

Original Article

Melissopalynological Evaluation of Honey from Ganja Gazakh Region of Azerbaijan

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ABSTRACT

This study provides a comprehensive melissopalynological analysis of honey samples from the Ganja Gazakh region of Azerbaijan, offering valuable insights into the diversity and abundance of plant species visited by honey bees in the area. A total of 23 honey samples were examined using light microscopy to identify and quantify the pollen types and their frequencies. The results revealed that the most abundant plant families in the honey samples were Asteraceae, Fabaceae, Rosaceae, and Lamiaceae. This finding suggests that these plant families are important sources of nectar and pollen for honey bees. Notably, the Fabaceae family was found to be the most prevalent, with pollen grains present in all 23 honey samples, ranging from 11.5% to 66% in frequency. Within the Fabaceae family, *Glycyrrhiza glabra* was the most dominant species, appearing in 16 samples. Other Fabaceae taxa, including *Astragalus* spp., *Lotus* spp., *Medicago* spp., *Melilotus* spp., *Onobrychis* spp., *Robinia* spp., *Trifolium* spp., and *Vicia* spp. were also identified at varying frequencies. These findings highlight the melliferous potential of the native flora in the Ganja Gazakh region and provide valuable information for consumers, beekeepers, and regulatory bodies to ensure the authenticity and traceability of the region's honey. The study contributes to the growing body of knowledge on the botanical origins of honey and the foraging preferences of honey bees in different geographical areas.

**Key words:** Palynological, honey bee, microscopic, pollen, botanical origin

Azerbaycan Gence Kazak Bölgesi Ballarının Melissopalinolojik Olarak Değerlendirmesi

Öz

Bu çalışma, Azerbaycan'ın Gence Kazak Bölgesi'nden alınan bal örneklerinin kapsamlı melissopalinolojik analizini sunmakta, bölgedeki bal arısı florası çeşitliliği ve bolluğu hakkında bilgiler vermektedir. Toplam 23 bal örneği, içeriğinde yer alan polen tiplerinin ve sıklıklarının belirlenmesi için ışık mikroskobu kullanılarak incelenmiştir. Sonuçlar, bal örneklerindeki en baskın bal arısı florası ailelerinin Asteraceae, Fabaceae, Rosaceae ve Lamiaceae olduğunu göstermiştir. Bu bulgu, bu bitki ailelerinin bal arıları için önemli nektar ve polen kaynakları olduğunu ortaya koymaktadır. Özellikle Fabaceae ailesi en baskın aile olarak öne çıkmış, bu familyaya ait polen taneleri 23 bal örneğinin tamamında toplamda %11,5 ile %66 arasında değişen sıklıklarda tespit edilmiştir. Fabaceae ailesi içinde *Glycyrrhiza glabra* belirlenen en baskın tür olup, 16 örnekte görülmüştür. *Astragalus* spp., *Lotus* spp., *Medicago* spp., *Melilotus* spp., *Onobrychis* spp., *Robinia* spp., *Trifolium* spp. ve *Vicia* spp. gibi diğer Fabaceae taksonları da değişen sıklıklarda tanımlanmıştır. Bu bulgular, Gence Kazak Bölgesi'ndeki doğal floranın arı florası potansiyelini vurgulamakta ve tüketiciler, arıcılar ve düzenleyici kurumlar için bölgenin balının izlenebilirliğini sağlamada değerli bilgiler sunmaktadır. Çalışma, farklı coğrafi alanlardaki bal arılarının beslenme

tercihlerinin belirlenmesine ve balın botanik kökenlerine ilişkin bilgi birikiminin geliştirilmesine katkı sağlamaktadır.

**Anahtar kelimeler:** Palinolojik, bal arısı, mikroskopik, polen, botanik kaynak

## INTRODUCTION

Honey is the most consumed and economically significant bee product by humans (El Sohaimy et al., 2015). Honey is produced by honey bees collecting nectar from floral or extrafloral nectaries found on various parts of plants (such as leaves, stems, etc.), or from secretions of insects feeding on living parts of plants (Karabagias et al., 2014), and then processing it by removing its moisture and adding enzymes through their bodies to produce a viscous and high-energy natural sweet product known as honey (Liu et al., 2013).

It is the most complex food found in nature and can be used by humans without processing (González-Miret et al., 2007). While honey serves as a sweetener in many foods (Ouchemoukh et al., 2010), it is also used as a raw material in the pharmaceutical and cosmetic industries (Mattonai et al., 2016). Honey has been accepted as a part of apitherapy since ancient times. Therefore, it is used in the treatment of burns, gastrointestinal diseases, chronic wounds, asthma, skin ulcers, cataracts, and other diseases due to its antimicrobial, antioxidant, antiviral, antiparasitic, anti-inflammatory, anticancer, and immune-enhancing activities (Küçük et al., 2007; Samarghandian, et al., 2017). The health benefits of honey are also mentioned in the holy text of various religions. Thus, honey is a product adopted by all cultural and religious beliefs (Nayik, et al., 2018).

