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Research Article

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The Investigation of Morphologic Analysis of Pollen Grains Which are Economically Important and Collected by *Apis mellifera* L.

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Abstract

Pollen samples were collected during from May to September of year 2001 from six regions where the most of the beekeeping process is realized in Bursa. Fourteen kinds of pollen taxa collected by *Apis mellifera* L. and which have the highest amount compared the others were selected as appropriate ones to study for morphological analysis because of their economical importance.

As a result of identifications which have been made in microscope, 14 kinds of pollen samples that belong to 8 family have been determined.

Key Words: Bursa, *Apis mellifera* L. , pollen samples, morphologic analysis.

Introduction

Honey, pollen and propolis are the bee products, which honeybees collect from the plants, and royal jelly, bee venom and beeswax are the bee products, which honeybees secrete themselves (Schmidt, 1996). Solving the nutrition and health problems of rapidly developing and growing world population, bee products are used both as nutrition and in apitherapy by people.

Beekeeping has been done for many years for the aim of benefiting from these products, which are very useful for people nutrition. The earliest records of man's harvesting from bees' nests are in the Mesolithic rock art of Europe and Asia, painted not more than 8000 years ago. (Crane, 1996). A lot of historical piece has been found that proves Hittites make beekeeping in Anatolia in B.C. 1300. Until A.D. 1850 beekeeping had been made with frameless hives and without technical intervention. After this date, first of all USA, has been passed modern beekeeping with first frame hives, which was made by American beekeeper Langstroth (İnci, 1985).

Pollen is an essential nutrition for honeybees for their development, reproduction and continue their other activities in hive. Also it is known that of various insects, birds, bats and some mammalians can digeste the pollen and use it for their nutrition (Stanley and Linskens, 1974; Schmidt and Buchmann, 1992). Pollen, which is an important nutrition for people and consumed with tons, is collected by honeybees.

At this study, has been done 14 kinds of pollen, which are economically important, were studied morphologically.

Material and Methods

Collection of materials

Pollen samples used in the research were collected from Cumalıkızık, Narlıdere, Akçalar, İkizce, Çekrice and Baraklı regions of Bursa, where beekeeping is widespread, during May, June, July, August and September of 2001.

Two healthy hives were settled in each of these regions between May and September for a total of twelve hives. Traps were fitted on the hives for *Apis mellifera* L. to collect pollen two days per week in the morning between the hours 8 and 12.

Pollen, which are accumulated in mixed colors in the hives' pollen traps, was put into glass jars right after collection from the traps, and the jars were brought in a refrigerated container to the laboratory. The total amounts of the collected pollen were then weighed on a sensitive scale and separated very carefully according to their colors.

To avoid any confusion, pollen samples which had been separated regarding their colors were checked under a microscope and weighed on a sensitive scale one more time, put into separate glass jars, then immediately closed. Data was written on the labels of the jars including the name of the region where the pollen had been collected, the date of collection, the name of the producer and the weight results. After weighing all the pollen samples which had been collected between May and September, the 14 most collected kinds of pollen samples were found appropriate for morphologic analysis studies, considering their economic importance.

Collection of plants from the region

During the field studies, there were regular trips to Bursa every 15 days to collect newly flowering plants, check the hives, and bring collected samples back to the laboratory.

Plants which had been brought to the laboratory were dried by pressing and after their diagnosis had been completed, referance pollen slides were prepared using glycerin gelatin. Thanks to this, diagnosis of the pollen samples studied was made using comparison and in a reliable way.

Preparation of slides from material

The investigation followed the Wodehouse method (Wodehouse, 1935) to prepare the slides in pollen samples.

Microscopic studies of pollen samples

Pollen slides were researched with Nikon Eclipse E400 microscope, and immersion objective(x100) was used in the description of pollen samples. In the researches all the area, which is 18x18mm², was checked. Relevant sources were used in the diagnosis of the pollens were from Erdtman, 1969; Aytuğ, 1971; Kapp et al., 2000; Markgraf and D'Antoni, 1978; Nilsson et al., 1983; Iwanami et al., 1988; Faegri and Iversen, 1989; Moore et al., 1991; Pehlivan, 1995; D'Albore, 1997 as well as prepared reference slides.

