MELLIFERA RESEARCH ARTICLE

Pollen Analysis of Chestnut Honey in Some Provinces of the Black Sea Region, Turkey

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A B S T R A C T

This study presents the pollen analysis of 16 natural chestnut honeys from the Black Sea Region of Turkey. Honey samples were collected from 12 different localities in 2018 around Samsun, Sinop, Kastamonu and Giresun. Honey samples were taken from a height of at least 10 and a height of 859 meters. The pollen analysis was carried out using microscopical analysis. All of the samples collected are monofloral honey.The highest number of pollen was observed in the samples from Bozkurt, Kastamonu in Turkey. The aim of the present study is to characterize the pollen grain association of monofloral honeys harvested in some provinces of the Black Sea Region. A second goal is to make a contribution to the knowledge of the pollen composition of monofloral honeys from northern Turkey, as has been extensively done for monofloral European honeys.

Keywords: melissopalynology, chestsnut honey, pollen analysis, Samsun, Kastamonu, Sinop, Turkey

Introduction

The variety of honey produced in a region depends on the variety of nectar sources in that region. Identification and classification of monofloral honeys are of scientific and commercial importance. Vegetation of the Black Sea Region allows the production of different types of honey. Studies of different types of honey in the Black Sea region have been carried out in previous years [1]. Chestnut honey is a type of honey obtained from the extracts that bees collect from the flowers of this tree during a certain period of the chestnut tree. This honey, which we can obtain thanks to the intensely working bees in June when chestnut trees begin to bloom, is considered among the more valuable honeys as it can be produced less than many other flower honeys.

The main method used in determining the geographical and herbal characteristics of honey is melissopalinology. Melissopalinology is based on microscopic examination of honey sediment. The first melissopalynological Turkey is based on studies in the 1980s [2-6]. Recently, studies identified nectariferous plants through pollen analysis in honey samples from Aegean region [7], Black Sea region [8] and the eastern and south-eastern regions [9]. There are several reports from

Turkey on the pollen spectrum of honeys [1,10]. All melissopalynological studies have been carried out in different phytogeographical regions of Turkey as reviewed by Öztürk et al. [10].

Melissopalynolgical, sensory and physico-chemical analyses together are needed for establishing the botanical denomination of a honey, because when considered individually, each one has its own limitations. Regarding melissopalynolgical analysis, whereas some honey types need high percentage of pollen to be considered as monofloral (90% for chestnut honey), for others only 15% is sufficient to declare their botanical origin (e.g., *Lavandula* spp.) [11].

In this study, although made with unifloral honey samples, pollen belonging to 23 different plant species was detected. The main purpose of the research is to analyse the pollen collection amount habits of honey bees in chestnut honey samples at different heights of the Black Sea Region.

Materials and Methods

Study Area

The Black Sea region is located in the north of Anatolian lands. The Black Sea climate is classified as humid climates. It is seen in the Black Sea Region and the Black Sea coastal zone of the Marmara Region. Every season of the year is rainy in this climate zone. This is because; winter and summer air masses affecting Turkey, the Black Sea region comes via the Black Sea. Maximum precipitation occurs in autumn and minimum precipitation occurs in spring. Annual rainfall is 1000-1500 mm. The annual average temperature is 13 - 15 0C. In places where the Black Sea climate is effective, natural vegetation is forest due to the high humidity and rainfall. Alpine meadows are seen in places where forests do not grow. The region, which constitutes 18% of the country's territory, is one of the largest regions of the country with its 141,000 square kilometers of land. According to the 2010 census, 7,540,000 people live in the Black Sea Region, mostly in rural areas. This is the region in Turkey, doing more than living in the city where one of those living in rural areas. The fact that the region consists of mountainous areas and rocky shores makes it difficult to establish large cities.

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Figure 1. Black Sea Region of Turkey

Samsun, Sinop, Kastamonu and Giresun, located on the coastline of the Black Sea region, both in terms of the number of honey bee colonies and honey production, it has an important place in the total of the country. Most beekeepers in the region have an average of 200 hives.

Figure 2. Black Sea Region

Vegetation of the Study Area

Lush forests are found on the Black Sea coastline, where every season is rainy and the temperature is sufficient. Climatic conditions caused forests to start from the coast. However, as a result of the destruction by people in order to settle on the coast and open fields, the lower limit of the forests was drawn to 200 - 300 meters in many places.

