The Plant Choices of Honey Bees to Collect Propolis in Tekirdag-Turkey

Tekirdağ-Türkiye'de Bal Arılarının Propolis Toplamak için Tercih Ettiği Bitkiler

Research Article

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ABSTRACT

The aim of this study is to investigate the preferences of honeybees to collect propolis in Tekirdağ city of Turkey. A total of 92 different propolis samples collected from Tekirdağ were examined under microscope to determine pollen contents and establish the botanical origin of Tekirdağ propolis. 38 different plant families and 51 taxa were identified by pollen analysis of the 92 propolis samples. Among these families Asteraceae family pollen were found most intensive. The most observed pollen in the investigated samples were belong to the taxa of *Asteracae*, *Boraginaceae*, *Brassicaceae*, *Fabaceae* and *Salicaceae* families.

Key words
Propolis, Tekirdağ, Pollen

ÖZET

Bu çalışmanın amacı Türkiye'nin bir ili olan Tekirdağ'da bal arılarının propolis toplamadaki tercihlerini incelemektir. Tekirdağ'dan toplanan 92 farklı propolis örneği, polen içeriği ve Tekirdağ propolisinin botanik orijinini saptamak için mikroskop altında incelenmiştir. 92 propolis örneğinin polen analizi ile 38 farklı bitki familyası ve 51 takson tanımlanmıştır. Bu familyalar arasından, Asteraceae familyasına ait polenler en yoğun olarak bulunmuştur. İncelenen örneklerde en çok gözlenen polenler Asteraceae, Boraginaceae, Brassicaceae, Fabaceae ve Salicaceae taksonlarına aittir.

Anahtar Kelimeler
Propolis, Tekirdağ, Polen

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INTRODUCTION

Propolis (bee glue) is a sticky dark-coloured material that honeybees collect from living plants, mix with wax and use in construction and adaptation of their nests [1]. The action against micro-organisms is an essential characteristic of propolis and it has been used by human beings since ancient times for its pharmaceutical properties. Propolis possesses antibacterial, antifungal and antiviral properties and many other benefical biological activities: anti-inflammatory, antiulcer, local anesthesic, hepatoprotective, antitumor, immunostimulating, etc. [2].

The chemical composition of bee glue is very complex and depends on the flora in the areas where it is collected [3]. To understand what causes the differences in chemical composition, it is necessary to keep in mind the plant origin of propolis. The knowledge about plant sources of propolis could be useful as a basis for the chemical standardization of propolis. Bee glue could be easily characterized using its plant source, which might be established by simple TLC, HPLC or GC comparison. As far as the composition of the corresponding plant exudates is known, this method gives information about the qualitative composition of the sample [1]. Nowadays, it is well documented that in the temperate zone all over the world, the main source of bee glue is the resinous exudate of the buds of poplar trees, mainly the black poplar *Populus nigra* [4]. For this reason, European propolis contains the typical 'poplar bud' phenolics: flavonoid aglycones (flavones and flavanones), phenolic acids and their esters. In the northern area of Russia propolis mainly comes from the exudate from birch buds (*Betula verrucosa*) and *P.tremula* [5]; in Mediterranean regions from black poplars and from the leaves of *Cistus* spp. [6]; in Brazil from the leaves of some species of *Baccharis dracunculifolia* [7].; in Venezuella and Cuba from the floral resin of the genus *Clusia* [8].

The presence of pollen in propolis has been reported by Jungkunz, who found *Lupinus, Robinia* and *Onobrychis sativa* polen grains in the insoluble portion of propolis [9]. Vanhaelen and Vanhaelen-Fastre presented microphotographs of propolis originating from different countries in the world, with pollen grains clearly visible in some of them [10]. Ricciardelli d'Albore attempted to determine the geographical origin of propolis by characterizing propolis samples from different countries on five continents [11]. In following years, pollen analysis of Polish propolis was made by Warakomska and Maciejewicz [12].