Measurements of pollen samples

One interval of micrometric ruler, which is used in pollen measurements, was calculated as a 1 μm. Polar, equatorial and AMB diameters was measured 50 times for every samples until Gausse curve occured. Also exine (sexine, nexine) thickness, intine thickness, longitude of colpus (Clg), latitude of colpus (Clt), longitude of porus (Plg), latitude of porus (Plt), height of spines (dh), base width of spines (dt) and distance of colpus peaks (t) were measured 50 times for every samples until Gausse curve occured. Plg, Plt etc. could not measured in some pollen samples because of difficulty in distinction.

Means of pollen measurements (M) and standard deviation (S) were calculated according to Sokal and Rohlf (1969). The formulas used are shown below.

Mean;

$$M = m + a1/n \sum xy$$

Standard deviation ;

$$S = \pm a\sqrt{1/n \sum x^2y - u^2}$$

(u = 1/n xy)

Results and Discussion

As a result of identifications which have been made in

Table 1. Genus or species name, region of collection, collection time, flowering time and collection amount of pollen samples.

Family	Genus or species name	Region of collection	Collection time	Flowering time (day)	Collection amount (g)
Asteraceae	*Carduus* L. type I	Akçalar Cumalıkızık İkizce Narlıdere	10.5.01 – 24.5.01	15	18.6
	Carduus L. type II	Narlıdere, Baraklı	11.6.01 – 16.7.01	36	
	Helianthus annuus L.	Çekrice İkizce	21.6.01 – 9.8.01	50	127.2
	Xanthium strumarium L.	Akçalar Çekrice İkizce	16.8.01 – 30.8.01	15	50.05
Brassicaceae	*Raphanus raphanistrum* L.	Akçalar Çekrice İkizce Narlıdere	3.5.01 – 25.6.01	54	411.1
Cistaceae	*Cistus creticus* L.	Akçalar Cumalıkızık Çekrice İkizce Narlıdere	3.5.01 – 18.6.01	47	582.5
	Cistus salviifolius L.	İkizce Baraklı	7.5.01 - 12.7.01	67	
Dipsacaceae	*Cephalaria transsylvanica* (L.) Schrader	Akçalar İkizce Çekrice Narlıdere Cumalıkızık	12.7.01- 30.8.01	50	90.1
	Scabiosa columbaria L.	Akçalar Çekrice Narlıdere	31.5.01- 19.7.01	50	52.5
Fabaceae	*Trifolium pratense* L.	Akçalar Baraklı Çekrice İkizce Narlıdere	31.5.01 – 5.7.01	36	233.0
	Trifolium repens L.	Akçalar Baraklı Cumalıkızık Çekrice	3.5.01 – 28.6.01	57	
Fagaceae	*Castanea sativa* Miller	Cumalıkızık	4.6.01 – 5.7.01	32	98.6
Papaveraceae	*Papaver rhoeas* L.	Akçalar Çekrice İkizce Narlıdere	30.4.01 – 4.6.01	36	111.3
Ranunculaceae	*Convolvulus arvensis* L.	Çekrice İkizce	14.6.01– 19.7.01	36	22.6

microscope, 14 kinds of pollen samples that belong to 8 family have been determined, which were collected during from May to September of year 2001. Family name, genus or species name, region of collection, collection time, flowering time and collection amount of these taxa were shown at Table 1.

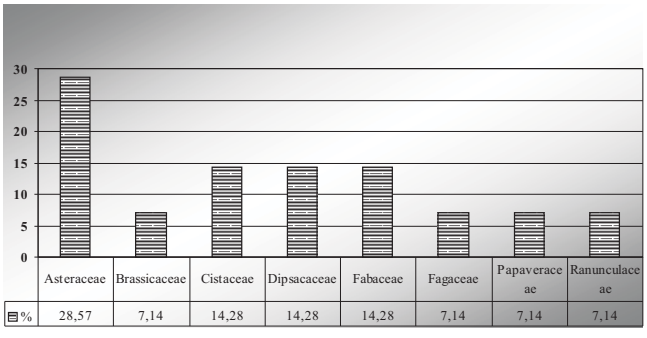

Figure 1. Distribution of pollen taxa according to families.

