



Botanical Origin and Diversity of Pollen in Honey Samples Collected from Uzbekistan Determined by the Melissopalynological Analysis

Sukjun Sun¹, Hyeonjeong Jang¹, Boymakhmat A. Kakhramanov², Chuleui Jung^{1,3,*}

¹Department of Plant Medicinals, Gyeongsuk National University, Andong 36729, Republic of Korea

²General Zoo and Veterinary Graduate School, Tashkent State Agrarian University, Tashkent, TK, 111218, Republic of Uzbekistan

³Agriculture Research Institute, Gyeongsuk National University, Andong 36729, Republic of Korea

Abstract

Beekeeping and honey production is important agricultural component in Uzbekistan. However, scientific verification of honey origin remains limited. Given increasing concerns about honey authenticity and labeling accuracy, this study aimed to evaluate the botanical origin of Uzbek honey using melissopalynological analysis. A total of 26 honey samples collected from supermarkets, markets, apiaries, and universities in the Tashkent region, examined to determine the correspondence between pollen composition and the floral source stated on product labels. Of the total, 20 samples were classified as monofloral honey based on the dominant pollen (>45%), while the remaining six were considered multifloral. Notably, 12 samples labeled as multifloral were reclassified as monofloral based on pollen dominance, revealing inconsistencies between actual content and labeling. Only 5 samples (1 *Tilia*, 3 *Alhagi*, 1 *Helianthus*) showed agreement between dominant pollen and the label claim. A total of 17 pollen taxa were identified, and hierarchical clustering analysis revealed distinct groupings based on dominant pollen types. These findings underscore the need for standardized labeling practices and demonstrate the value of melissopalynology as a reliable tool for verifying the botanical origin of honey and supporting the development of quality standards for Uzbekistan honey.

Keywords

Apis mellifera, Palynology, Uzbekistan, Melliferous flower, Honey

INTRODUCTION

Uzbekistan, located in Central Asia, offers a unique environment for honey research due to its diverse topography and climate. The majority of the country's territory consists of desert landscapes (85%), while mountainous areas and foothills occupy only about 15% of the land. This distinct ecological setting contributes to the diversity of both cultivated and wild melliferous plants in Uzbekistan, which in turn reflects the environmental specificity of local apiculture (Mamadaliyeva *et al.*, 2017; Atamuratova *et al.*, 2021). Despite the ecological and apicultural importance of these plants, studies

on their taxonomy and the characteristics of the honey produced from them remain limited (Atamuratova *et al.*, 2021).

Honey is defined as a natural sweet substance produced by honey bees from the nectar of plants or from honeydew, which they collect and store within the hive (EC, 2001). After foraging, bees bring nectar back to the colony, where it is processed into honey primarily through the activity of the enzyme invertase. Honey is generally classified into monofloral and multifloral types: monofloral honey is derived predominantly from the nectar of a single plant species, while multifloral honey is produced from the nectar of multiple floral

sources (Mureşan *et al.*, 2022). The botanical origin of honey significantly affects its biochemical composition, including amino acid profiles, carbon isotope ratios, and organoleptic properties such as taste and aroma (Khan *et al.*, 2024).

Honey naturally contains pollen, and the quantity and composition of pollen present can vary depending on the plant species and the foraging behavior of honey bees (Louveaux *et al.*, 1978). The scientific analysis of pollen grains found in honey to determine its botanical origin is known as mellisopalynology. This method enables the identification of the geographical and floral origin of honey and helps elucidate the floral preferences of honey bees (Dórea *et al.*, 2010). Based on the relative abundance of pollen types in a honey sample, dominance categories are established: a pollen taxon comprising more than 45% of the total pollen count is classified as predominant, 16–45% as secondary, and 3–15% as minor (Forcone, 2008). When a single pollen type exceeds 45% of the total, the honey is generally considered monofloral. This classification provides valuable insight into the surrounding vegetation and plays a critical role in identifying melliferous plants favored by honey bees (Von Der Ohe *et al.*, 2004).

Uzbekistan is known for its high levels of honey production and consumption. However, scientific investigations into the botanical origins of Uzbek honey remain scarce. Several previous studies have already proposed standards regarding pollen content in honey (El-Sofany *et al.*, 2020). Moreover, in some European countries, regulations require the indication of pollen composition on honey labels (Thrasyvoulou *et al.*, 2018; Greek Decision 127, 2004). Given the importance of accurate labeling and botanical traceability in ensuring honey quality, there is a pressing need to evaluate the floral composition of locally produced honey.

To address this gap, the present study employs mellisopalynological techniques to analyze the pollen content of honey samples produced in Uzbekistan. The primary objective is to assess the consistency between the identified botanical origins and the floral information provided on consumer labels. By doing so, this research aims to establish a foundational reference for future studies and standardization efforts related to Uzbek honey.

MATERIALS AND METHODS

1. Honey samples

A total of 26 honey samples were used in this study. The samples were collected from four different sources in Tashkent: supermarkets ($n=5$), local markets ($n=7$), apiaries ($n=9$), and Tashkent State Agrarian University ($n=5$). All honey was produced in Tashkent in 2022. Detailed information on each sample is provided in Table 1.

2. Pollen extract

The experimental procedure was conducted with slight modifications to the method described by Von Der Ohe *et al.* (2004), as outlined below. Crystallized, spreadable honey samples were first liquefied by placing them in a water bath at 60°C until fully dissolved and homogenized. A 15 g aliquot of honey was then mixed with 30 mL of distilled water at room temperature, and the mixture was vortexed to ensure uniform dispersion. The resulting solution was centrifuged at $1080 \times g$ for 10 minutes, after which the supernatant was discarded. This washing step was repeated at least twice to ensure complete removal of sugar crystals from the mixture.

