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Research Article

Melissopalynological Analysis for Geographical Marking of Kars Honey [1]

Ömür GENÇAY ÇELEMLİ [1,2] Çiğdem ÖZENİRLER [1,2] Nesrin ECEM BAYRAM [3]
Golshan ZARE [4] Kadriye SORKUN [1,2]

[1] *This study is supported by Serhat Development Agency and Beekeepers Association of Kars*

[1] Hacettepe University, Faculty of Science, Department of Biology, TR-06800 Beytepe, Ankara - TURKEY
[2] Hacettepe University Bee and Bee Products Research and Application Center, TR-06800 Beytepe, Ankara - TURKEY
[3] Bayburt University, Aydıntepe Vocational College, Department of Food Processing, TR-69500 Aydıntepe, Bayburt - TURKEY
[4] Hacettepe University, Faculty of Pharrmacy, Department of Pharmaceutical Botany, TR-06100 Sıhhiye, Ankara - TURKEY

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Abstract

In this research, the melissopalynological analysis of honey samples collected from Kars city located in the East Anatolian Region of Turkey was conducted for geographical marking. Within this context, melissopalynological analyses of 100 honey samples determined by sampling method were collected from eight districts of Kars in Eastern Anatolia Region of Turkey were done, to determine the nectarous source plants of Kars honey. As a result of melisopalynological analyses carried out in 100 honey samples; pollens of the taxa belonging to Apiaceae, Asteraceae, Berberidaceae, Betulaceae, Brassicaceae, Boraginaceae, Campanulaceae, Caryophyllaceae, Chenopodiaceae, Cistaceae, Cyperaceae, Dipsacaceae, Ericaceae, Fabaceae, Iridaceae, Lamiaceae, Liliaceae, Malvaceae, Onagraceae, Papaveraceae, Plantaginaceae, Poaceae, Polygonaceae, Ranunculaceae, Rhamnaceae, Rosaceae, Rubiaceae, Rutaceae, Salicaceae and Scrophulariaceae families were detected at different rates. Almost in all of the honey samples, *Lotus corniculatus* (in 99 samples), *Onobrychis radiata* (in 99 samples), *Trifolium nigrescens* (in 88 samples) from Fabaceae family and pollens of *Echium vulgaris* (81 samples) and *Myosotis lithoospermifolia* (15 samples) taxa from the Boraginaceae family, were found in honey samples. *Onobrychis radiata* pollen was the most intensely observed one among these samples (in dominant, secondary, minor, trace amounts). The total number of pollens (TPN-10) in 10 grams of honey were also detected during the melissopalynological analyses. TPN-10 values minimum: 226, maximum: 481157 and mean: 31678 were detected and the pollen abundance of the honeys are classified as good category. Kars is an important province for beekeeping with floral variety. As a result of this study, the first step of the geographical marking studies of Kars' honey was completed.

Keywords: *Kars, Melissopalynology, Honey, TPN-10*

Kars Balının Coğrafi İşaretlemesi İçin Melissopalinolojik Analiz

Özet

Bu çalışmada, Türkiye'nin Doğu Anadolu Bölgesi'nde bulunan Kars İli'nde üretilen balların coğrafi işaretlenmesi için gerekli bir aşama olan melissopalinolojik analizleri yapılmıştır. Bu kapsamda sekiz ilçeden, örnekleme metoduna göre yapılan istatistiksel analizlerle tespit edilen 100 bal örneğinin mikroskobik analizleri gerçekleştirilerek Kars balına kaynaklık eden nektarlı bitkiler tespit edilmiştir. Bu amaçla melissopalinolojik analizleri yapılan 100 adet örnek balda; Apiaceae, Asteraceae, Berberidaceae, Betulaceae, Brassicaceae, Boraginaceae, Campanulaceae, Caryophyllaceae, Chenopodiaceae, Cistaceae, Cyperaceae, Dipsacaceae, Ericaceae, Fabaceae, Iridaceae, Lamiaceae, Liliaceae, Malvaceae, Onagraceae, Papaveraceae, Plantaginaceae, Poaceae, Polygonaceae, Ranunculaceae, Rhamnaceae, Rosaceae, Rubiaceae, Rutaceae, Salicaceae ve Scrophulariaceae familyalarına ait taksonların polenleri değişik oranlarda tespit edilmiştir. Fabaceae familyasından *Lotus corniculatus* (99 örnek), *Onobrychis radiata* (99 örnek), *Trifolium nigrescens* (88 örnek), Boraginaceae familyasından *Echium vulgaris* (81 örnek) ve *Myosotis lithoospermifolia* (15 örnek) taksonlarına ait polenlere hemen hemen tüm bal örneklerinde rastlanılmış (dominant, sekonder, minör, eser) olmakla birlikte bu türler içinde de en yoğun olarak *Onobrychis radiata* polenleri gözlenmiştir. Ayrıca, melissopalinolojik analizler sırasında, ballarda polen teşhisinin yanı sıra 10 gram baldaki toplam polen sayısı (TPS-10) değerleri de hesaplanmıştır. Hesaplamalar sonucunda minimum: 226, maximum: 481157 ve ortalama: 31678 TPS-10 değerleri elde edilerek balların polence zenginlikleri belirtilmiştir. Sonuç olarak, bu çalışma ile arıcılık için floral zenginliğiyle önemli bir il olan Kars'ın ballarına ait coğrafi işaret çalışmalarının ilk basamağı gerçekleştirilmiştir.