Distribution of pollen taxa according to families were shown at Figure 1, collection amount of pollen taxa According to families were shown Figure 2 and flowering time of pollen taxa according to families were shown Figure 3.

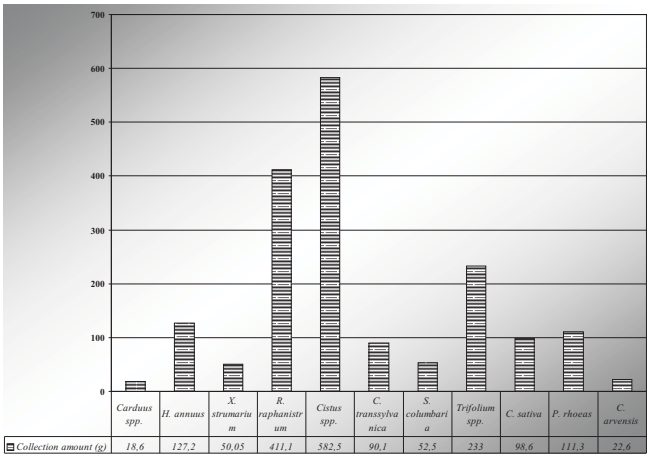

Figure 2. Collection amount of pollen taxa according to families (g).

Table 2. Morphologic characters and measurements results of pollen taxa.

No	Pollen taxa	Pollen type	Pollen view	P/E	Polar axis(µm)		Equatorial diameter (µm)		L (µm) (AMB diameter)		Clg (µm)	Clt (µm)	Plg (µm)	Plt (µm)	Exine (µm)	Intine (µm)	t (µm)	dh (µm)	ds (µm)
					M	σ	M	σ	M	σ									
1	*Carduus* L. type I	Tricolporate	Oblate Spheroid	0.90	35.98	±4.062	39.98	±3.602	40.12	±4.827	25.12	7.38	10.14	9.94	2.57	0.725	16.5	3.58	4.96
2	*Carduus* L. type II	Tricolporate	Oblate Spheroid	0.92	43.72	±2.474	47.32	±2.65	45.54	±2.692	31.62	17.48	14.96	15.58	5.485	0.73	13.68	2.98	5.1
3	*Helianthus annuus* L.	Tricolporate	Oblate Spheroid	0.95	30.08	±1.354	31.7	±1.486	31.24	±1.530	23	5.38	7	7.14	1.035	0.475	-	4.9	2.36
4	*Xanthium strumarium* L.	Tricolporate	Oblate Spheroid	1.00	25.82	±1.558	25.78	±1.253	25.46	±1.459	4.06	3.08	-	-	1.865	0.515	-	-	-
5	*Raphanus raphanistrum* L.	Tricolpate	Oblate Spheroid	0.94	27.18	±2.951	28.74	±2.575	28.24	±2.413	24.02	10.3	-	-	2.16	0.875	3.42	-	-
6	*Cistus creticus* L.	Tricolporate	Prolate Spheroid	1.02	38.8	±1.99	38.1	±2.012	38.72	±1.918	30.9	3.9	8.1	7.8	1.72	0.615	7.82	-	-
7	*Cistus salviifolius* L.	Tricolporate	Oblate Spheroid	0,98	44.18	±1.976	45.12	±2.094	45.42	±1.650	34.24	23.5	8.24	7.76	2.48	0.665	11.82	-	-
8	*Cephalaria transsylvanica* (L.) Schrader	Triporate	Sub oblate	0.82	58.68	±3.524	71.114	±4.086	68.78	±2.752	-	-	15.6	12.42	3.42	1	-	-	-
9	*Scabiosa columbaria* L.	Tricolpate	Prolate Spheroid	1.07	65.36	±2.2	60.72	±1.855	59.72	±1.662	20.3	10.22	-	-	5.02	0.92	-	-	-
10	*Trifolium pratense* L.	Tricolporate	Prolate Spheroid	1.11	33.54	±2.434	30.18	±2.233	30.44	±2.523	28.72	4.72	10.6	9.92	1.09	0.975	12.06	-	-
11	*Trifolium repens* L.	Tricolporate	Prolate Spheroid	1.11	27.4	±1.523	24.58	±1.167	24.28	±1.562	23.04	4.38	9.04	9.34	0.935	0.74	9.62	-	-
12	*Castanea sativa Miller*	Tricolporate	Sub prolate	1.16	14.48	±0.727	12.46	±0.78	11.88	±0.84	11.62	3.04	4.28	4.8	1	0.66	2.96	-	-
13	*Papaver rhoeas* L.	Tricolpate	Oblate Spheroid	0.91	22.86	±1.341	25.02	±1.048	24.58	±0.961	18.62	8.74	-	-	1.235	0.25	4.84	-	-
14	*Convolvulus arvensis* L.	Tricolpate	Sub oblate	0.86	54.92	±3.005	63.36	±2.52	62.78	±2.802	42.54	19.68	-	-	3.65	0.85	14.12	-	-