Depending on the plant source, honey contains approximately 200 different substances. Sugar and water are the main components of honey. Additionally, honey contains minerals, proteins, free amino acids, enzymes, vitamins, organic acids, flavonoids, phenolic acids, and other phytochemicals (Bentabol Manzanares et al. 2011; Karabagias et al. 2014). The composition of honey depends on factors such as its botanical source, processing of honey, environmental conditions such as climate and temperature of the region where honey is obtained (Nayik et al. 2018). Considering the diversity of nectar-bearing plants, it is understandable that the contents of honeys vary greatly from one another.

The botanical and geographical origin of honey have become a significant focus for ensuring its quality and authenticity (Alghamdi et al., 2020). In many parts of the world, the only official procedure used to determine the floral source of honey is melissopalynological analysis, which involves microscopic examination of pollen grains contained in honey. Honey's purity, geographical origin, and botanical source are all been ascertained through the melissopalynology, an efficient procedure (Shakoori et al., 2023). Melissopalynology, a specialized sub-discipline, sheds light on the environmental interactions and feeding behaviors of honey bees. It also plays a key role in assessing the purity of honey. Melissopalynology enables the identification of pollen grains in the honey by analyzing various morphological features such as pollen diameter, exine thickness, pollen type, class, aperture, and exine ornamentation. These characteristics observed using with different microscopy techniques, allow for precise differentiation of pollen at the family, genus, and species levels. This method provides pollens as a key that distinguishes different honey varieties based on their botanical and geographical origins. Additionally, it offers a comprehensive profile of the pollen and nectar plants consumed by honeybees, aiding in the evaluation of a region's melliferous potential.

Due to its geographical location, Azerbaijan have nine of the eleven climate zones found globally (Mehtiyeva and Zeynalova, 2013). The diversity of Azerbaijan's soil and climatic conditions supports the variety of plant genetic resources. Azerbaijan have approximately 4500 naturally occurring plant species, 4.74% of them are endemic (Musayev and Akparov, 2015). Additionally, 720 plant species found in the country are known as nectariferous plants (Kamboj et al., 2013). The climate conditions of the Ganja-Gazakh Economic Region vary due to differences in elevation. In the plains (below 500 meters), the climate is arid and hot, while the slopes experience a mild and semi-arid climate. In contrast, the mountainous areas are characterized by a humid and cold climate (Seyidov, 2003). Due to its climate and variations in elevation, the Ganja-Gazakh Economic Region is one of the most suitable areas for beekeeping, boasting an abundance of diverse and high-quality nectar-producing plants. Melliferous flora from the taxa *Ajuga* spp., *Amygdalus communis*, *Arctium lappa*, *Astragalus* spp., *Aster* spp., *Brassica oleracea*, *Calamintha clinopodium*, *Centaurea* spp., *Cynoglossum officinale*, *Echium* spp., *Echinops* spp., *Epilobium* spp., *Helianthus cultus*, *Lamium* spp., *Leonurus villosus*, *Lonicera* spp., *Lythrum salicaria*, *Melilotus officinalis*, *Mentha longifolia*, *Nepeta* spp., *Onobrychis* spp., *Origanum vulgare*, *Phlomis* spp., *Robinia pseudoacacia*, *Symphytum* spp., *Teucrium* spp., *Thymus* spp., and *Trifolium* spp. have been reported earlier (Qualiyev, 2014) .

Understanding the botanical and geographic origins of honey is a fundamental requirement for identification of it's authenticity, emphasizing the importance of this research. The aim of the study was to