Anahtar sözcükler: *Kars, Melissopalinoloji, Bal, TPS-10*

İletişim (Correspondence)
+90 546 2987529
ecem.nesrin@gmail.com

INTRODUCTION

Honey is a unique food product consisting of carbohydrates, amino acids, proteins, organic acids, vitamins, minerals and various phytochemicals. It is produced by bees from the nectar collected from a large variety of flowers, and its chemical composition, physical, sensory and biological properties depend on the nectar source [1]. Honey bees select their forage plants primarily on the basis of the sugar content of the plant nectar which is the raw material of honey [2].

Melissopalynology is of great importance for quality control of honey. Honey always includes numerous pollen grains and honeydew elements, so these contents provide a good fingerprint of the environment where honey comes from. Pollen analysis can therefore be useful to determine and control the geographical and botanical origin of honeys [3]. Multifloral honey can never be derived from a single botanical source. On the contrary, the term "unifloral" honey is used to describe honey produced mostly from one species. Generally, the pollen content for a honey to be called "unifloral," the percentage should be at least 45% of the total pollen count [4].

Due to the location of Turkey, different climatic conditions and plant cover can be observed in this country. Turkey includes three phyto-geographical and seven geographical regions. Turkey has a rich and interesting floristic structure. It has more than 10.000 plant species naturally and culturally grown and nearly 450 species are nectary plants which are known to be important in apiculture [5]. There are 9222 naturally grown species in Turkey and 3.000 of these are endemic [6]. Because of its climatic conditions and flora, Turkish honey is quite valuable.

Turkey has an important place among honey producing countries in the world. In Turkey, production of honey amounted to 105727 tons in 2016 (http://www.tuik.gov.tr). Kars is located in East Anatolia region of Turkey and also beekeeping in Kars is over average in Turkey's ratings of honey production per hive.

Pollen analysis of Turkish honey was firstly done by Sorkun and İnceoğlu [7]. Subsequently, more research about microscopic analysis of Turkish honey was carried out by other researches parallel to world literature [7-11]. By this study, we aimed to analyse honey samples produced in Kars to make geographical marking of Kars honey. These results will be a step towards further studies.

MATERIAL and METHODS

Collection Of Plant Materials for Reference Pollen Slides

In field study, 138 plants were collected from surrounding beehives that honey samples are collected from. After the identification of plants, pollen slides of these plants were prepared as reference slides.

Statistical Methods

Firstly, all the number of stable beehives in Kars were determined. It was observed that 399 beehives are stable in Kars region. Random sampling method were used to determine the number of beehives to collect honey samples instead of collecting from all 399 beehives. According to the statistical results analyzing 100 samples of were sufficient to form an opinion about Kars honey.

Collection of Honey Samples

Honey samples were collected from eight towns of Kars. The number of beehives for each town, that the samples were collected from, are determined according to the random sampling method-statistical analysis. The towns and the samples collected from them are given in *Fig. 1*.