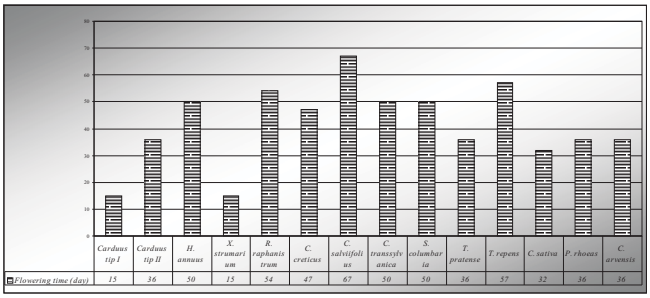

Figure 3. Flowering time of pollen taxa according to families (day).

Morphologic characters and measurements results of pollen taxa were shown at Table 2 and pollen photographs of pollen taxa were shown on Figure 4-17.

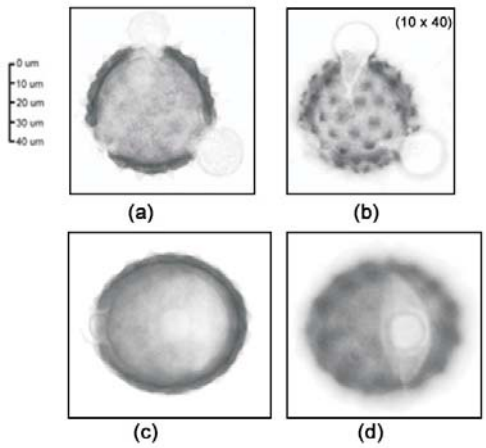

Figure 4. Asteraceae: *Carduus* L. type I. **a.** *Carduus* L. type I, AMB view; **b.** *Carduus* L. type I, exine ornemantation; **c.** *Carduus* L. type I, equatorial view; **d.** *Carduus* L. type I, aperture.

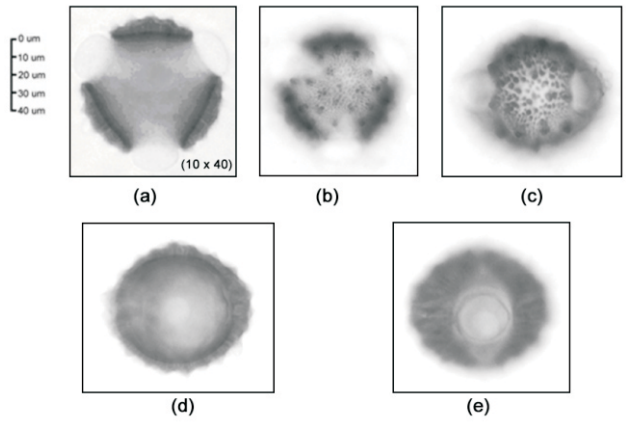

Figure 5. Asteraceae: *Carduus* L. type II. **a.** *Carduus* L. type II, AMB view; **b and c.** *Carduus* L. type II, AMB and equatorial exine ornemantation; **d.***Carduus* L. type II, equatorial view; **e.** *Carduus* L. type II, aperture.