In Turkey, the first scientific research about propolis was published by Sorkun and Bozcuk [13]. Although many studies were accomplished about Turkey propolis, most of them were made about chemical composition and biological effects of propolis. The first detailed microscopic study of Turkish propolis was made by Gençay and Sorkun in 2006. They investigated the plant choices of honey bees for collecting propolis by pollen analysis. They determined the pollen contents of 30 propolis samples collected from Kemaliye-Erzincan region of Turkey [14].

Turkey has a geography characterized by different climatic conditions in three phyto-geographical regions (Mediterranean, Irano-Turanian and Europe- Siberian). This is the basis of rather interesting vegetation cover in country. There are 9222 naturally grown plant species in Turkey and 3000 of these are endemic [15]. Turkey is also like a bridge between Asia and European continent. The part of Turkey which is situated in Europe continent is called as Thracian. Tekirdağ is a city of Turkey that located in Thracian part of the country. We choosed Tekirdağ owing to its location and to represent the Thracian part of Turkey. Also Tekirdağ is located in Europe-Siberian phytogegraphic region.

The aim of the present work was to determine the plants that form the botanical origin of propolis in Tekirdağ by characterizing the pollen.

MATERIALS AND METHODS

Collecting of propolis samples

In 2007-2008 the propolis samples were collected from the hives of Tekirdağ. The hives from eight towns (Çerkezköy, Çorlu, Hayrabolu, Malkara, Merkez, Muratlı, Saray, Şarköy) of Tekirdağ choosed according to the sampling method. By this method 92 bee farms were choosen to collect propolis. So the study carried on with 92 propolis samples. The number of beehives choosen by sampling method is given in Table 1 and Figure 1. Propolis samples were

Table 1. The number of collected propolis samples and collecting areas

Number	Towns	The number of registered beehives (Nh)	The number of samples that must be collect (nh)	The number of collected samples
1	Çerkezköy	35	7	7
2	Çorlu	44	8	8
3	Ereğli	23	2	-
4	Hayrabolu	59	9	9
5	Malkara	85	14	14
6	Merkez	164	31	31
7	Muratlı	45	9	9
8	Saray	65	12	12
9	Şarköy	11	2	2
TOTAL	9 TOWNS	497 BEEHIVES	94	92

collected from the edges of frames by scraping with a spatula.

Preparation of propolis samples for microscopic analysis

The materials were prepared for examination under the microscope according to the method of Warakomska and Maciejewicz [12].

Samples were ground to a powder. After grinding, powder was mixed with ethanol-ether-acetone (1:1:1) and vortexed. Mixture was filtered through a strainer with 0.3 mm holes. The suspension centrifuged at 3500-4000 rpm for 20 min. After centrifugation, the supernatant was poured and from the residual sediment slides were prepared by using glycerin gelatin. The slides were investigated simultaneously and the pollen count was made.

RESULTS AND DISCUSSION

In Tekirdağ the plant choices of honeybees to collect propolis were found very limited and similiar, though 445 plant taxa are existing in the city [16]. Totally 38 plant families (Aceraceae, Apiaceae, Asteraceae, Betulaceae, Boraginaceae, Brassicaceae, Campanulaceae, Caryophyllaceae, Chenopodiaceae, Cucurbitaceae, Cistaceae, Cupressaceae, Cyperaceae, Dipsecaceae, Ericaceae, Fabaceae, Fagaceae, Juglandaceae, Geraniaceae, Lamiaceae, Liliaceae, Oleaceae, Onagraceae, Malvaceaea, Moraceae, Pinaceae, Plantaginaceae, Platanaceae, Poaceae, Polygonaceae, Rhamnaceae, Rutaceae, Rosaceae, Rubiaceae, Salicaceae, Scrophulariaceae, Solanaceae, Tiliaceae) and 51 plant taxa were determined by microscopic analysis of propolis samples. Owing to similiar pollen content of propolis samples, it is expected that chemical compositions will show similarities too.