Preparation of Pollen Slides for Botanical Origin

The floral sources of honey samples were determined by the mellisopalynological method. The materials were prepared for examination under the microscope according to the method of Louveaux et al.[12] and Sorkun [13]. Accordingly, 10 g of stock honey samples thoroughly mixed with a sterile glass rod were taken and transferred to the test tube and then 20 mL of distilled water was added. For dissolution of the honey sample in water, the tubes were placed in a water bath at about 45°C for 10-15 min and then each tube was shaken by a stirrer. The solution is then centrifuged at 3500 rpm for 45 min and the supernatant fraction is poured off. The precipitate remaining at the bottom of the tube was infused with a quantity of basic-fucose added glycerin-gelatin taken from the needle tip, and this material was then transferred onto the slide. The slide was heated at 30-40°C to allow the dissolution of basic fuchsine, and was added glycerin gelatin. Then, 18x18 lamella was covered on top of it. The preparation was left to stand for about 12 h upside down, and then it became available for examination under microscobe. In the diagnosis of pollen grains, the microphotographs of pollens in literature and reference preparations were used [13]. And then, observed pollen types were classified into four categories: dominant pollen (≥45%, D), secondary pollen (16-44%, S), important minor pollen (>3-15%, M) and rare polen (3%<). When one pollen type represented >45% of the total number of pollen grains, the sample was classified as a monofloral honey [14]. Besides the determination of botanical origin, the total pollen number (TPN-10) of all samples were calculated according to the Moar [15].

Preparation of Slides for Total Number of Pollens

In order to determine the Total Number of Pollen types (TNP in 10 g honey), pollen preparations were prepared according to the method that was described by Sorkun

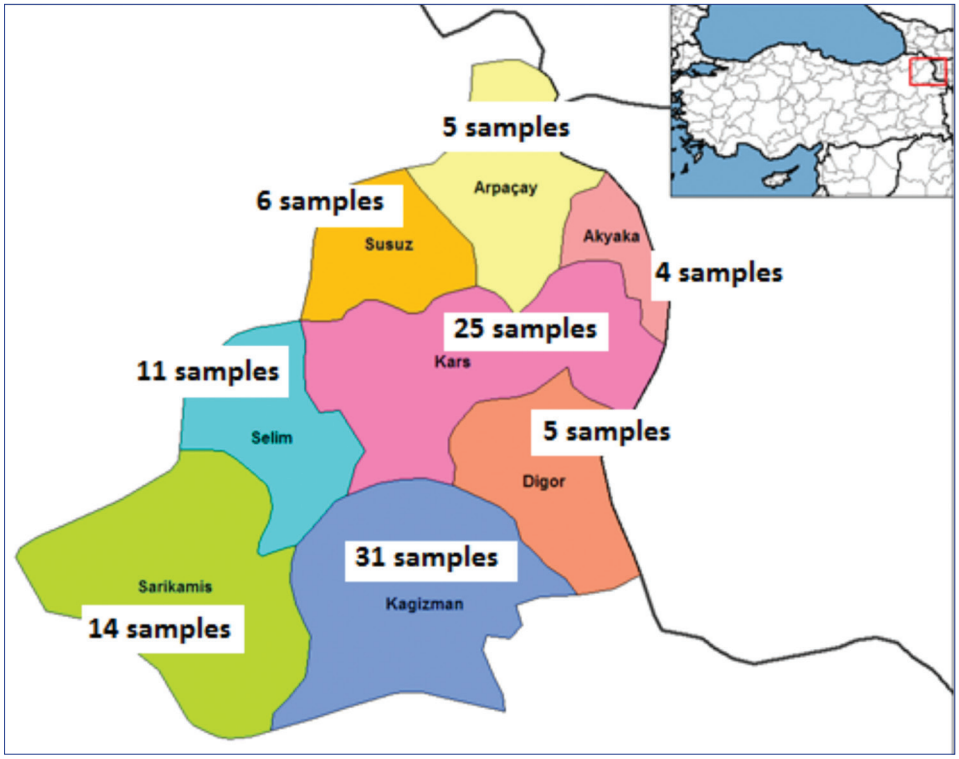

Fig 1. Map of Kars province (http://tr.wikipedia.org/wiki/Kars_(il) 2014)