Broad-leaved forests take place up to about 700 meters from the shore. Beech, chestnut, alder, oak, hornbeam are the main tree species in this belt. There are mixed-leaf forests between 700 and 1500 meters. In this belt, there are beech from broad-leaved, yellow pine and fir from coniferous. There are coniferous forests between 1500 - 2200 meters. The main tree species in this belt are fir, spruce, scotch pine and larch. Under-forest plants are also very rich in these forests located on the slopes of the mountain facing the sea. On the southern slopes of the mountains, forests become sparse due to the decrease in precipitation. Black Sea forests, Turkey constitute about 14% of the forest.

Forest areas along the coasts of this region, which receives abundant rainfall, especially start from sea level and reach 2000 m. It continues to include some maquis (*Santalum* sp., *Arbutus* sp., *Pistacia* sp., *Phillyrea* sp.) at the lower levels in the form of a strip that narrows from place to place, in areas up to heights, in a louder way than the West section in the East. Then, along the northern slopes, 200 m. It is encountered with a generation continuing until.

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This is also the area known as leafy grove forests. Forest areas in the Black Sea Region are 1200 m. It gives a different appearance in the West, Middle and East parts after the. Accordingly, while larch and firs are concentrated on the coast in the West and Central parts, fir, scotch pine and spruce are concentrated in the East. Dry forests dominate the southern slopes of the Black Sea mountains and lower levels on the second rows. There are various types of oak in the lower levels, while the leaves (beech, hornbeam, alder, larch, linden) in the upper levels are shedding from 600-800 meters. The upper limit of forest in the North Anatolian mountains is 2000 m. is around. After this height, the trees disappear. Alpine meadows begin in the field. Black forest in Turkey shows a value of 32%, which is one third of all our forest areas.

Data Analysis

A total 16 natural chestnut honeys samples (Table 1), were collected from non-migratory beekeepers (members of the Samsun Beekeepers Association) which different altitudes in the Black Sea Region during 2018. The preparation of honey samples was carried out according to the standardized method of Loveaux (1970) [12]., Pendleton [13], Silici and Gökçeoğlu [1]. To analysis the pollen content of the honey samples methods outlined in detail by Ozturk et al. [10,14], and Sorkun [15] were followed. Honey samples (500 gr) were collected from different altitudes was put into sterile jars.

After the honey is mixed thoroughly and homogenized, 10gr is weighed and transferred to the tube. 20 ml of distilled water was added to the test tube, as a witness in the tube. 12 542 Lycopodium spp. the tablet containing the spore was thrown. Tubes 10- It was kept in a 45 ° C water bath for 15 minutes. Add basic fuchsine to solution It was centrifuged at 3 500 rpm for 45 minutes. The supernatant portion of the solution in the tubes was poured out. Later, 0.1 ml of 50% glycerine was added to the tube, 0.01 ml of this mixture taken into another tube in which 0.09 ml of 50% glycerine was previously placed. has been transferred and then 0.01 ml of the solution in this tube was taken and examined under a microscope. Each preparation is 18x18 mm^2 starting from the upper left corner. Scanning of the area completely It was examined microscopically with. All pollen in this area has been determined. Counting was made in two preparations prepared from two separate tubes and It has been

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applied to the formula shown below by taking the average.

TPS-10 = (Number of Pollen Counted x 12 542 *) / Counted Lycopodium spp. spores

*Amount of *Lycopodium* spp contained in one *Lycopodium* spore tablet.

Percentage of every pollen type in the pollen sediment was calculated for all chestnut honey samples. Pollen types were allocated to one of four frequency classes; (*) predominant pollen types (>45% of the total pollen content); (**) secondary pollen types (16-45%); (***) important minor pollen types (3-15%); and (****) minor pollen types (<3%) [16] (Table 1). The total amount of pollen grains in a honey sample was determined and the results were compared with the classification proposed by Louveaux et al. [17]. The five classes according to the pollen content in 10 g of honey were: **1**: less than 20.000 pollen grains; **2**: 20.000 to 100.000 pollen grains; **3**: 100.000 to 500.000 pollen grains; **4**: 500.000 to 1.000.000 pollen grains and **5**: over 1.000.000 pollen grains.