The most abundant identified pollen was belong to the taxa of the Asteraceae family. The pollen of this family were determined in investigated all 92 samples and in ratio of 1.8-65.7%. The pollen amount of Brassicaceae (3.8-62.1%), Boraginaceae (0.4-49.7%), Fabaceae (2-38.4%) families followed pollen amount of Asteraceae family. Although the pollen belong to taxa of Salicaceae family that is indicated as an important propolis source, were found approximately in all 92 samples, they were existing with lesser amounts compare to these identified families (0.4-23.8%).

Small quantities of pollen from other plants were found in all preparations (*Acer, Pimpinella, Alnus, Betula, Corylus, Carpinus, Echium, Cerinthe, Juniperus, Carex, Scabiosa, Castanea sativa, Juglans, Teucrium, Thymus, Nepeta, Salvia, Ornithogalum, Olea, Epilobium, Fraxinus, Morus, Plantago, Platanus, Zea mays, Rumex, Polygonum, Rhamnus, Zyzyphus, Palirus spina-christi, Galium, Tilia, Nicotiana, Linaria*).

After microscopic analysis, the pollen identified in the 92 samples were compared according to their families on the basis of towns. With regard

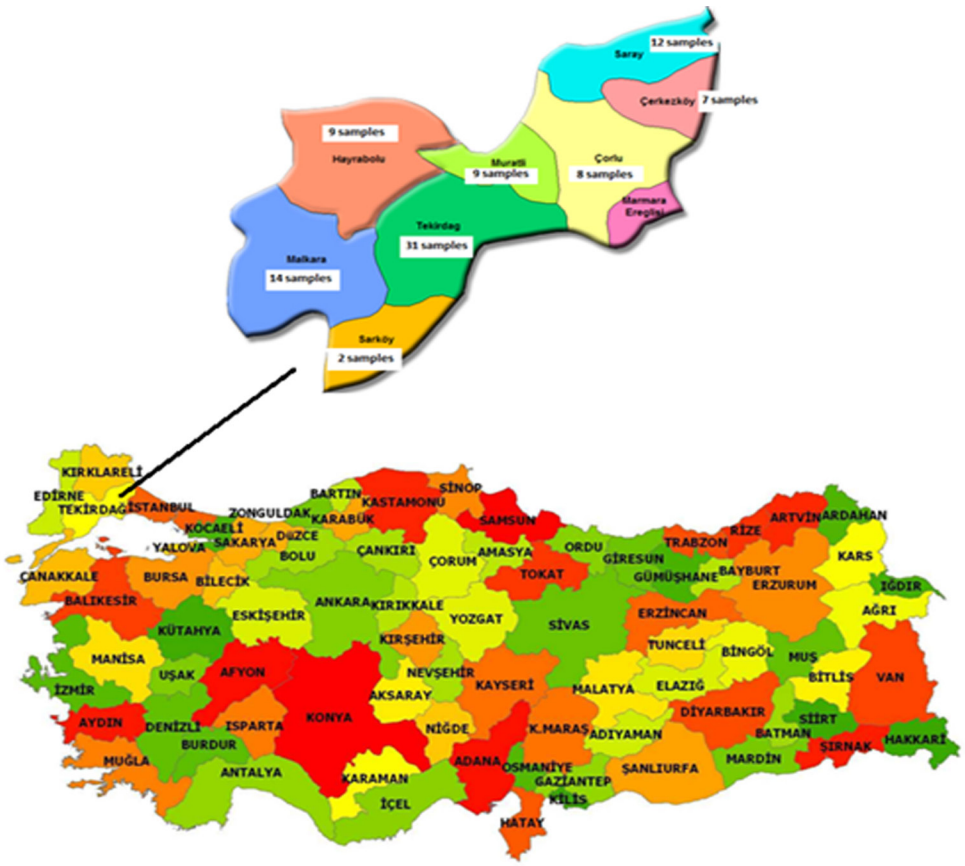

Figure 1. The location of behives and the numbers of collected propolis samples

Table 2. The statistical comparing of eight towns of Tekirdağ city according to the Brassicaceae pollen amounts