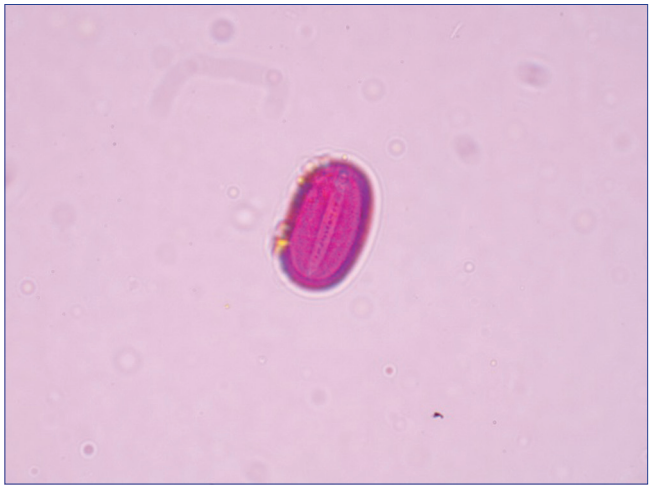

Fig 2. Microphotograph of *Onobrychis radiata* pollen

and Dogan [16]. According to this, 10 g from the stock honey was homogenized by mixing it thoroughly with a sterile glass rod. Then, 20 mL distilled water was added and a tablet containing 12542 *Lycopodium* spores was also put into the tube to control. After the tablet dissolved in the water, the tube was centrifuged at 3500-4000 rpm for 30 min. And then, the supernatant liquid was then poured off. To strain the water completely out of the tubes, the tubes were turned upside down onto a drying paper. Glycerine and precipitate were mixed homogeneously by adding 0.1 mL 50% of glycerine and a very little amount of bazic fuksin into the tube. 0.01 mL was taken from this mixture and put on a microscope slide, and the material was covered with 18x18 mm^2 of lamella. And then, the TNP-10 g preparations were examined under a light microscobe. At this stage, 10X objective was used for pollen counting. Finally, pollen classifications were made according to Moar et al.[15] and Maurizio and Hodges [17].

RESULTS

The pollens of the plants belonging to the family Apiaceae, Asteraceae, Berberidaceae, Betulaceae, Brassicaceae, Boraginaceae, Campanulaceae, Caryophyllaceae, Cheno podiaceae, Cistaceae, Cyperaceae, Dipsacaceae, Ericaceae, Fabaceae, Iridaceae, Lamiaceae, Liliaceae, Malvaceae, Onagraceae, Papaveraceae, Plantaginaceae, Poaceae, Polygonaceae, Ranunculaceae, Rhamnaceae, Rosaceae, Rubiaceae, Rutaceae, Salicaceae and Scrophulariaceae were found at different rates in the honey samples of the Kars region. Especially, pollens belonging to Fabaceae, Boraginaceae and Asteraceae families were frequently observed in honey samples. Pollens belonging to *Lotus corniculatus, Onobrychis radiate*, *Trifolium nigrescens* from Fabaceae family, *Echium vulgaris* and *Myosotis lithoospermifolia* from Boraginaceae family were observed frequently (dominant, secondary, minor, rare) nearly in all the investigated samples. The microphotograph of *Onobrychis radiata* pollen is shown in *Fig. 2*.

The pollen of the following taxa was found in the samples; *Carum* spp., *Eryngium billardieri*, *Malabaila dasyantha* from Apiaceae; *Achillea* spp., *Carduus nutans*, *Centaurea depressa*, *Centaurea triumfetti*, *Tussilago* spp., *Xanthium* spp., *Taraxacum* spp. from Asteraceae; *Sisymbrium elatum*, *Sinapis arvensis* from Brassicaceae; *Echium vulgaris*, *Cerinthe minör*,

Myosostis lithoospermifolia, *Rindera lanata*, *Silene vulgaris* from Boraginaceae; *Scabiosa columbaria* from Dipsacaceae; *Astragalus* spp., *Astragalus lagurus*, *Coronilla varia*, *Hedysarum* spp., *Lotus corniculatus*, *Medicago falcata*, *Trifolium repens*, *Trifolium nigrescens*, *Onobrychis radiata*, *Vicia sativa*, *Melilotus officinalis*, *Trifolium pratense*, *Trifolium ochrleucum*, *Onobrychis oxyodonta*, *Onobrychis tournefortii*, *Onobrychis* spp., *Lathyrus rotundifolius* from Fabaceae; *Iris* spp. from Iridaceae; *Salvia* spp., *Teucrium chamaedrys*, *Teucrium orientalis*, *Thymus longicaulis*, *Teucrium* spp., *Teucrium polium* from Lamiaceae; *Allium* spp. *Ornithagalum* spp. from Liliaceae; *Epilobium* spp. from Onagraceae; *Plantago lanceolata* from Plantaginaceae; *Rumex* spp. from Polygonaceae; *Nigella arvensis*, *Consolida orientalis* from Ranunculaceae; *Galium* spp. from Rubiaceae; *Salix* spp. from Salicaceae; *Linaria genistifolia* from Scrophulariaceae.