Preparation of Pollen Slides from Honey Samples and Identification

For pollen analysis, the pollen preparations were prepared as recommended by the International Bee Research Assosiation (Louveaux et al.1970) [12] and modified by Sorkun and Doğan (2002) [18]. Accordingly, 10 grams of each honey was dissolved in 20 ml of distilled water in the sterile test tube. The solution was centrifuged for 45 min. at 3500-4000 rpm. The supernatant solution was poured and small quantities of each pellet at the bottom of the tubes were mounted with basic fuchsine added glycerin gelatin on permanent glass slides.

For microscopic analysis of the pollen taxa of honey samples, two slides were prepared from each sample. Pollen identification and count were carried out using a light microscope (Zeiss Axiolab) with 400× and 1000× objectives, the latter being used when greater detail was required for the morphological identification. For each honey sample, we counted and analyzed a minimum of 1000 pollen grains. Frequency classes were determined twice for each sample and designated as dominant pollen (>45% of a specific pollen type), secondary pollen (16–45%), important minor pollen (3–15%) and minor pollen (<3%).

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Results

During the research, a total of 23 pollen types belonging to 25 families were identified from 16 chestnut honey samples. Although honey samples are unifloral, the plant taxa (pollen types) in each honey sample varies between 7 and 22 (Table 1). Since the honey samples studied are unifloral, the dominant taxon has been identified as Fagaceae (*Castanea sativa*, *Quercus* sp. *Coronilla* sp. *Trifolium* sp.).

Based on the absolute pollen content per 10 g of chestnut honey samples, % 68.75 (n=11) of the samples were found to belong to Group 1 (<20.000 pollen grains per 10 g honey), %18.75 (n=3) to Group 2 (20.000 to 100.000 pollen grains per 10 g honey) and %12.5 (n=2) Group 3 (100.000 to 500.000 pollen grains per 10 g honey) (Table 1).

The number of pollen grains per 10 g of honey, extended from the "very poor" (<20.000) to the "very rich" category (500.000 to 1.000.000) [19]. In our study, honey samples generally "poor" in grains represented % 68.75 of all the samples (Table 1).

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Table 1. Pollen spectra and TNP 10g values obtained from the honey samples collected from various localities in the Black Region

Sample	Locality	Altitude	Pollen spectra	TNP-10g
Sample 1	Samsun Aycacık	76 m	**Castanea sativa* ** *** *****Quercus* sp., *Platanus* sp. *Artemisia* sp., *Robinia* sp., *Salix* sp. Rocaceae, Poaceae, Pappaveraceae, *Acer* sp., Lamiaceae, Chenepodiaceae/Amaranthaceae, *Taraxacum* sp., Asteraceae, *Xanthium* sp.	136 691
Sample 2	Samsun Aycacık	76 m	**Castanea sativa* ** *** *****Platanus* sp., *Artemisia* sp. *Quercus sp.*, Poaceae, Chenepodiaceae/Amaranthaceae, *Robinia sp.*, Rosaceae, Asteraceae, *Pinus sp.*	77 713
Sample 3	Samsun Çarşamba Ordubaşı Village	300 m	**Castanea sativa* ** ****Quercus* sp. *****Platanus* sp., *Artemisia* sp. *Xanthium sp., Salix sp., Robinia sp., Ulmus sp., Inga*, Asteraceae, Brassicaceae, Apiaceae, Chenepodiaceae/Amaranthaceae, Rosaceae, Fabaceae, Poaceae, *Ailanthus* sp., *Convolvulus* sp., Papaveraceae	45 625
Sample 4	Samsun Terme	10 m	**Castanea sativa* ** ****Coronilla* sp ****Apiaceae, *Rubus sp., Quercus sp., Artemisia sp.*, Papaveraceae, Asteraceae, Brassicaceae,*Trifolium sp., Sambucus sp.Platanus sp., Xanthium sp., Salix sp.*, Rosaceae, Convolvulus *sp.*, Poaceae, *Tilia sp., Plantago sp., Lotus sp.*, Lamiaceae	27 898