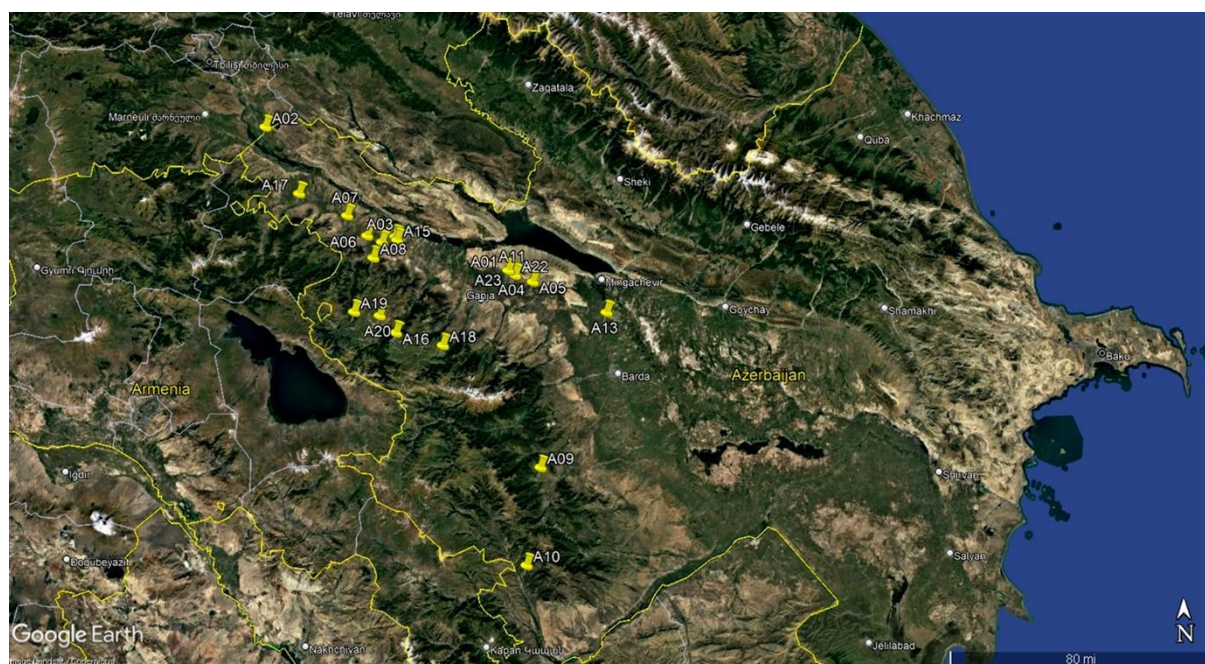
evaluate 23 honey samples from Aghstafa, Dashkasan, Gadabay, Goranboy, Gazakh, Samukh, Shamkir, and Tovuz administrative districts of Azerbaijan Ganja-Gazakh Economic Region based on melissopalynological analyses. This study represents the first scientific investigation of melissopalynological analysis of Azerbaijan Ganja Gazakh Economical Region, providing novel insights about bee flora of this region. Background and significance of the problem by using the most recent publications should be provided.

## MATERIALS AND METHODS

### MATERIALS

#### Honey Samples

Honey samples were obtained from eight different administrative district (Aghstafa, Dashkasan, Gadabay, Goranboy, Gazakh, Samukh, Shamkir, and Tovuz) of Azerbaijan Ganja-Gazakh Region (Table 1., Figure 1.). Samples were provided directly from beekeepers in clean glass jar and stored in the dark +4 °C until analysis.



**Figure 1.** Map of the sampling area

**Table 1.** Honey samples' name, administrative district, municipality and altitude

| Samples name | Administrative district | Municipality | Altitude(m) |
|--------------|-------------------------|--------------|-------------|
| A02          | Aghstafa                | Sadıqlı      | <500        |
| A18          | Dashkasan               | Destaphour   | 500-750     |
| A19          | Gadabay                 | Kelaman      | >750        |
| A20          | Gadabay                 |              | >750        |
| A04          | Goranboy                | Fexralı      | <500        |
| A10          | Goranboy                | Kushchular   | <500        |
| A11          | Goranboy                | Fexralı      | <500        |
| A13          | Goranboy                | Semedabad    | <500        |
| A21          | Goranboy                | Fexralı      | <500        |
| A22          | Goranboy                | Fexralı      | <500        |
| A23          | Goranboy                | Fexralı      | <500        |
| A17          | Qazax                   | İzlazam      | <500        |
| A01          | Samux                   | Aghasibayli  | <500        |
| A03          | Shamkir                 | Zeyem        | 500-750     |
| A08          | Shamkir                 | Tatarlı      | >750        |

**Table 1.** Honey samples' name, administrative district, municipality and altitude

| Samples name | Administrative district | Municipality   | Altitude(m) |
|--------------|-------------------------|----------------|-------------|
| A09          | Shamkir                 | Dashbulag      | 500-750     |
| A12          | Shamkir                 | Zeyem          | 500-750     |
| A14          | Shamkir                 | Chanlibel      | >750        |
| A15          | Shamkir                 | Zeyem          | <500        |
| A16          | Shamkir                 | Gyneykend      | <500        |
| A05          | Tovuz                   | Ehmedabad      | 500-750     |
| A06          | Tovuz                   | Ibrahim Hacili | 500-750     |
| A07          | Tovuz                   | Hutu Meseligi  | 500-750     |

