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POLLEN MORPHOLOGY OF SOME *TRIFOLIUM* SPECIES WHICH ARE FAVORITE PLANTS OF HONEY BEES IN ISTANBUL

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SUMMARY

The genus *Trifolium* L. is one of the most important genera of the family *Fabaceae* and for the honey bees. This agriculturally valuable genus is represented by 100 species in Turkey of which and 54 occur in Istanbul. In this study, 6 districts of Istanbul have been chosen where honey is produced by beekeepers and the plant sources were identified and kept in ISTE (the Herbarium of the Faculty of Pharmacy of Istanbul University) herbarium. According to our observations, *Trifolium* species are the favorite plants for honey bees in Istanbul. The pollen morphology of 16 *Trifolium* taxa have been examined using Wodehouse (1935) method.

ÖZET

Trifolium cinsi hem Fabaceae familyası içinde, hem de bal arıları için en önemli cinslerden biridir. Ekonomik değeri olan bu cins, 54'ü İstanbul'da gözlenen, Türkiye'de 100 türle temsil edilir. Bu çalışmada İstanbul'un arıcılık yapılan 6 ilçesi seçilerek bitkisel kaynakları teşhis edildi ve örnekler ISTE (İstanbul Üniversitesi, Eczacılık Fakültesi Herbaryumu)'de koruma altına alındı. Gözlemlerimize göre İstanbul'da bal arısının en sevdiği bitkiler *Trifolium* türleridir.

Key words: *Trifolium,* honey bee, pollen, Istanbul, Turkey

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INTRODUCTION

The Fabaceae (Leguminosae) is the third largest family of flowering plants (727 genera and ca. 19,325 species (1). The clover genus, *Trifolium* L., is one of the largest genera in the family, with ca. 255 species (2, 3, 4). Clovers are widely grown as livestock forage and green manure crops, many native species are also utilized by grazing animals (5). At least 16 species of *Trifolium* are actively cultivated (2). The native distribution of *Trifolium* encompasses the temperate and, to a lesser extent, subtropical regions of the Northern and Southern Hemispheres. Native clovers are absent from Southeast Asia and Australia. The greatest species diversity is found in three geographic regions: the Mediterranean basin, western North America, the highlands of eastern Africa (1). *Trifolium* species occur in a wide range of habitats; including meadows and prairies, open woodlands, semi-deserts, mountains, and alpine peaks. A common feature of these diverse habitats is high solar radiation; few clover species tolerate shade. The genus *Trifolium* is represented by 100 species in Turkey of which 16 are endemics and it is grouped into 10 sections (6). It is represented by 71 taxa (54 species with 2 subspecies and 15 varietes) belonging to 9 sections in Istanbul.

The species of genus *Trifolium* are the favorite plants of *Apis mellifera*, because; 1) *Trifolium* species have long flowering time so they can use during four seasons by honey bees. Additionally they can germinate easily again and again in suitable condition during a year. 2) Their inflorescence have many flowers, so when honey bees visited an individual, they could reach many flowers and many pollen grains. 3) *Trifolium* species live as dense populations. 4) Honey bees can take both pollen grains and nectars from *Trifolium* species. 5) Many *Trifolium* species can live at the same habitats, for example 54 taxa distribute between Alemdağ to Nişantepe (Istanbul), so pollen diversity is provided by bees (6).

MATERIAL AND METHODS

In the study, 6 districts (Silivri, Şile, Sarıyer, Beykoz, Çekmeköy, Çatalca) have been chosen where honey is produced by beekeepers in Istanbul. Field trips have been done during 2011, and approximately 713 flowered plants have been collected. They have been kept in ISTE (Herbarium of Istanbul University, Faculty of Pharmacy). The plant sources were identified used for the production of wildflower honey in Istanbul.