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Sample	Locality	Altitude	Pollen spectra	TNP-10g
Sample 5	Samsun Salıpazarı	859 m	**Castanea sativa* ** *** *****Coronilla* sp., Quercus sp., Poaceae, Artemisia sp. Apiaceae, *Tilia* sp., Asteraceae, *Papaver* sp. *Trifolium* sp., *Robinia* sp., *Rubus* sp., *Xanthium* sp., Rosaceae, *Salix* sp., *Echium* sp., Lamiaceae, Brassicaceae	39 246
Sample 6	Samsun Salıpazarı	859 m	**Castanea sativa* ***Salix* sp. ***Brassicaceae, *Papaver* sp., *Robinia* sp., *Coronilla* sp., *Quercus* sp. *****Sambucus* sp., *Rubus* sp., Asteraceae, Apiaceae, *Rumex* sp., *Artemisia* sp., *Tilia* sp., *Xanthium* sp.	17 380
Sample 7	Samsun Ayvacık	76 m	**Castanea sativa* ** ****Trifolium* sp. *Salix* sp. *****Papaver* sp., *Sambucus* sp., *Artemisia* sp. Poaceae, *Tilia* sp., *Quercus* sp., Apiaceae, Asteraceae, Caryophyllaceae, Rosaceae, Brassicaceae, *Xanthium* sp., *Robinia* sp.	25 873
Sample 8	Samsun Salıpazarı	859 m	**Castanea sativa* ** ***Asteraceae, *Robinia* sp., Lamiaceae *****Echium* sp., *Quercus* sp., , *Trifolium sp., Rosaceae, Salix sp., Artemisia sp., Papaver sp., Tilia sp.*	99 442
Sample 9	Samsun Tekkeköy	240 m	**Castanea sativa* ** ***Asteraceae, Lamiaceae, *Echium* sp. **** *Quercus* sp., *Trifolium* sp., Rosaceae,*Xanthium* sp., *Salix sp.*, Poaceae, *Artemisia sp., Papaver sp.*, Caryophyllaceae, Chenepodiaceae/Amaranthaceae, *Rumex* sp.	45 960

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Sample	Locality	Altitude	Pollen spectra	TNP-10g
Sample 10	Samsun Salıpazarı	859 m	**Castanea sativa* ** *** *****Echium* sp., *Papaver* sp., *Trifolium* sp., Lamiaceae, Rosaceae, *Salix* sp., *Quercus* sp.,	10 937
Sample 11	Kastamonu Çatalzeytin	74 m	**Castanea sativa* ** *** *****Quercus sp., Trifolium sp., Salix sp., Papaver sp.*, Poaceae, Lamiaceae, Rosaceae, Apiaceae, Asteraceae, *Xanthium sp.*, Chenepodiaceae/Amaranthaceae	52 832
Sample 12	Giresun Yağlıdere	50 m	**Castanea sativa* ** ***Lamiaceae, *Quercus* sp. ****Caryophyllaceae, Apiaceae, *Salix* sp., *Trifolium* sp., *Tilia* sp., Poaceae, *Coronilla* sp., *Robinia* sp., Rosaceae, *Artemisia* sp., *Papaver* sp., Asteraceae	50 470
Sample 13	Kastamonu Günebakan	100 m	**Castanea sativa* ** *** *****Salix sp., Papaver sp.*, Poaceae, *Quercus sp., Trifolium sp.*, Caryophyllaceae, *Robinia sp., Coronilla sp.*, Lamiaceae, *Artemisia sp.*, Apiaceae	133 823
Sample 14	Kastamonu Demirci	220 m	**Castanea sativa* ** *** *****Quercus* sp., *Papaver* sp., *Tilia* sp., Rosaceae, *Trifolium* sp., Caryophyllaceae, Lamiaceae, *Salix* sp., *Robinia* sp.	86 153

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Sample	Locality	Altitude	Pollen spectra	TNP-10g
Sample 15	Kastamonu Bozkurt	10 m	**Castanea sativa*	167 772
			**	
			***	
			*****Tilia sp.*, *Quercus sp.*, Poaceae, *Trifolium sp.*, Caryophyllaceae, *Rumex sp.*, Asteraceae, Apiaceae, Betulaceae	
Sample 16	Sinop Erfelek	300 m	**Castanea sativa*	61 457
			**	
			***	
			*****Salix sp.*, Lamiaceae, *Trifolium sp.*, Poaceae, *Artemisia sp.*, Ericaceae	

(*) predominant pollen types (>45% of the total pollen content); (**) secondary pollen types (16-45%); (***) important minor pollen types (3-15%); and (****) minor pollen types (<3%) [16]

Discussion

Pollen content ant the diversity is most important factor to determine the quality level of honey [20]. Pollen present in the dominant and secondary group are primary contributors to the formation of honey while pollen content in quantities less than other pollen are added to the honey generally by external factors such as wind [21]. The dominant and secondary groups determine the honey content and quality. According to Lieux (1972) [22], the diversity of trace and minor groups has always bigger than diversity of dominant group pollen taxa.