## METHODS

### Melissopalynological Analysis

#### Botanical Origin

Thoroughly mixed ten g honey was transferred to a test tube, followed by the addition of 20 mL of distilled water. The tubes were incubated in a 45°C water bath for 10–15 minutes, then centrifuged at 3500 rpm for 45 minutes. The supernatant was discarded, and the sediment was collected a small amount using a sterile needle tip with (1-2 mm<sup>3</sup>) basic fuchsin glycerin-jelly. The sample was transferred onto a slide. The microscope slides were evaluated (100x, 40x) magnification with Leica DM500 light microscope. Different morphological characteristics were used to identify the pollen grains at the species, genus and family level. In this study, 200 pollen grains were counted for the calculation of frequency. Although some researchers have counted between 500 and 1000 pollen grains, recent studies have reported that counting excessive numbers of pollen grains increases error and leads to unnecessary time loss. Therefore, the ideal number has been recorded as 200. And we have included nectarless pollen species for melissopalynological studies because of these taxa may contribute to composition of honey. Pollen taxa were divided into four main categories according to frequency in honey predominant pollen ( $P > 45\%$ ), secondary pollen (16-45%), important minor pollen (3-15%) and minor pollen (less than 3%) (Louveaux et al., 1978; Bobiş et al., 2020).

#### Total Pollen Number (TPN-10)

The total pollen number (TPN) analysis for 10 g honey was conducted as follows: After homogenizing, 10 g honey was weighed and placed in a test tube, followed by the addition of 20 ml distilled water and a Lycopodium spp. tablet containing 12 542 spores as a control. The tubes were incubated in a 45°C water bath for 10–15 minutes, added basic fuchsin, and centrifuged at 3500 rpm for 45 minutes. The supernatant was discarded, and 0.1 ml of 50% glycerin was added. A 0.01 ml sample of this solution was transferred to another tube containing 0.09 ml of 50% glycerin. Finally, 0.01 ml of the solution was examined under light microscope. Honeys were categorized into classes based on the total pollen number per 10 g of honey (Louveaux et al., 1978). The pollen classes are as follows: Class I: Less than 20000 (unifloral honeys with under-represented pollen), Class II: 20000–100000 (multifloral honeys, honeydew honeys, and mixtuers of flower and honeydew honeys), Class III: 100000–500000 (unifloral honeys with over- represented pollen and honeydew honey), Class IV: 500000–1000000 (unifloral honeys with strongly over-represented pollen and some pressed honeys), Class V: More than 1000000 (only pressed honey).

## RESULTS AND DISCUSSION

The melissopalynological analysis of honey samples from the Azerbaijan Ganja Gazakh Region revealed many types of pollen in total of 23 samples of honey. The variety of pollen types reflects the floristic diversity of the region. Qualitative analysis identified 70 plant taxa from 34 plant family present in the selected site (Table 2., Figure 2.).