As a result of field studies, *Trifolium* species which are mostly visited by honey bees in Istanbul around were collected during field trips. Our object was to examine the pollen morphology of 16 of *Trifolium* taxa by using the data obtained from LM observations. Pollen grains of specimens for LM investigations were prepared according to the methods of Wodehouse in 1935. Measurements were taken from at least 15 grains of each sample. Photographs were taken on Nikon 80i Eclipse Trilocular Research Microscope as 1000x size, dimensions of all species were measured with Clemex Vision Profesional Version 4.0 imaging system as 200x size. Also scanning electron microscopy photographs of nine *Trifolium* taxa were taken in Erciyes University, Technology Research and Application Center.

RESULTS AND DISCUSSION

The pollen morphology of 16 *Trifolium* taxa in Istanbul by using the data obtained from LM observations (Fig. 1, 2) which of 9 taxa were taken by using SEM (Fig. 3,). Their numerical values were given in Table. Also the pollen grains of the analyzed taxa were divided into 3 groups according to their shape and their size as follows:

According to pollen shape:

1) Prolate: *T. angustifolium, T. pannonicum* subsp. *elongatum, T. stellatum*

2) Subprolate: *T. arvense* var. *arvense, T. campestre, T. constantinopolitanum, T. echinatum* var. *echinatum, T. lingusticum, T. purpureum* var. *purpureum, T. striatum, T. subterraneum*

3) Spheroidal: *T. nigrescens* subsp. *petrisavii, T. pallidum, T. patens, T. pratense* var. *pratanse, T. repens* var. *repens*

According to pollen size:

1) Small (10-25 µm): *T. arvense* var. *arvense, T. campestre, T. patens*

2) Medium (25-50 µm): All of the other taxa.

The shape of *Trifolium* pollen grains in equatorial view ranges from subprolate to prolate. Size of polar axis varies from 37.83 µm to 20.41µm (Fig. 4) while size of equatorial axis varies from 41.25 µm to 22.10 µm (Fig. 5). They are 3-colporate. The colpus generally extends not fully to the end of the grain. Exine thickness varies from 2 µm to 1.1 µm, its ornamentation is reticulate and scabrate. Aperture membrane is smooth, aperture surface without exine elements. Pollen grain with elongated apertures situated at the equator.

Mellisopalynology of the genus *Salix*, *Thymus*, *Allium*, *Malcolmia* and *Genista* have carried out recently (7, 8, 9, 10, 11). The analytical study of honey provides a basis for identifying the origins of a honey in terms of locality and floral source. It is determined that pollen of nectariferous plants are dominant inside honey in Adapazarı province, for instance *Fagaceae, Ericaceae, Rosaceae, Asteraceae, Cistaceae, Lamiaceae* and *Fabaceae* (12). The pollen content changes according to various plant taxa. So, various pollen grains cause different increase in hemoglobin amount in blood (13). Therefore, the information should be used to develop analytical standards among honey and medicinal plants.

These results show that there are several pollen characters of taxonomic significance (shape, size, ornamentation) in the genus *Trifolium*. The main palynological differences have not been registered at the species level. These results are almost similar to the earlier studies on the pollen morphology of *T. repens* and *T.pratense* (14).