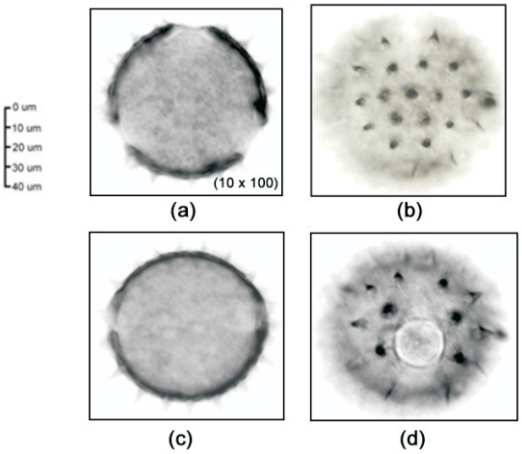

Figure 6. Asteraceae: *Helianthus annuus* L. **a.** *H.annuus* L., AMB view; **b.** *H.annuus* L., exine ornemantation; **c.** *H.annuus* L., equatorial view; **d.** *H.annuus* L., aperture.

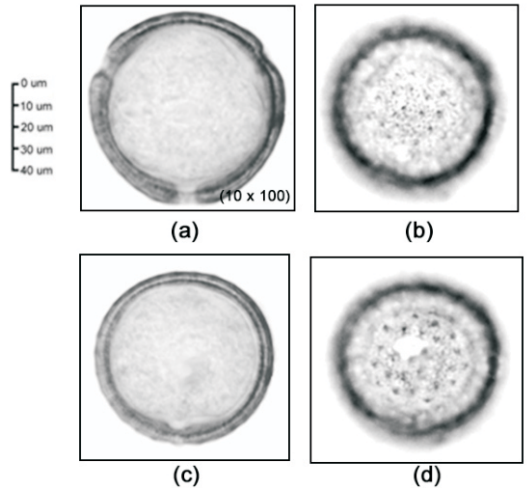

Figure 7. Asteraceae: *Xanthium strumarium* L. **a.** *X.strumarium* L., AMB view; **b.** *X.strumarium* L., exine ornemantation; **c.** *X.strumarium* L., equatorial view; **d.** *X.strumarium* L., aperture.

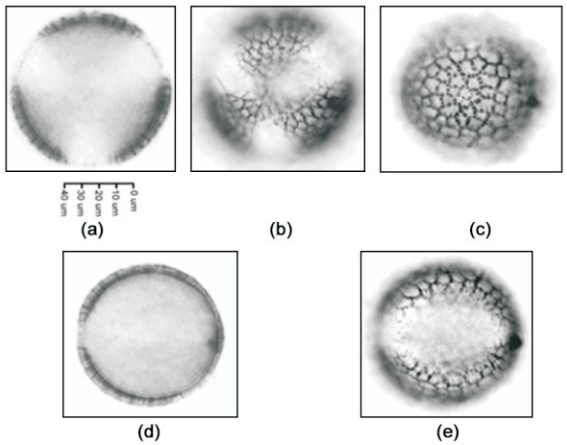

Figure 8. Brassicaceae: *Raphanus raphanistrum* L. **a.** *R.raphanistrum* L., AMB view; **b. and c.** *R.raphanistrum* L., AMB and equatorial exine ornemantation; **d.** *R.raphanistrum* L., equatorial view; **e.** *R.raphanistrum* L., aperture.

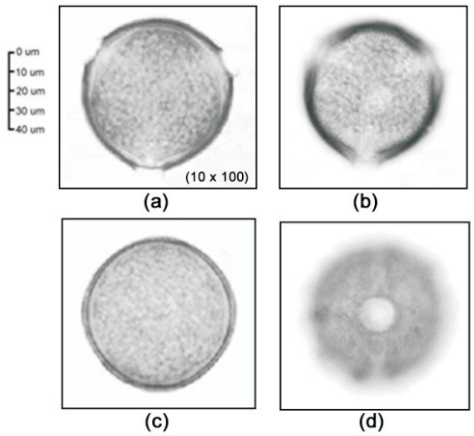

Figure 9. Cistaceae: *Cistus creticus* L. **a.** *C. creticus* L., AMB view; **b.** *C. creticus* L., exine ornemantation; **c.** *C. creticus* L., equatorial view; **d.** *C. creticus* L., Aperture.