In short, the pollens identified by microscopic analysis of honey samples reflect the flora of Kars city. Plus, it is observed that the plants collected from the surroundings of the beehives show a resemblance with the melisso-playnological results.

TPN-10 values were calculated after mellisopalynological analysis and 226 was found as minimum, 481157 as maximum, 31 678 as mean value. The TPN-10 values and groups of honey samples are presented in *Table 1*. Classification of honey samples according to TPN-10 values was done according to Maurizio [18]. Accordingly, honey samples based on TPN-10 values were classified as group I (<20.000 pollen grains per 10 g honey), group II (20.000-100.000 pollen grains per 10 g honey), group III (100.000-500.000 grains per 10 g honey), group IV (500.000 -1.000.000 grains per 10 g honey), group V (>1.000.000 grains per 10 g honey). Also, honeys with very low pollen content, normal-pollen honeys and honeys with very rich pollen, were included in Group I, Group II and Group III, respectively [19].

DISCUSSION

As a result of the melissopalynologic analysis, it is possible to determine from which plants the honey is produced. In our study, as a result of the melissopalynologic analysis, 54 plant taxa belonging to 30 families were diagnosed in honey samples at different rates in the honey samples of the Kars region. Especially, pollens belonging to Fabaceae, Boraginaceae and Asteraceae families were frequently observed in honey samples. Consequently, important information on the nectar resources of the region has been obtained. These results indicate that honey samples from Kars are highly varied in terms of pollen content. It was an expected result that there was to be a lot of pollen diversity in honey samples from Kars province due to its climate, geographical position and rich plant cover of this region. Of the 100 samples analyzed, 21 were identified as unifloral and 79 as multiforal honey. Also, the pollens from

Table 1. *TPN-10 values of honey samples*

Town	Sample No	TPN-10	Groups
AKYAKA	28	25808	II
AKYAKA	53	9648	I
AKYAKA	54	4515	I
AKYAKA	60	15869	I
ARPAÇAY	9	481157	III
ARPAÇAY	27	26517	II
ARPAÇAY	39	24770	II
ARPAÇAY	64	25681	II
ARPAÇAY	65	55231	II
DİGOR	1	42303	II
DİGOR	10	5664	I
DİGOR	35	17311	I
DİGOR	46	22804	II
KAĞIZMAN	5	10034	I
KAĞIZMAN	19	6394	I
KAĞIZMAN	42	28920	II
KAĞIZMAN	43	10033	I
KAĞIZMAN	44	10750	I
KAĞIZMAN	45	36058	II
KAĞIZMAN	47	8026	I
KAĞIZMAN	48	29045	II
KAĞIZMAN	49	17366	I
KAĞIZMAN	50	16723	I
KAĞIZMAN	51	21038	II
KAĞIZMAN	52	17917	I
KAĞIZMAN	55	15305	I
KAĞIZMAN	56	7066	I
KAĞIZMAN	57	16461	I
KAĞIZMAN	58	58909	II
KAĞIZMAN	59	11208	I
KAĞIZMAN	87	23383	II
KAĞIZMAN	88	30691	II
KAĞIZMAN	89	17482	I
KAĞIZMAN	90	9345	I
KAĞIZMAN	91	16230	I
KAĞIZMAN	92	11328	I
KAĞIZMAN	93	6601	I
KAĞIZMAN	94	8466	I
KAĞIZMAN	95	16917	I
KAĞIZMAN	96	24883	II
KAĞIZMAN	97	20784	II
KAĞIZMAN	98	20381	II
KAĞIZMAN	99	6532	I
KAĞIZMAN	100	15241	I
MERKEZ	2	41807	II