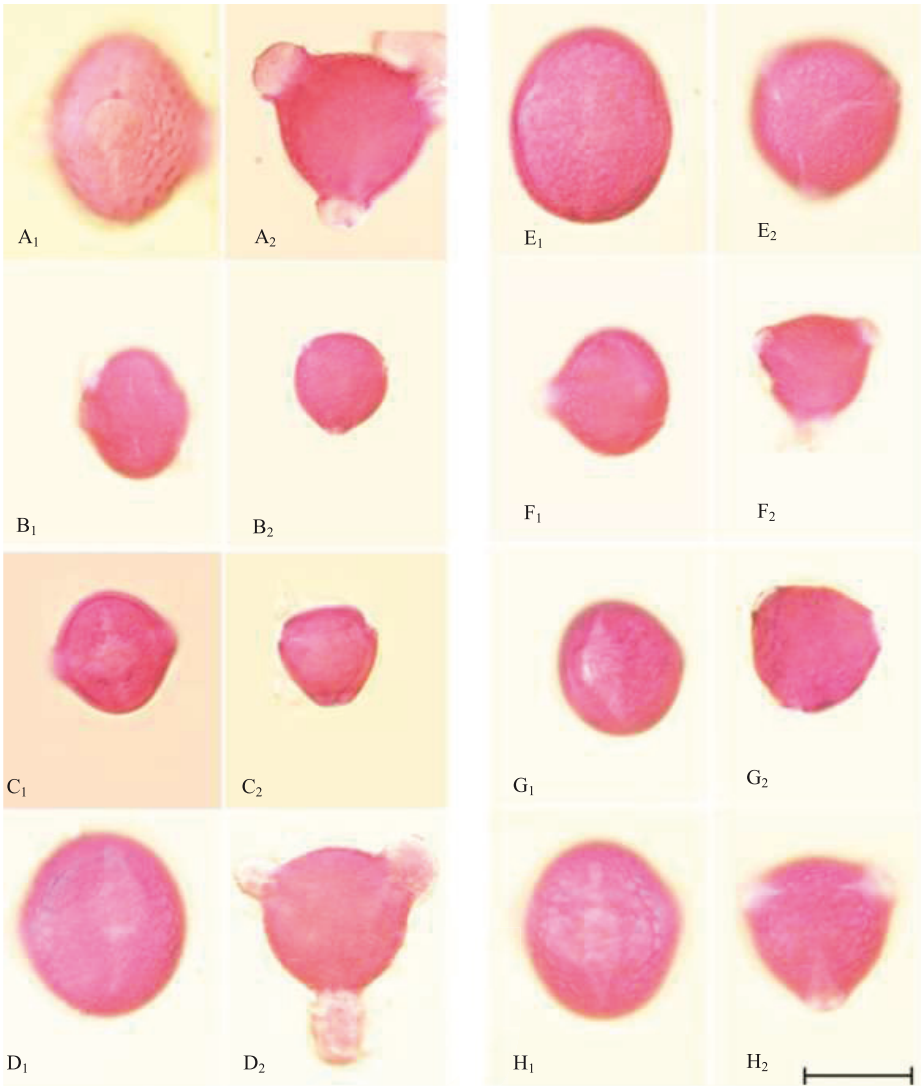

Figure 1: Pollen grains in LM: 1: Equatorial view, 2: Polar view;
A) *Trifolium angustifolium*, B) *T. arvense*, C) *T. campestre*,
D) *T. constantinopolitanum*, E) *T. echinatum*, F) *T. lingusticum*,
G) *T. nigrescens* subsp. *petrisavii*, H) *T. pallidum*
(Scale bar=20 μm).

Figure 2: Pollen grains in LM: 1: Equatorial view, 2: Polar view;
A) *Trifolium pannonicum* subsp.*elongatum*, B) *T. patens*,
C) *T. pratense* var. *pratanse*, D) *T. purpureum*, E) *T. repens* var. *repens*,
F) *T. stellatum*, G) *T. striatum*, H) *T. subterraneum*
(Scale bar=20 μm).