			Brassicaceae		
			Subset for alpha = 0.05		
Town		N	1	2	3
Duncan [a,b]	7.00	12	10.0667		
	1.00	7	10.5714		
	5.00	31	17.8645	17.8645	
	3.00	9	18.4556	18.4556	
	6.00	9		23.3222	
	2.00	8		25.3750	
	4.00	14		25.7429	
	8.00	2			46.0000
	Sig.		0.152	0.171	1.000

Means for groups in homogeneous subsets are displayed.

[a] Uses Harmonic Mean Sample Size = 6.796

[b]The gorup sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guarenteed.

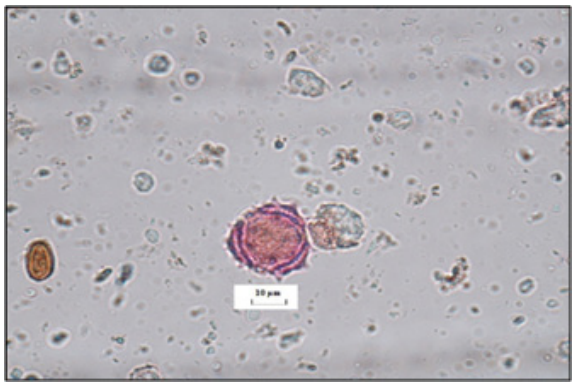

Figure 2. Asteraceae pollen (x100)

Figure3. Brassicaceae pollen (x100)

to the results of microscopic analysis, mostly Asteraceae, Fabaceae, Brassicaceae, Boraginaceae and Salicaceae pollens were observed in propolis samples. In fact the variety of pollen observed in propolis shows similarities with polen existing in honey [17].

The pollen intensities of identified families were calculated for each town. As a result in Çerkezköy, Çorlu, Hayrabolu, Merkez and Saray, the botanical origin of propolis mostly sourced from the taxa belong to the Asteraceae family. In Malkara, Fabaceae and Asteracae pollen values were very close but Fabaceae species were most dense in these samples. Only in Şarköy samples a distinct difference were observed compare to the other seven towns. Unlike the other towns in Şarköy samples Asteraceae polen were found in lower amounts (with an average amount of 7.8%) and Brassicaceae polen presented the highest amount only in Şarköy samples (with an average amount of 46%).

If we look at the other samples for Brassicaceae polen amount, it showed a minimum average value in Saray (10.06%) and a maximum average value in Malkara (25.7%). Compare to these values Şarköy samples has a considerably high amount of Brassiceae pollen. To confirm these results the pollen ratios of Brassicaeae family of 8 towns were compared by ANOVA-Duncan test (Table 2). The test

Table 3. The statistical comparing of eight towns of Tekirdağ city according to the Salicaceae pollen amounts

			Salicaceae		
			Subset for alpha = 0.05		
Town		N	1	2	3
Duncan [a,b]	1.00	7	4.3429		
	7.00	12	5.1083	5.1083	
	2.00	8	6.0625	6.0625	
	3.00	9	6.8778	6.8778	
	5.00	31	6.9484	6.9484	
	6.00	9	7.0667	7.0667	
	4.00	14		10.9929	10.9929
	8.00	2			13.7300
	Sig.		0.408	0.070	0.335

Means for groups in homogeneous subsets are displayed.

[a] Uses Harmonic Mean Sample Size = 6.796

[b] The gorup sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guarenteed.

results showed the difference between the Şarköy samples and the other samples.

An other difference existing in Şarköy samples was the amount of Salicaceae pollen.

The percentage of pollen of taxa belong to the Salicaceae family was found maximum in Şarköy and minimum in Çerkezköy samples (Table 3). Owing to the location of Şarköy near coast of Marmarean Sea, frequently come accrossing to Salicaceae plants is not suprising.

