Melissopalynological Analysis for Geographical Marking of Kars Honey

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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 in Eastern Anatolia Region of Turkey were done, to determine the nectarous source plants of Kars honey. As a result of melissopalynological 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, Melissopalinology, Honey, TPN-10

Kars Balının Coğrafi İşaretlemesi İçin Melissopalinolojik Analiz

Özet

Bu çalışmada, Türkiye’nin Doğu Anadolu Bölgesi’nde bulunan Kars ilinde üretilen balların coğrafi işaretlenmesi için gerekli bir aşama olan melissopalinolojik analizleri yapılmıştır. Bu kapsamda sezik ilçede toplanan bal örnekleri ile 100 bal örneklemesi hazırlanmıştır. Bu örneklemelerde, 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, Rubiaceae, Rutaceae, Salicaceae ve Scrophulariaceae familyalarına ait polenler tespit edilmiştir. Bu polenlerin sıklığına bakıldığında, Kars balının florasi ve coğrafi işaretlenmesi açısından önemlidir. Bu çalışmada, Kars balının coğrafi işaretlenmesi için melissopalinolojik analiz uygulandı.

Anahtar sözcükler: Kars, Melissopalinoloji, Bal, TPN-10

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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 3000 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 melissopalynological 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 fuchsin, 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 microscope. 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 pollen (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.
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 basic fuchsins into the tube. 0.01 mL was taken from this mixture and put on a microscope slide, and the material was covered with 18x18 mm² of lamella. And then, the TNP-10 g preparations were examined under a light microscope. At this stage, 10X objective was used for pollen counting. Finally, pollen classifications were made according to Moar et al. and Maurizio and Hodges.

RESULTS

The pollen of the following taxa was found in the samples; Carum spp., Eryngium billardieri, Malabailla 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,

Fig 2. Microphotograph of Onobrychis radiata pollen
Melissopalynological Analysis for ...  

**Myosostis lithoospermifolia**, *Rindera lanata*, *Silene vulgaris* from Boraginaceae; *Scabiosa columbaria* from Dipsacaceae; *Astragalus* spp., *Astragalus lagurus*, *Coronilla varia*, *Hedysarum oxydonta*, *Onobrychis tournefortii*, *Onobrychis* spp., *Lotus corniculatus*, *Medicago falcata*, *Trifolium repens*, *Trifolium nigrescens*, *Vicia sativa*, *Mellolus officinalis*, *Triofolium pratense*, *Triofolium ochreulum*, *Onobrychis* spp., *Lathyrus rotundifolius* from Fabaceae; *Iris* spp. from Iridaceae; *Salvia* spp., *Teucrium chamaedrys*, *Teucrium orientalis* from Lamiaceae; *Allium* spp., *Ornithagalam* spp. from Liliaceae; *Epilobium* spp. from Onagraceae; *Plantago lanceolata* from Plantaginaceae; *Rumex* spp. from Polygonaceae; *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 bee hives show a resemblance with the melissopalynological results.

TPN-10 values were calculated after melissopalynological analysis and 226 was found as minimum, 481157 as maximum, 31678 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 multifloral honey. Also, the pollens from

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**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     |
| KAŞIZMAN | 5         | 10034   | I      |
| KAŞIZMAN | 19        | 6394    | I      |
| KAŞIZMAN | 42        | 28920   | II     |
| KAŞIZMAN | 43        | 10033   | I      |
| KAŞIZMAN | 44        | 10750   | I      |
| KAŞIZMAN | 45        | 36058   | II     |
| KAŞIZMAN | 47        | 8026    | I      |
| KAŞIZMAN | 48        | 29045   | II     |
| KAŞIZMAN | 49        | 17366   | I      |
| KAŞIZMAN | 50        | 16723   | I      |
| KAŞIZMAN | 51        | 21038   | II     |
| KAŞIZMAN | 52        | 17917   | I      |
| KAŞIZMAN | 55        | 15305   | I      |
| KAŞIZMAN | 56        | 7066    | I      |
| KAŞIZMAN | 57        | 16461   | I      |
| KAŞIZMAN | 58        | 58909   | II     |
| KAŞIZMAN | 59        | 11208   | I      |
| KAŞIZMAN | 87        | 23383   | II     |
| KAŞIZMAN | 88        | 30691   | II     |
| KAŞIZMAN | 89        | 17482   | I      |
| KAŞIZMAN | 90        | 9345    | I      |
| KAŞIZMAN | 91        | 16230   | I      |
| KAŞIZMAN | 92        | 11328   | I      |
| KAŞIZMAN | 93        | 6601    | I      |
| KAŞIZMAN | 94        | 8466    | I      |
| KAŞIZMAN | 95        | 16917   | I      |
| KAŞIZMAN | 96        | 24883   | II     |
| KAŞIZMAN | 97        | 20784   | II     |
| KAŞIZMAN | 98        | 20381   | II     |
| KAŞIZMAN | 99        | 6532    | I      |
| KAŞIZMAN | 100       | 15241   | I      |
| MERKEZ   | 2         | 41807   | II     |
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 ochroleucum, Onobrychis spp., Onobrychis tournefortii, Trifolium repens, Trifolium pratense, Onobrychis oxyodonta and Vicia sativa taxa were found as secondary, minor and rare. Similarly, 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 Fabaceae, 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

| 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  | I      |
| 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     |
| SELIM    | 7         | 6055   | I      |
| SELIM    | 15        | 15402  | I      |
| SELIM    | 20        | 143774 | III    |
| SELIM    | 23        | 6482   | I      |
| SELIM    | 26        | 21395  | II     |
| SELIM    | 32        | 55635  | II     |
| SELIM    | 37        | 7378   | I      |

| Town     | Sample No | TPN-10 | Groups |
|----------|-----------|--------|--------|
| SELIM    | 41        | 27519  | II     |
| SELIM    | 61        | 11901  | I      |
| SELIM    | 62        | 11288  | I      |
| SELIM    | 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      |
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 produced in this region. Sorkun and Yuluğ also did melissopalynological 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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