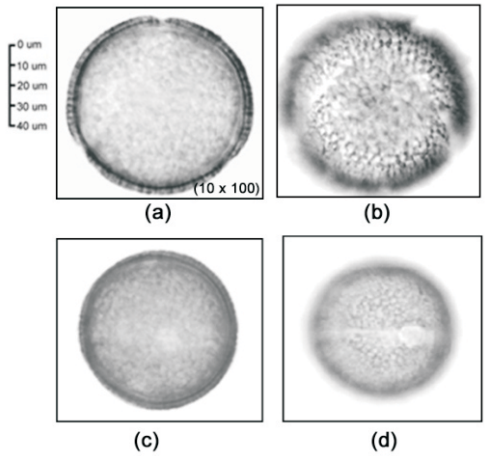

Figure 10. Cistaceae: *Cistus salviifolius* L. **a.** *C. salviifolius* L., AMB view; **b.** *C. salviifolius* L., exine ornemantation; **c.** *C. salviifolius* L., equatorial view; **d.** *C. salviifolius* L., Aperture.

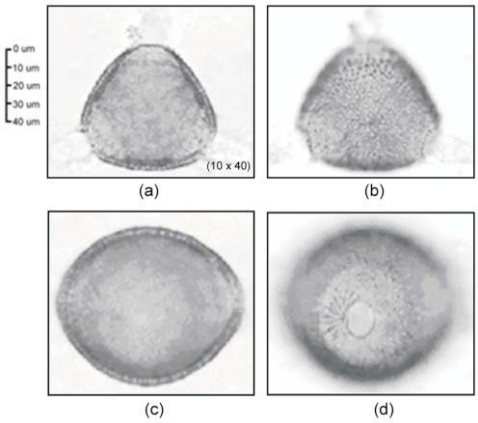

Figure 11. Dipsacaceae: *Cephalaria transsylvanica* (L.) Schrader. **a.** *C.transsylvanica* (L.) Schrader, AMB view; **b.** *C.transsylvanica* (L.) Schrader, exine ornemantation; **c.** *C. transsylvanica* (L.) Schrader, equatorial view; **d.** *C. transsylvanica* (L.) Schrader, aperture.

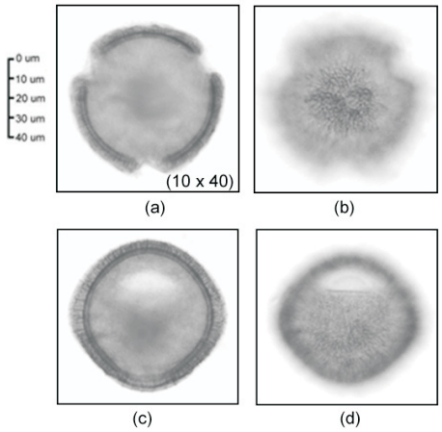

Figure 12. Dipsacaceae: *Scabiosa columbaria* L. **a.** *S. columbaria* L., AMB view; **b.** *S. columbaria* L., exine ornemantation; **c.** *S. columbaria* L., equatorial view; **d.** *S. columbaria* L., aperture.

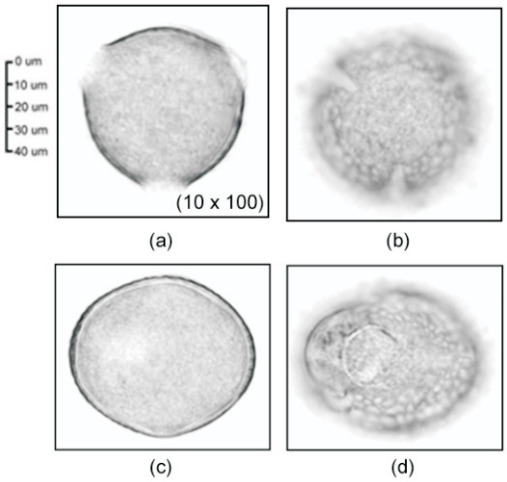

Figure 13. Fabaceae : *Trifolium pratense* L. **a.** *T. pratense* L.*,* AMB view; **b.** *T.pratense* L.*,* exine ornemantation; **c.** *T.pratense* L., equatorial view; **d.** *T.pratense* L., *a*perture.