Table 1. *TPN-10 values of honey samples (Continue)*

Town	Sample No	TPN-10	Groups
MERKEZ	3	2675	I
MERKEZ	4	46063	II
MERKEZ	11	226	I
MERKEZ	12	14165	I
MERKEZ	13	11825	I
MERKEZ	14	10091	I
MERKEZ	18	4561	I
MERKEZ	21	19462	I
MERKEZ	22	12425	I
MERKEZ	24	8710	I
MERKEZ	25	8361	I
MERKEZ	29	51804	II
MERKEZ	30	28832	II
MERKEZ	33	55635	II
MERKEZ	67	37009	II
MERKEZ	68	13159	I
MERKEZ	69	33369	II
MERKEZ	70	14856	I
MERKEZ	71	31260	II
MERKEZ	72	140440	III
MERKEZ	73	85442	II
MERKEZ	74	8640	I
MERKEZ	75	134516	III
MERKEZ	76	20839	II
SARIKAMIŞ	6	19020	I
SARIKAMIŞ	16	16278	I
SARIKAMIŞ	34	75252	II
SARIKAMIŞ	36	33905	II
SARIKAMIŞ	77	32426	II
SARIKAMIŞ	78	38364	II
SARIKAMIŞ	79	25762	II
SARIKAMIŞ	80	34620	II
SARIKAMIŞ	81	47158	II
SARIKAMIŞ	82	44266	II
SARIKAMIŞ	83	23154	II
SARIKAMIŞ	84	10083	I
SARIKAMIŞ	85	929	I
SARIKAMIŞ	86	38382	II
SELİM	7	6055	I
SELİM	15	15402	I
SELİM	20	143774	III
SELİM	23	6482	I
SELİM	26	21395	II
SELİM	32	55635	II
SELİM	37	7378	I

Table 1. *TPN-10 values of honey samples (Continue)*

Town	Sample No	TPN-10	Groups
SELİM	41	27519	II
SELİM	61	11901	I
SELİM	62	11288	I
SELİM	63	13259	I
SUSUZ	8	28035	II
SUSUZ	17	6689	I
SUSUZ	31	44655	II
SUSUZ	38	35776	II
SUSUZ	40	22234	II
SUSUZ	66	11208	I

Lotus corniculatus, Onobrychis radiata, Trifolium nigrescens taxa of family Fabaceae and *Echium vulgaris* taxa of family Boraginaceae were frequently found in almost all honey samples (as dominant, secondary, minor and trace) and among these taxa, *Onobrychis radiata* pollens were the most intense. It can be said that the taxa, which are determined to be predominant in honey samples, play a very important role in the composition of honey.

In our study, the pollen of Fabaceae was detected at different rates in all of the samples, dominant in 16 samples. On the other hand, the pollens of *Lotus corniculatus* (in 1 sample), *Trifolium nigrescens* (in 3 samples) and *Onobrychis radiata* (in 12 samples) were determined as dominant. The pollen of *Onobrychis radiata* from Fabaceae was detected in 99 of 100 samples as dominant (in 12 samples) and secondary (in 48 samples). These results suggest that *Onobrychis radiata* pollen could be a marker for Kars honey. Also, we have found pollen of *Lotus corniculatus* from Fabaceae in 99 samples as dominant (in 1 sample), secondary (in 38 samples), minor (in 50 samples) and rare (in 10 samples). *Trifolium nigrescens* pollen was detected in 80 samples as dominant (in 3 samples) and secondary (in 7 samples). In addition, pollens of *Astragalus* spp., *Astragalus lagurus, Coronilla varia, Hedysarum, Lathyrus rotundifolius, Medicago falcata, Medicago sativa, Melilotus officinalis, Trifolium ochrleucum, Onobrychis spp., Onobrychis tournefortti, Trifolium repens, Trifolium pratense, Onobrychis oxyodonta* and *Vicia sativa* taxa were found as secondary, minor and rare. Similary, Silici ve Gökçeoğlu [11] found that pollens of *Trifolium* spp. (in 3 samples) and *Astragalus* spp. (in 1 sample) were secondary in Antalya honeys. Plants such as *Trifolium, Lotus* (trefoil), and *Astragalus*, which have a long flowering period and are used as sources of pollen and nectar by bees, were also frequently observed.The results of our study indicate that these plants are also used as source of nectar in Kars region. On the other hand, in a different study it was reported that pollen of Fabacaea, *Castanea sativa* and Euphorbiaceae taxa were observed as secondary in honey samples from Kars region [19]. Contrary to these results, in our study, the pollen of *Castanea*

sativa and Euphorbiaceae taxa were not found in any sample. These results may be due to plant flora where the samples are collected from, which indicate that there is no distribution area of these plants in the regions where the samples examined in the scope of our study are collected.

