groups; 3- colpate apertures and 6-colpate apertures. This was a follow up to Erdtman (1952) when he studied 100 species of 55 genus where the forms of Oblate-prolate, divided into two groups depending on the number of colpates are :

- 1- Pollen grains 2-nucleate usually 3-colpate, sometime 4- colpate in subfamily : Ajugoideae (except Rosmarineae), Prostantheroidea, Prasioideae, Scutellarioideae, and stachyoidea (marrubieae, perillomieae) stachyeae (melittinae, Brunellinae-*Brazoria* only), Pogostemoneae (*Pogostemon* only)
- 2- Pollen grains 6-nucleate usually 3-colpate in subfamily: Ajugoideae (Rosmarineae), Lavanduloideae, stachyoidea (Nepeteae), stachyeae Brunellinae (Brunellinae except *Brazoria*), Salviaeae, Meriandreae, Monnardeae, Hormineae, Glechoneae, Saturejeae, Pogostemoneae (except *Pogostemon*), Ocimoideae and Catopherioideae

The study findings concord with Bazarragch (2012) in his study for the shape of pollen grains in 16 species of Labiatae family in Mongolia and (Azzazy 2016) in Egypt.

Regarding the *Lycopus europaeus* where our results shown Prolate -spheroidal shape, 6-coplate apertures and small size, While this finding study disagreed with Moon and hong (2003) who mentioned that a species of *Lycopus* oblate-prolate, medium size and often small, and 6-coplate apertures in Korea, AL-Saadi and AL-Mayah (2012) also mentioned that pollen morphology of *L.europaeus* in Southern Marshes of Iraq oblate-spheroidal. ornamentation recorded of the Perforate types, While our study disagreed with (Moon and hong, 2003) and (AL-Saadi and AL-Mayah, 2012) Who mentioned that Reticular ornamentation in *Lycopus europaeus*.

Results showed that the pollen grains of *Marrubium vulgare* are Oblate–spheroidal, 3-coplate apertures and Perforate ornamentation, this agreed with Akgul *et al.* (2008) Which studied 19 species of *Marrubium* genus, it was pollen grains Oblate-spheroidal and Prolate-spheroidal, Tetracoplate-tricoplate apertures in

M.latescens and *M.calriifolium* and Syncoplate-tricoplate apertures in *M. cordatum*, ornamentation it were Reticulate, Granuale-perforate and Psilate-perforate.

The pollen grains of *Mentha longifolia* was Oblate-spheroidal, 6-coplate apertures, small-medium sizes and Reticulate Ornamentation, this agreed with Celenk *et al.* (2008) Which studied 10 species of family in Turkey where it was pollen grains Oblate-spheroidal in *M. longifolia* and subspecies to which they belong, Spheroidal, Prolate-spheroidal and suboblate in other species, either Ornamentation are Reticulate and Bireticulate. also this agreed with Azzazy (2016) Which studied 6 species of labiatae family.

Regarding *Stachys* genus, the shape of pollen grains was Oblate-spheroidal in *S.lavandualifolia* and Prolate-spheroidal in *S. sylvatica* and 3-Coplate apertures, Reticulate Ornamentation in both species, While the records of Salmaki (2008) where Subprolate in *S.sylvatica* and Oblate–spheroidal in *S.lavandualifolia*, The Ornamentation were microreticulate in those species. Salmaki *et al.* (2012) also mentioned that most species prolate-spheroidal and few of them are shaped subprolate, spheroidal and Oblate-spheroidal, 3-Coplate apertures except *S. iberica* and *S.atherocalyx* were they were Tetracoplate, and Ornamentation were Reticular, Microreticulate, perforate, foveolate-psilate and Foveolate in the studied species.

Concerning the genus *Thymus*, It was oblate shaped pollen grains, 6-colpate apertures, small-medium size and Reticulate Ornamentation, This fact agreed with Azzazy (2016), While Bazarragch (2012) recorded Prolate pollen grains, small size and Microreticulate ornamentation in *Thymus gobicus* species and Subprolate pollen grains and Bireticulate ornamentation in *Thymus palustris*.

However, the pollen grains of *Teucrium polium* where subprolate, 3-colpate apertures, medium size and verrucate ornamentation, this agreed with Marzouk *et al.* (2017) Who studied 11 species belonged to *Teucrium* in Libya.

Characters of pollen grains such as: shape, size, number and size of apertures, exine ornamentation are important and deciding factor for the systematic study of various genera under *Lamiaceae* family. We recommend further comparative studies on the other species belong to other genera of *Lamiaceae*.

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