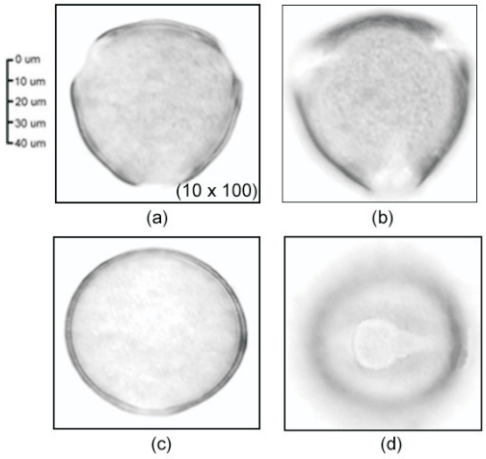

Figure 14. Fabaceae : *Trifolium repens* L. **a.** *T. repens* L., AMB view; **b.** *T. repens* L., exine ornemantation; **c.** *T. repens* L., equatorial view; **d.** *T. repens* L., *A*perture.

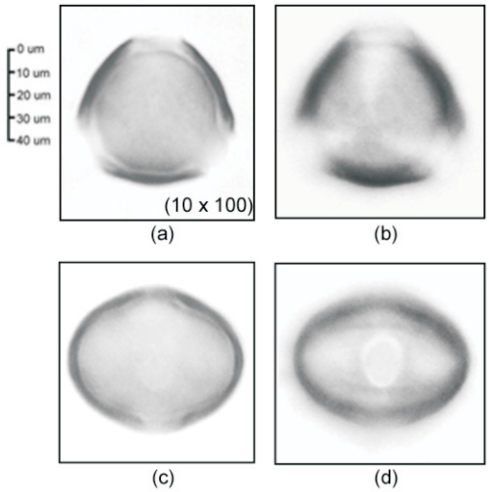

Figure 15. Fagaceae : *Castanea sativa* Miller. **a.** *C.sativa* Miller*,* AMB view; **b.** *C.sativa* Miller, exine ornemantation; **c.** *C.sativa* Miller, equatorial view; **d.** *C.sativa* Miller, aperture.

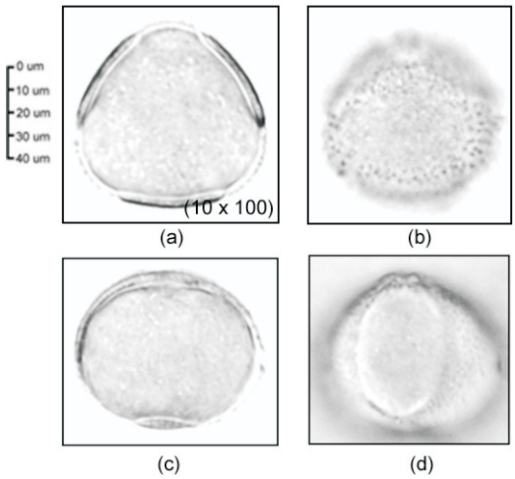

Figure 16. Papaveraceae : *Papaver rhoeas* L. **a.** *P. rhoeas* L., AMB view; **b.** *P. rhoeas L.,* exine ornemantation; **c.** *P. rhoeas* L., equatorial view; **d.** *P. rhoeas* L., *a*perture.

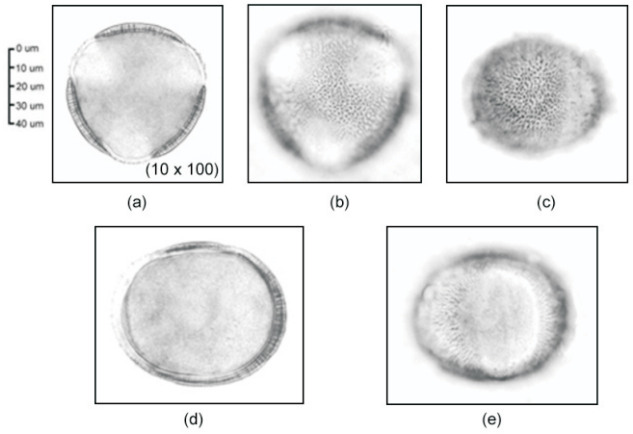

Figure 17. Ranunculaceae : *Convolvulus arvensis* L. **a.** *C. arvensis* L., AMB view; **b and c.** *C. arvensis* L.*,* AMB and equatorial exine ornemantation; **d.** *C. arvensis* L., equatorial view; **e.** *C. arvensis* L., *a*perture.