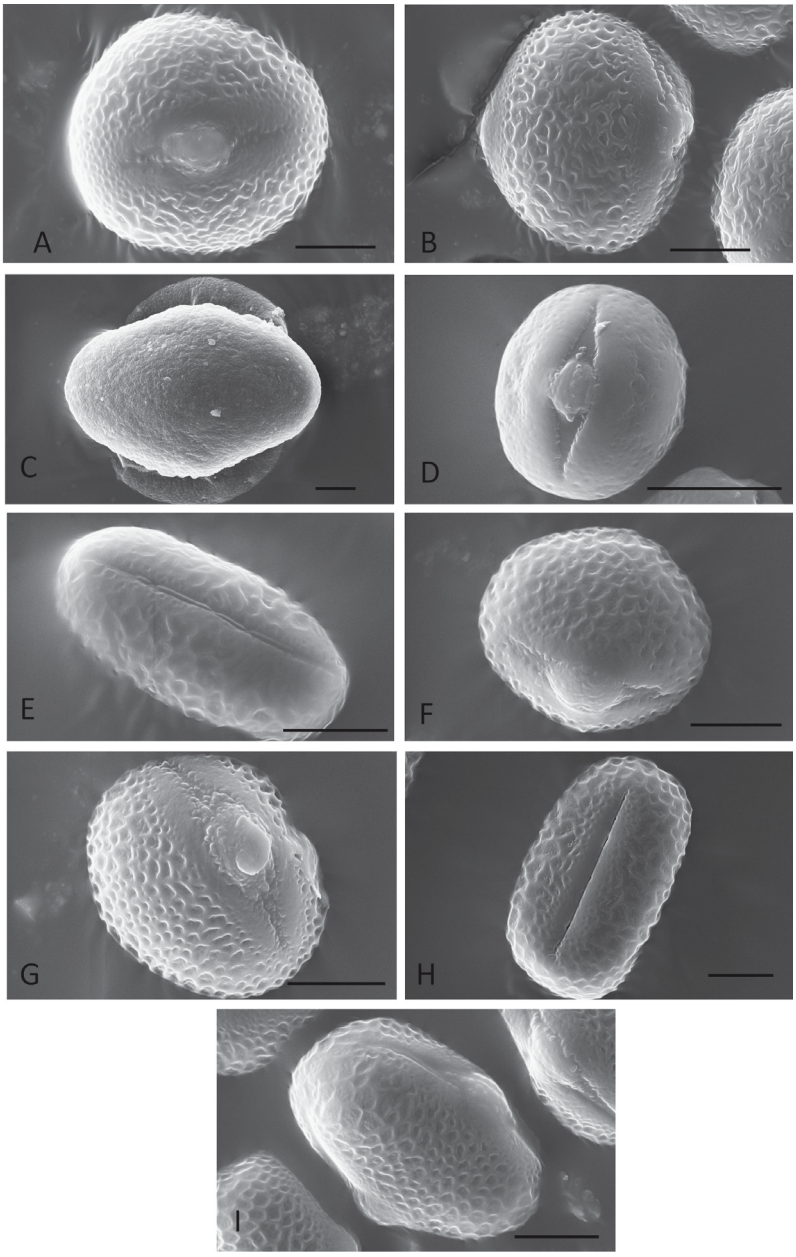

Figure 3: Pollen grains in SEM: A) *Trifolium angustifolium*, B) *T. arvense*, C) *T. constantinopolitanum*, D) *T. nigrescens*, E) *T. pallidum*, F) *T. pannonicum*, G) *T. pratense*, H) *T. purpureum*, I) *T. repens*
(Scale bar 10 µm).

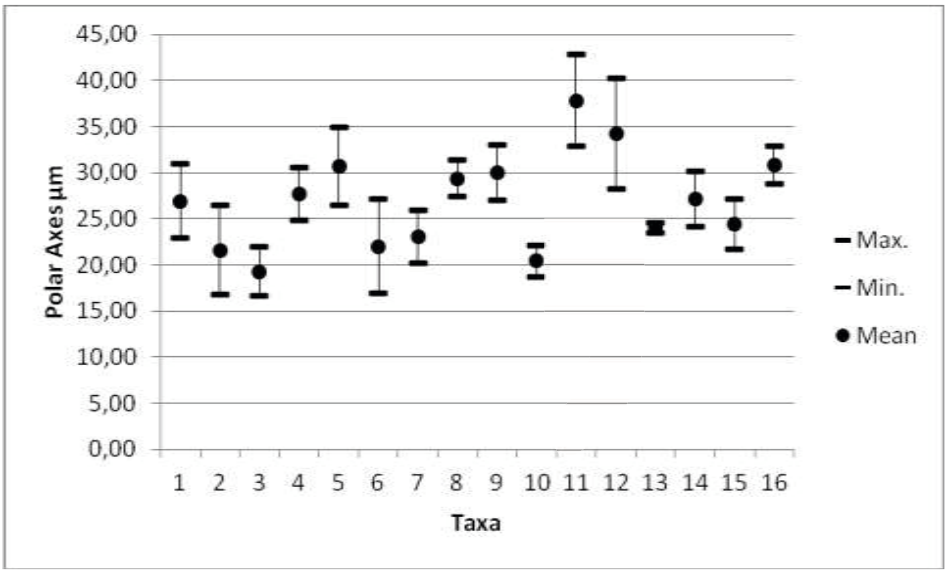

Figure 4: Polar axes lengths (µm) in the *Trifolium* species examined.