We found pollens of Boraginace in all the samples. Pollens of *Echium vulgaris* (in 81 samples), *Cerinthe minör* (in 42 samples), *Myosotis lithoospermifolia* (in 15 samples), *Bunglossoides arvernsis* (in 8 samples), *Rindera lanata* (in 1 sample), *Anchusa* (in 1 sample) taxa from Boraginacaea found in honey samples. Among these, pollens of *Echium vulagris* (in samples 6, 37 and 72) and *Myosotis lithoospermifolia* (in samples 11 and 48) were detected as dominant.

From Asteraceae, pollen of *Achillea* spp. (in 24 samples), *Carduus nutans* (in 3 samples), *Centaurea depressa* (in 26 samples), *Centaurea triumfetti* (in 33 samples), *Tussilago* spp. (in 1 sample), *Xanthium* spp. (in 3 samples), *Taraxacum* spp. (in 8 samples) taxa were determined as minor and rare in honey samples. Similarly, many studies have been conducted to determine the origin of honey in samples collected from different origins [20, 21]. A group of researchers reported that they found pollens of plants belonging to the families Apiaceae, Asteraceae, Fabaceae and Rosaceae in honey samples as a result of their analysis of 25 honey samples [11]. In another study, it was reported that pollen of the *Hedera helix, Gossypium, Trifolium* (Kırıkkale), *Sophora, Rhododendron, Castanea sativa, Peganum harmala, Helianthus* taxa were identified as dominant in 13 floral honey collected from different regions of Turkey [22]. Similar to this study, we have detected pollen of *Trifolium nigrescens* was dominant in 3 honey samples. Temizer et al.[23] reported that Fabaceae taxa were common in honey samples collected from Giresun region. Similarly, Can et al.[24] reported that Fabaceae, *Trifolium* and *Rubus* taxa as predominant were found in honey samples collected from Kars province.

On the other hand, honey samples classified according to TPN-10 values. The TPN-10 value of 100 honey samples analyzed in the course of this study was determined between 226 and 481157. It was determined that 52 samples belong to group I (honey samples with low pollen content), 44 samples belong to group II (honey samples with pollen content at normal levels), and 4 samples belong to group III (honey samples with a very rich pollen content). It was detected that the honey sample with the least amount of pollen was sample 11 from Merkez (Group I) and the honey sample with the highest amount of pollen was sample 9 from Arpaçay (Group III). According to analysis carried out by Başoğlu et al.[25] on 25 honey samples collected from different regions of Turkey, it was detected that TPN-10 was between 400 and 12.400 in the 7 honey samples that were thought to be artificial, while it was between 14.800-37.800 in the 16 honey samples that were designated as pure honey. Moreover, it was found that TPN-10 was above the limit of 1.000.0000 in two honey samples, one of which was collected from Kars province. In another study, it was reported that the total number of pollen in two honey samples collected from Kars province was between 22713 and 6685 [19]. Similarly, in the study conducted by Sorkun and Doğan [16], it was reported that among 127 samples of natural flower honey samples collected from various regions of Turkey, TPN-10 was between 54383 and 38112 and TPN-10 in 42 artificial honey was between 954 and 4983. Researchers have reported that the value of TPN-10 in natural honey should be between 20.000 and 100.000 but this value can get below 20.000 in honey samples of Lamiaceae and Boraginacea families. Consistent with these results, we have determined that *Myosotis lithosppermifolia* (Boraginaceae) was the honey with the lowest TPN-10 value. Similar results with our study were also found in honey samples from diverse origins [23,26].

The honey samples were obtained from Kars province, located in Northeast Turkey and part of the Irano-Turanian phytogeographical region. The area is a pass between Caucasia and Anatolia. In addition, due to its geological, morphological and climatological differences, Kars region is also very rich in terms of plant diversity, which is the main source of beekeeping activities. For these reasons, it is not surprising that there is a rich content of honey samples produced in this region [27]. Sorkun and Yuluğ [9] also did melisopalynological investigations in this region with a narrower scope and found that *Onobrychis radiata* pollens are the most frequent plant. It is understood by this research that the 28 years of process between the two studies did not cause any serious change in the flora and vegetation.

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Conflict in Interest

The authors declare no competing financial interest.

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