Pollen samples were collected during May to September of year 2001 from Akçalar, Baraklı, Cumalıkızık, Çekrice, İkizce and Narlıdere regions in Bursa, where one of the most important beekeeping center in Turkey because of its floral richness. 14 kinds of pollen taxa, which belong to Asteraceae, Brassicaceae, Cistaceae, Dipsacaceae, Fabaceae, Fagaceae, Papaveraceae and Ranunculaceae families, have been determined as a plants which have the highest amount pollen and the most preferable plants by honeybees. Asteraceae family has 4 taxa and has biggest portion by %28.57. Cistaceae, Dipsacaceae and Fabaceae families' portion is %14,28. Fagaceae, Papaveraceae and

Ranunculaceae families' portion is %7.14 (Fig.1). Pollen analysis in the 94 honey samples, which look like our results, have been determined by Sorkun and İnceoğlu (1984). Especially they reported that Asteraceae and Fabaceae families are widespread in Turkey and important for beekeeping. Also Davis informed that Asteraceae and Fabaceae families have very rich plant varieties and shows spread widely in Turkey (Davis, 1965-1985).

Cistus salviifolius L., which belongs to Cistaceae family, have been collected during 67 days from İkizce and Baraklı regions by the honeybees and thus it was the most preferable pollen type by the honeybees. Ortiz (1994) reported that *Cistus* L. and *Helianthemum* Adans taxa pollen grains, which are belong to Cistaceae family, are important as a honeybee nutrition and also determined that honeybee collects pollen instead of nectar from *Cistus* flowers. *Trifolium repens* L. follow the pollen of *Cistus salviifolius* L. with 57 flowering days. *Carduus* L. type I and *Xanthium strumarium* L. pollen grains have the least flowering days with 15 days (Fig.3). However *Cistus creticus* L. and *Cistus salviifolius* L. have the most collection amounts with 582.5 g. *Raphanus raphanistrum* L. is the second with 411.1 g. *Carduus* L. type I and *Carduus* L. type II have the least collection amounts with 18.6 g (Fig. 2). In this case, as Cistaceae family has the most flowering time and collection amount, taxa belong to this family were determined suitable for the studies aiming production.

As a result of morphologic analysis of pollen samples, these results have been determined: *Carduus* L. type I, *Carduus* L. type II, *Helianthus annuus* L. and *Xanthium strumarium* L. have echinate ornemantation and tricolporate pollen type, *Cistus creticus* L., *Cistus salvifolius* L., *Trifolum pratense* L., *Trifolium repens* L. and *Castanea sativa* Miller have reticulate ornemantation and tricolporate pollen type, *Raphanus raphanistrum* L. has reticulate ornemantation and tricolpate pollen type, *Cephalaria transsylvanica* (L.) Schrader has microechinate ornemantation and triporate pollen type, *Scabiosa columbaria* L. has echinate ornemantation and tricolpate pollen type, *Papaver rhoeas* L. has microechinate ornemantation and tricolpate pollen type, *Convolvulus arvensis* L. has scabrate(granulate) ornemantation and tricolpate pollen type.

Also pollen measurements have been found. According to this; *Cephalaria trassylvanica* L. (P=58.68 m; E=71.114m; L=68.78m), *Scabiosa columbaria* L. (P=65.36 m; E=60.72 m; L=59.72 m) and *Convolvulus arvensis* L. (P=54.92 m; E=63.36 m; L=62.78 m) have been measured respectively polar, equatorial and AMB diameters bigger than other 11 pollen samples. However *Castanea sativa* Miller, which belong to Fagaceae family (P=14.48 m; E=12.46 m; L=11.88 m) has been measured respectively polar, equatorial and AMB diameters smaller than other 13 pollen samples.

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