An other difference was observed in Saray samples. In these samples the pollen amounts of Ericaceae family (0.8-53%) were close to the Asteraceae polen amounts (10.9-57%). Unlike Saray samples, in the other samples except Şarköy, Ericaceae pollens were observed in lower ratios (0.3-13.8%). In Şarköy samples no Ericaceae pollens were observed. The taxa belong to Ericaceae family are existing mostly in Black Sea Region in Turkey. As shown in Figure 1 Şarköy is so far both Saray and the Black Sea Region. Owing to the nearliness of Saray to Black Sea Region, Ericaceae pollens were observed densely compare to the other towns.

As a result we can say that there can be some differences for botanical origin of propolis even though the samples collected from the same city. Because Tekirdağ is like a board between Asia and Europe, it has an edge both Marmarean Region an Black Sea Region. Some of its towns has an edge to the sea but the others don't have. Because of these, the climatic conditions and the plant cover of the city can show variabilty from town to town. Differences in the plant cover cause the variability in propolis samples. As a result the preferences of honeybees to collect propolis in Tekirdağ are mostly Asteraceae, Boraginaceae, Brassicaeae, Fabaceae and Salicaceae.

The studies about Turkey propolis are mostly about its chemical composition and uses. On the other hand microscobic researches about propolis are very limited in Turkey as well as in the world. When we scan the microscobic studies of Turkey propolis, Gençay and Sorkun (2006) investigated Erzincan propolis under microscope to determine botanical origin of samples. According to the results of the analysis in 30 propolis samples they determined the pollens of 32 plant families. Among these families Apiaceae, Asteraceae, Campanulaceae, Fabaceae, Fagaceae, Lamiaceae, Liliaceae, Pinaceae, Rosaceae, Salicaceae, Rhamnaceae, Scrophulariaceae were the most observed families. Our study shows similarities with this study.

In the studies about pollen analysis of propolis around the world, Warakomska and Maciejewicz analysed the Polish propolis and they found some taxa belong to the Asteraceae family that existed most abundant in our samples, in ratios of 30-100% in ten Polish propolis samples. Also they found some *Apiaceae (Anthriscus), Betulaceae (Betula, Alnus), Brassicaceae, Fabaceae (Trifolium repens, Trifolium pratense, Vicia), Boraginacae, Gramineae, Hipocastanaceae (Aesculus), Papaveraceae (Papaver), Pinaceae (Pinus), Plantaginaceae (Plantago), Polygonaceae (Fagopyrum) Rosaceae (Malus, Prunus, Rubus), Tiliaceae (Tilia)* and Salicaceae (Salix) pollens as we observed.

D'albore (1979) indicated the botanical origins of propolis samples of some countries(Swiss, France, Italy, Germany, Scotland, Finland, Israel, Fez, Japan, Brasil, Peru, Argentine, Cuba, Zambie, Australia, Tanzania, Kenya, Canada,) [11]. Among these countries polen spectrum of France, Germany and Scotland propolis samples are the most similiar to our results.

In an other research about Austruia, Germany, Israel, England propolis, the characteristic compounds of *Populus* bud exudates were observed in these samples [18]. In Tekirdağ samples we observed *Populus* pollen approximately in all samples but in minor amounts.

As similiar to these studies, Gençay (2004) encountered *Salix* spp. pollen densely in propolis samples of Erzincan-Turkey. The results of GC-MS analysis of *Salix* bud exudates and propolis samples were compared. The contents of bud exudates and propolis samples exhibited the paralellisms [19]. As Erzincan-Turkey samples, in Şarköy-Tekirdağ samples, Salicaceae pollen were observed densely.

To understand what causes the differences in chemical composition, it is necessary to keep in

mind the plant origin of propolis. Whereby this study chemical composition of Tekirdağ propolis can be compare with botanical origin of Tekirdağ propolis. So this study will be a basis for further studies about Turkish propolis.

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