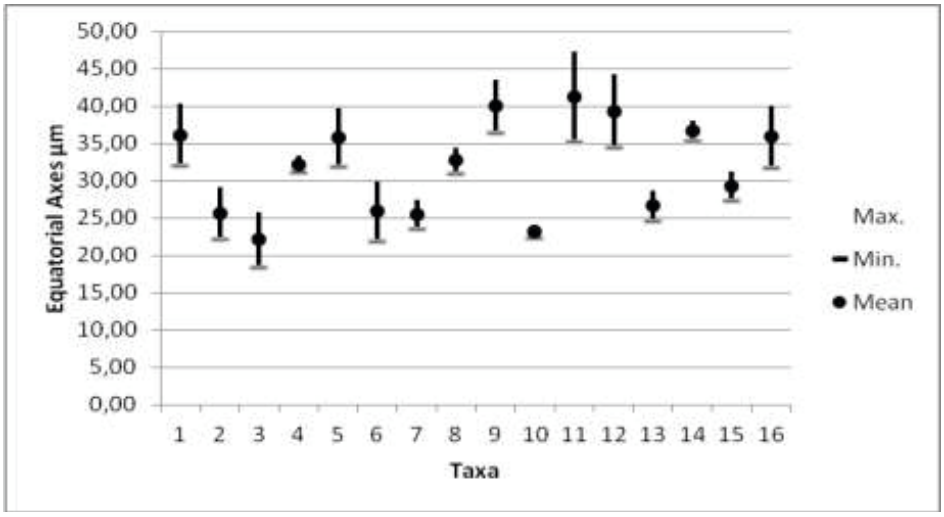

Figure 5: Equatorial axes lengths (µm) in the *Trifolium* species examined.

Table: Pollen characteristics of the examined taxa.

	Equatorial Axis µm (E)	Polar Axis µm (P)	P/E	Exine µm	Shape
(1) *T. angustifolium*	36.15±2.32	26.94±0.91	1.34	2	Prolate
(2) *T. arvense* var. *arvense*	25.62±1.49	21.58±0.82	1.19	1.3	Subprolate
(3) *T. campestre*	22.10±1.53	19.30±1.13	1.15	1.4	Subprolate
(4) *T. constantinopolitanum*	32.17±1.77	27.70±1.43	1.17	1.3	Subprolate
(5) *T. echinatum* var. *echinatum*	35.81±1.4	30.72±2.01	1.17	1.4	Subprolate
(6) *T. lingusticum*	25.89±1.78	22.04±1.54	1.18	1.2	Subprolate
(7) *T. nigrescens* subsp. *petrisavii*	25.46±1.11	23.09±0.94	1.10	1.1	Spheroidal
(8) *T. pallidum*	32.70±1.58	29.37±1.04	1.11	1.4	Spheroidal
(9) *T. pannonicum subsp.elongatum*	39.96±1.75	30.04±1.28	1.33	1.6	Prolate
(10) *T. patens*	23.21±0.65	20.41±1.17	1.14	1.3	Spheroidal
(11) *T. pratense var. pratanse*	41.25±1.33	37.83±1.23	1.09	1.2	Spheroidal
(12) *T. purpureum* var. *purpureum*	39.28±0.71	34.22±1.44	1.15	1.4	Subprolate
(13) *T. repens var. repens*	26.65±2.14	24.00±1.39	1.11	1.5	Spheroidal
(14) *T. stellatum*	36.68±1.31	27.14±0.34	1.35	1.6	Prolate
(15) *T. striatum*	29.24±0.74	24.11±1.25	1.22	1.1	Subprolate
(16) *T. subterraneum*	35.91±0.85	30.83±1.24	1.17	1.4	Subprolate

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