**Table 2.** Pollen composition of honey samples

| Honey samples | Administrative district | Altitude (m) | Pollen composition                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|---------------|-------------------------|--------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| A01           | Samux                   | <500         | <p>*<i>Plantago</i></p> <p>**<i>Helianthus annuus</i></p> <p>***Brassicaceae, <i>Astragalus</i>., <i>Eryngium</i>, <i>Populus</i></p> <p>****Lamiaceae, Poaceae, Rosaceae, <i>Aster</i>, <i>Echium</i>, <i>Cornus</i>, <i>Medicago</i>, <i>Onobrychis</i>, <i>Trifolium</i>, <i>Vicia</i>, <i>Castanea sativa</i></p> <p>**<i>Malus</i></p> <p>***Brassicaceae, Fabaceae, Poaceae, Scrophulariaceae, Rosaceae, <i>Ailanthus</i>, <i>Echium</i>, <i>Lamium</i>, <i>Medicago</i>, <i>Onobrychis</i>, <i>Trifolium</i>, <i>Teucrium</i>, <i>Glycyrrhiza glabra</i>, <i>Plantago lanceolata</i></p> |
| A02           | Aghstafa                | <500         | <p>****Apiaceae, Myrtaceae, Solanaceae, <i>Populus</i> spp., <i>Castanea sativa</i>,</p> <p>**Lamiaceae, <i>Echium</i>, <i>Onobrychis</i>,</p> <p>***Fabaceae, Rhamnaceae, Rosaceae, <i>Astragalus</i></p>                                                                                                                                                                                                                                                                                                                                                                                      |
| A03           | Shamkir                 | 500-750      | <p>****Asteraceae, Campanulaceae, Convolvulaceae, Lamiaceae, Pinaceae, <i>Centaurea</i>, <i>Cichorium</i>, <i>Fragaria</i>, <i>Melilotus</i>, <i>Plantago</i>, <i>Teucrium</i>, <i>Trifolium</i>, <i>Glycyrrhiza glabra</i></p> <p>**<i>Astragalus</i>, <i>Helianthus annuus</i></p>                                                                                                                                                                                                                                                                                                            |
| A04           | Goranboy                | <500         | <p>***<i>Cichorium</i></p> <p>****Apiaceae, Brassicaceae, Cornaceae, Pinaceae, <i>Centaurea</i>, <i>Lamium</i>, <i>Medicago</i>, <i>Robinia</i></p> <p>**Rhamnaceae, <i>Eucalyptus</i></p>                                                                                                                                                                                                                                                                                                                                                                                                      |
| A05           | Tovuz                   | 500-750      | <p>*** Fabaceae, Lamiaceae, Sapindaceae, <i>Epilobium</i>, <i>Onobrychis</i>, <i>Teucrium</i>, <i>Glycyrrhiza glabra</i></p> <p>****Alliaceae, Pinaceae, <i>Campanula</i>, <i>Vicia</i>, <i>Castanea sativa</i>,</p> <p>*Fabaceae</p>                                                                                                                                                                                                                                                                                                                                                           |
| A06           | Tovuz                   | 500-750      | <p>**<i>Eucalyptus</i></p> <p>***Rhamnaceae, Rosaceae, <i>Teucrium</i></p> <p>****Asteraceae, Apiaceae, Brassicaceae, Poaceae, <i>Ailanthus</i>, <i>Onobrychis</i>, <i>Plantago</i>, <i>Castanea sativa</i></p> <p>**Fabaceae, Rhamnaceae</p>                                                                                                                                                                                                                                                                                                                                                   |
| A07           | Tovuz                   | 500-750      | <p>***Cistaceae, Lamiaceae, Rosaceae, <i>Astragalus</i>, <i>Teucrium</i>, <i>Tilia</i>, <i>Trifolium repens</i></p> <p>****Apiaceae, Campanulaceae, Myrtaceae, <i>Ailanthus</i>, <i>Convolvulus</i>, <i>Echium</i>, <i>Geranium</i>, <i>Medicago</i>, <i>Plantago</i>, <i>Ranunculus</i>, <i>Glycyrrhiza glabra</i></p> <p>**Rosaceae</p>                                                                                                                                                                                                                                                       |
| A08           | Shamkir                 | >750         | <p>***Boraginaceae, Fabaceae, Lamiaceae, <i>Ailanthus</i>, <i>Helianthemum</i>, <i>Onobrychis</i>, <i>Plantago</i>, <i>Teucrium</i>, <i>Trifolium</i>, <i>Glycyrrhiza glabra</i></p> <p>****Ranunculaceae</p>                                                                                                                                                                                                                                                                                                                                                                                   |

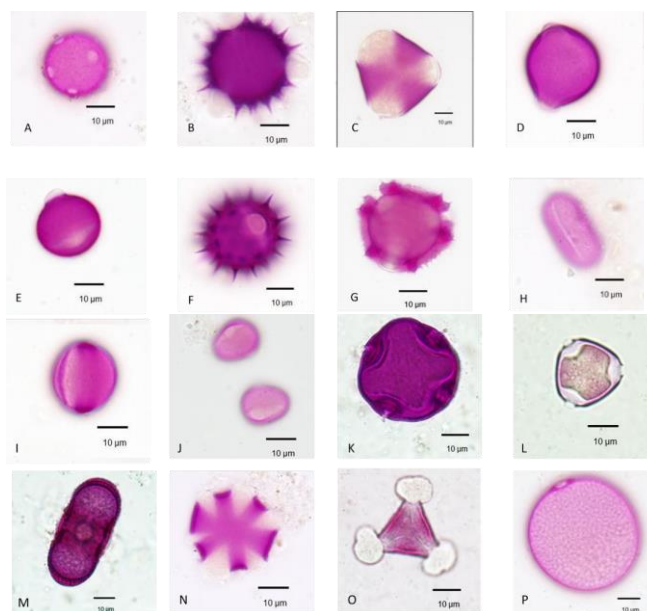
**Table 2.** Pollen composition of honey samples