Monofloral honeys are predominantly from a single botanical source (numerous plants and contains only one plant source predominantly) it is. Generally, the taste of monofloral honey is specific [23].

In monofloral honey, the sensory properties of nectar of the dominant plant species are intense. It is felt and the contribution of nectar of other plant species it contains is minor or can also be a work [24]. Honey, produced in a natural environment containing different plant species, is never a single plant. It is impossible to control the honey bee behavior in such an environment is generally accepted. So scientifically

unifloral honey producing is much more difficult than producing multifloral honey [25].

It has been reported that certain types of monofloral honeys are beneficial for human health. Due to its antibacterial properties, such honeys in the treatment of wounds and diseases it is used. Therefore, recently, plant source of monofloral honeys interest in verification is increasing [26].

Melissopalinological analysis is still considered as a suitable method for honey evaluation. Many workers think that acidity and humidity are not the only significant parameters for honey quality; in addition, pollen analysis gives important knowledge about the geographical and botanical origin [27-29, 1], especially- if the plant is an endemic plant [30]. As emphasised by Mandić et al. [31] Europa has more than 100 unifloral honeys but the honeys have local importance and people produce them periodically. Geographical and botanical properties play critical roles about their quality [32]. About 500 plants in Turkey are important nectar and pollen offering beekeeping plants. It is reported that all of this plants are important for beekeeping also 50-60 of them are economically dominant nectar and pollen yields [10, 15].

This study was done with completely monofloral honey samples (Chestnut honey) in the Black Sea Region. The locations of the producers differ in terms of the flora characteristics of both the altitude and the regions where the hives are located. Although the localities where honeys are collected vary in height, no significant result has been observed in terms of the amount and concentration of pollen in honey. The reason for this can be considered as the vegetation in the vicinity where the beehives are located. The fact that there is no classification of pollen amounts specific to chestnut honey has caused the use of general classification principles instead of making a special classification. However, we know that for honey samples such as chestnut honey, when the pollen concentrations are 90% and above, it is defined as monofloral honey.

Conclusion

Black Sea Region, Turkey's economy in terms of major tea, nuts, a region where the cultivation of major agricultural products such as honey. However, due to the wrong agriculture and energy policies

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of the governments, the floral features of the region have been at risk of disappearing. In particular, Hydroelectric Power Plant projects will lead to the drying of freshwater resources in the region, which will cause the destruction of natural vegetation. In this case, quality and especially honey production in the region will be compromised.

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Karadeniz Bölgesi (Türkiye)'nin Bazı Yörelerinde Üretilen Kestane Ballarında Polen Analizi

ÖZ: Bu çalışma, Türkiye'nin Karadeniz Bölgesi'nde bulunan farklı şehirlerden 16 doğal kestane balının polen analizini içermektedir. 2018 yılında Samsun, Sinop, Kastamonu ve Giresun çevresinde 12 farklı bölgeden bal örnekleri toplanmıştır. Bal örnekleri deniz seviyesinden en az 10 metre, en fazla 859 metre yükseklikten alınmıştır. Balların mikroskobik analizi Toplam Polen Sayısı (TPS) kullanılarak gerçekleştirildi. Toplanan örneklerin tamamı unifloral baldır. Çalışmada en fazla polen Bozkurt, Kastamonu'dan alınan örneklerde görülmüştür.

Çalışmanın amacı, Karadeniz Bölgesinin bazı şehirlerinde üretilen monofloral balların polen miktarını ve tayinini yapmaktır. İkinci olarak da Avrupa' da yapılan geniş kapsamlı monofloral bal analizlerini, Karadeniz Bölgesi ve ülkemiz için de yaygınlaştırmaktır.

Anahtar kelimeler: melisopalinoloji, kestane balı, polen anlizi, Samsun, Kastamonu, Sinop, Türkiye

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