| Honey samples | Administrative district | Altitude (m) | Pollen composition                                                                                                                                                                                                                                                                                           |
|---------------|-------------------------|--------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| A09           | Shamkir                 | 500-750      | <p>*Scrophulariaceae</p> <p>***Asteraceae, Brassicaceae, Lamiaceae, Violaceae</p> <p><i>Onobrychis, Trifolium</i></p> <p>****Myrtaceae, Poaceae, <i>Ailanthus, Allium, Astragalus, Cichorium, Echium, Teucrium, Vicia,</i></p> <p>**<i>Helianthus annuus, Trifolium</i></p>                                  |
| A10           | Goranboy                | <500         | <p>*** Scrophulariaceae, <i>Viola, Cichorium, Glycyrrhiza glabra, Plantago</i></p> <p>****Apiaceae, Asteraceae, Brassicaceae, Fabaceae, Lamiaceae, Myrtaceae, Pinaceae, <i>Lamium, Medicago, Populus, Teucrium</i></p> <p>**Fabaceae</p>                                                                     |
| A11           | Goranboy                | <500         | <p>*** Hydrangaceae, Pinaceae, Poaceae, Rosaceae, Violaceae, <i>Plantago, Trifolium, Helianthus annuus</i></p> <p>****Apiaceae, Lamiaceae, Myrtaceae, Scrophulariaceae, <i>Cichorium, Medicago, Populus, Teucrium, Glycyrrhiza glabra</i></p> <p>**<i>Echium, Onobrychis</i></p>                             |
| A12           | Shamkir                 | 500-750      | <p>*** Fabaceae, Lamiaceae, Rhamnaceae, Rosaceae, <i>Melilotus</i></p> <p>****Apiaceae, Asteraceae, Brassicaceae, Campanulaceae, Convolvulaceae, Myrtaceae, Pinaceae, <i>Astragalus, Cichorium, Lamium, Medicago, Poaceae, Salix, Symphytum, Teucrium, Glycyrrhiza glabra</i></p> <p>**<i>Astragalus</i></p> |
| A13           | Goranboy                | <500         | <p>***<i>Helianthus, Plantago, Teucrium, Viola, Trifolium pratense</i></p> <p>**** Asteraceae, Brassicaceae, Fabaceae, Myrtaceae, Poaceae, Rosaceae, <i>Artemisia, Castanea, Cichorium, Vicia, Glycyrrhiza glabra</i></p> <p>**<i>Onobrychis</i></p>                                                         |
| A14           | Shamkir                 | >750         | <p>*** Fabaceae, Lamiaceae, Rosaceae, <i>Astragalus, Echium, Lamium,</i></p> <p>****Acanthaceae, Apiaceae, Astearaceae, Campanulaceae, Myrtaceae, <i>Castanea, Centaurea, Cichorium, Plantago Teucrium, Trifolium, Vicia, Glycyrrhiza glabra</i></p> <p>**<i>Helianthus annuus</i></p>                       |
| A15           | Shamkir                 | <500         | <p>***Brassicaceae, Fabaceae, Lamiaceae, Rosaceae, <i>Echium, Onobrychis, Vicia</i></p> <p>****Apiaceae, Asteraceae, Campanulaceae, Pinaceae <i>Castanea, Cichorium, Teucrium, Viola, Glycyrrhiza glabra,</i></p> <p>not detected</p> <p>** Fabaceae, <i>Onobrychis</i></p>                                  |
| A16           | Shamkir                 | <500         | <p>***Brassicaceae, Castanea, Echium, Trifolium, Lamiaceae, <i>Viola</i></p> <p>**** Apiaceae, Asteraceae, <i>Cynoglossum,</i> Campanulaceae, <i>Astragalus, Trifolium, Teucrium,</i> Myrtaceae, Poaceae, Rosaceae</p> <p>* Rosaceae</p>                                                                     |
| A17           | Gazakh                  | <500         | <p>***Caprifoliaceae, Fabaceae, Lamiaceae, <i>Fragaria, Medicago, Viola, Glycyrrhiza glabra,</i></p> <p>**** Hydrangaceae, Poaceae, <i>Allium, Elaeagnus, Teucrium, Trifolium</i></p>                                                                                                                        |

**Table 2.** Pollen composition of honey samples

| Honey samples | Administrative district | Altitude (m) | Pollen composition                                                                                                                                                                                                                                                                                  |
|---------------|-------------------------|--------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| A18           | Dashkesen               | 500-750      | **Fabaceae, <i>Teucrium</i><br>***Brassicaceae, Lamiaceae, Pinaceae, Rosaceae, <i>Echium</i> ,<br><i>Onobrychis</i> , <i>Glycyrrhiza glabra</i> , <i>Castanea sativa</i> ,<br>****Tiliaceae                                                                                                         |
| A19           | Gedebey                 | >750         | ** <i>Lotus</i> , <i>Ziziphora</i><br>*** Campanulaceae, Fabaceae, Lamiaceae, <i>Echium</i> ,<br><i>Medicago</i> , <i>Plantago</i> , <i>Trifolium</i> , <i>Vicia</i><br>Asteraceae, Pinaceae, Rosaceae, <i>Astragalus</i> , <i>Onobrychis</i> ,<br><i>Glycyrrhiza glabra</i>                        |
| A20           | Gedebey                 | >750         | ***Apiaceae, Brassicaceae, Rosaceae, Lamiaceae,<br><i>Astragalus</i> , <i>Echium</i> , <i>Onobrychis</i> , <i>Trifolium</i> , <i>Glycyrrhiza glabra</i>                                                                                                                                             |
| A21           | Goranboy                | <500         | **** Fabaceae, Pinaceae, <i>Plantago</i> , <i>Symphytum</i> , <i>Teucrium</i> ,<br>* Fabaceae<br>***Brassicaceae, Pinaceae, Rosaceae. <i>Plantago</i><br>**** Poaceae, <i>Cichorium</i> , <i>Helianthus</i> , <i>Astragalus</i> , <i>Lotus</i> ,<br><i>Malva</i><br>*Fabaceae<br>** <i>Plantago</i> |
| A22           | Goranboy                | <500         | *** Brassicaceae, Pinaceae, <i>Helianthus annuus</i><br>****Cistaceae, Fabaceae, Poaceae<br>*Fabaceae<br>** <i>Plantago</i> , <i>Helianthus annuus</i>                                                                                                                                              |
| A23           | Goranboy                | <500         | **** Brassicaceae, Hydrangaceae, Malvaceae, Myrtaceae,<br>Rosaceae, <i>Cichorium</i> , <i>Trifolium</i> , <i>Viola</i>                                                                                                                                                                              |

\*>45%; \*\*15-45%; \*\*\*3-15%; \*\*\*\*<3%



**Figure 2.** Light microscope (100x) image of : A: *Plantago* spp.; B: Asteraceae, C: Rosaceae; D: *Glycyrrhiza glabra*; E: *Astragalus* spp., F: *Helianthus annuus*; G: *Cichorium* spp., H: *Onobrychis* spp.; I: *Teucrium* spp.; J: *Echium* spp.; K: Tiliaceae; L: Rhamnaceae; M: Apiaceae; N: Lamiaceae; O: Myrtaceae; P: Poaceae

The most frequently identified plant families in all analyzed honey samples were Fabaceae (100% of samples) (including *Astragalus* spp., *Glycyrrhiza*, *Trifolium* spp., and *Onobrychis* spp.), followed by Lamiaceae

(87% of samples), Rosaceae (78% of samples), Plantaginaceae (74% of samples), and Asteraceae (70% of samples). Results showed that Acanthaceae and Solanaceae were the least frequent families. Fabaceae family with 12 taxa had the maximum species contribution followed by Asteraceae, Boraginaceae, Lamiaceae, and Rosaceae. The Fabaceae family, commonly known as the legume family, is one of the most diverse and ecologically significant plant families in Azerbaijan. In this study, Fabaceae, Asteraceae, and Rosaceae families, which showed high nectar and pollen flow, have been previously reported as melliferous families in Kazakhstan, Mexico and Pakistan ( Moldakhmetova et al., 2023; Balvino-Olvera et al., 2024; Mushtaq et al., 2024). Similar to our study, Asteraceae, Fabaceae and Lamiaceae families were found to be the most frequently used plants by bees in West and East Azerbaijan regions (Khosroshahi and Lotfalizadeh, 2011). Studies on Iranian honey have indicated that the honey is derived from plant families most commonly observed in this research, including Asteraceae, Fabaceae, Rosaceae, and Lamiaceae (Mehti, 2023). Sorkun et al. (2014), conducted pollen analysis on 76 honey samples from Türkiye Ardahan province and found that the most common pollen taxa belonged to the Fabaceae and Boraginaceae families. In this study, pollen from the Fabaceae family was observed in all 23 honey samples, with a frequency ranging from 11.5% to 66%. Among these, *Glycyrrhiza glabra* was the most prevalent species, discovering in 16 samples. Additionally, taxa such as *Astragalus* spp., *Lotus* spp., *Medicago* spp., *Melilotus* spp., *Onobrychis* spp., *Robinia* spp., *Trifolium* spp., and *Vicia* spp. were identified at varying frequencies.

**Table 3.** TPN-10 results of honey samples

| Samples | District  | Altitude | TPN-10    | Group |
|---------|-----------|----------|-----------|-------|
| A01     | Samux     | <500     | 22 076    | II    |
| A02     | Aghstafa  | <500     | 43 895    | II    |
| A03     | Shamkir   | 500-750  | 10 021    | I     |
| A04     | Goranboy  | <500     | 21 431    | II    |
| A05     | Tovuz     | 500-750  | 23 158    | II    |
| A06     | Tovuz     | 500-750  | 23 124    | II    |
| A07     | Tovuz     | 500-750  | 58 567    | II    |
| A08     | Shamkir   | >750     | 1 394 578 | V     |
| A09     | Shamkir   | 500-750  | 64 557    | II    |
| A10     | Goranboy  | <500     | 11 652    | I     |
| A11     | Goranboy  | <500     | 8 291     | I     |
| A12     | Shamkir   | 500-750  | 9 353     | I     |
| A13     | Goranboy  | <500     | 64 794    | II    |
| A14     | Shamkir   | >750     | 28 536    | II    |
| A15     | Shamkir   | <500     | 25 600    | II    |
| A16     | Shamkir   | <500     | 14 146    | I     |
| A17     | Gazakh    | <500     | 82 385    | II    |
| A18     | Dashkasan | 500-750  | 8 398     | I     |
| A19     | Gedebey   | >750     | 2 582     | I     |
| A20     | Gedebey   | >750     | 16 403    | I     |
| A21     | Goranboy  | <500     | 18 956    | I     |
| A22     | Goranboy  | <500     | 20 743    | II    |
| A23     | Goranboy  | <500     | 20 858    | II    |

In this study, the most frequent plant taxa were *Plantago* spp., *Echium* spp, *Eucalyptus* spp., *Helianthus annuus*, *Malus* spp., *Onobrychis* spp., *Astragalus* spp., *Teucrium* spp., and *Trifolium* spp. had high pollen frequency percentage (> 45% or 16-45% )

*Plantago* spp. was identified as the dominant pollen in sample A01; Fabaceae in samples A06, A21, A22, and A23; Scrophulariaceae in sample A09; and Rosaceae in sample A17. In this study, the frequency of occurrence Plantaginaceae family was determined 47% in A01 sample, Fabaceae family over 50% in A06, A21, A22, and A23, Scrophulariaceae family 51.5% in A09, and Rosaceae family 57.5% in A17.

Anemophilous pollen grains like *Plantago* spp. was highly represented in the studied honeys, whereas is lower represented in the honeys of Argetina (Forcone et al., 2005). Several studies have found that pollen from non-melliferous plants can be present in honey samples, in addition to the predominant melliferous plant pollen (Bobış et al., 2020; Layek et al., 2020) For instance, a study on acacia honey found the presence of pollen from non-melliferous plants such as *Plantago* like this study (Bobış et al., 2020). Furthermore, the presence of non-melliferous plant pollen in honey can provide valuable information about the foraging behavior of honey bees

and the overall floral diversity in the surrounding environment (Layek et al., 2020). This information can be useful for conservation efforts, habitat management, and sustainable beekeeping practices.

The quantitative analysis of the 23 honey samples revealed a normal pollen content. 13 of the samples were classified into Group II and showed moderate amount of pollen. One of sample (A08) was Group V according to the Maurizio classification system, while the remaining nine samples were categorized into Group I and had a low pollen count. We didn't detect honeydew elements in any honey (Table 3).

## CONCLUSION

Given the limited palynological research on Azerbaijan honey, it is strongly recommended that this study be expanded to cover a broader area. Expanding the scope of this research would significantly contribute to filling existing knowledge gaps and provide valuable insights for the development and sustainability of the apiculture sector in these regions. This study not only enhances our understanding of the bee flora in the Ganja-Gazakh region of Azerbaijan but also holds the promise of transforming beekeeping practices, promoting environmental conservation, and advancing scientific knowledge

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## Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

## Author Contributions

**1<sup>st</sup> Author Duygu Nur-Çobanoğlu:** Conceptualization; data curation; formal analysis; investigation; methodology; writing— original draft; writing—review and editing.

**2<sup>st</sup> Author Kadriye-Sorkun:** Conceptualization; data curation; acquisition; writing— original draft; writing—review and editing.

**3<sup>st</sup> Author Duygu Nur-Çobanoğlu :** Conceptualization; methodology; writing— original draft; writing—review and editing.

## Author's Note Section:

This publication is based on the thesis titled “Determination of Contents of the Honey Samples Collected from Azerbaijan Ganja Gazakh Region by Microscopic, HPLC And GC-MS Analysis” submitted to Hacettepe University for the fulfillment of the PhD degree in 2017.

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