To enhance the visibility of pollen grains under the microscope, 10 mL of a 10% Safranin-O staining solution was added to the residue and vortexed thoroughly. The stained mixture was then centrifuged at $1080 \times g$ for 10 minutes, and the supernatant was carefully removed.

Subsequently, 10 mL of absolute ethanol (100%) was added to the pellet to remove excess stain and moisture, followed by a third centrifugation under the same conditions. After discarding the ethanol supernatant, 1 mL of the resulting concentrate was transferred to a 2 mL microcentrifuge tube using a micropipette.

The concentrate was mixed with glycerol and left to stand at room temperature for 24 hours to allow ethanol evaporation. This process yielded the final pollen preparation for microscopic analysis.

3. Identification

Pollen grains were counted in three randomly selected fields within a single cover glass area (18 mm \times 18 mm), and the average was calculated. For each replicate, a minimum of 300 pollen grains were identified. Pollen

Table 1. Melissopalynological analysis of honey samples collected from Uzbekistan in 2022 summer

Source	Label	Pollen type			
		Dominant pollen	Frequency (%)	Subdominant pollen	Frequency (%)
Apiary	Multifloral*	<i>Medicago</i> (alfalfa)	47.06 ± 3.88	<i>Artemisia</i>	15.09 ± 0.94
	Multifloral*	<i>Salvia</i> (sage)	69.67 ± 2.47	<i>Medicago</i> (alfalfa)	15.74 ± 0.47
	Multifloral*	<i>Alhagi</i> (camelthorn)	85.16 ± 1.11	<i>Artemisia</i>	2.4 ± 0.94
	Multifloral*	<i>Medicago</i> (alfalfa)	45.83 ± 0.59	<i>Artemisia</i>	14.65 ± 2.87
	Multifloral*	<i>Salvia</i> (sage)	52.17 ± 2.16	<i>Populus</i>	13.16 ± 1.25
	Multifloral*	<i>Alhagi</i> (camelthorn)	85.19 ± 0.43	<i>Populus</i>	10 ± 0.94
	Multifloral*	<i>Helianthus</i> (sunflower)	73.68 ± 2.77	<i>Medicago</i> (alfalfa)	25.33 ± 3.4
	Multifloral	<i>Helianthus</i> (sunflower)	37.78 ± 0.88	<i>Alhagi</i> (camelthorn)	26.97 ± 3.3
	Multifloral*	<i>Helianthus</i> (sunflower)	70.59 ± 1.69	<i>Medicago</i> (alfalfa)	21.05 ± 1.41
Supermarket	<i>Gossypium</i> (cotton)**	<i>Helianthus</i> (sunflower)	37.77 ± 4.19	<i>Artemisia</i>	23.18 ± 2.16
	<i>Helianthus</i> (sunflower)*	<i>Helianthus</i> (sunflower)	83.15 ± 5.56	<i>Medicago</i> (alfalfa)	4.49 ± 5.56
	<i>Tilia</i> (linden)*	<i>Tilia</i> (linden)	95.61 ± 13.27	<i>Medicago</i> (alfalfa)	6.82 ± 1.63
	Multifloral*	<i>Onobrychis</i> (sainfoin)	69.56 ± 4.11	<i>Salvia</i> (sage)	7.84 ± 1.41
	Multifloral*	<i>Alhagi</i> (camelthorn)	85.94 ± 0.50	<i>Helianthus</i> (sunflower)	7.45 ± 1.25
Market	<i>Alhagi</i> (camelthorn)**	<i>Alhagi</i> (camelthorn)	26.23 ± 2.79	<i>Helianthus</i> (sunflower)	25 ± 5.19
	<i>Alhagi</i> (camelthorn)*	<i>Alhagi</i> (camelthorn)	85.9 ± 0.71	<i>Populus</i>	4.33 ± 2.05
	<i>Psoralea</i> *	<i>Alhagi</i> (camelthorn)	86.42 ± 14.52	<i>Medicago</i> (alfalfa)	6.17 ± 4.03
	Multifloral*	<i>Medicago</i> (alfalfa)	48.42 ± 1.00	<i>Psammochloa</i>	10 ± 0.5
	Multifloral	<i>Calligonum</i>	37.5 ± 0.76	<i>Salvia</i> (sage)	10.67 ± 1.25
	Multifloral*	<i>Alhagi</i> (camelthorn)	77.42 ± 3.78	<i>Artemisia</i>	16.22 ± 2.94
	Multifloral*	<i>Helianthus</i> (sunflower)	51.43 ± 0.55	<i>Populus</i>	18.32 ± 0.82
TSAU	<i>Ferula</i> **	<i>Ferula</i>	38.81 ± 3.71	<i>Alhagi</i> (camelthorn)	15.98 ± 1.25
	<i>Salvia</i> **	<i>Onobrychis</i>	39.44 ± 1.63	<i>Medicago</i> (alfalfa)	33.07 ± 1.25
	<i>Trifolium</i> (clover)**	<i>Ferula</i>	53.33 ± 5.30	<i>Helianthus</i> (sunflower)	19.48 ± 1.41
	Multifloral*	<i>Medicago</i> (alfalfa)	41.18 ± 2.07	<i>Psammochloa</i>	14.71 ± 2.05
	Multifloral*	<i>Alhagi</i> (camelthorn)	59.57 ± 0.92	<i>Onobrychis</i>	11.69 ± 2.16

Monofloral honey was considered as having dominant pollen frequency, >45%. Frequency (%) of dominant and subdominant pollen were indicated as mean ± SD

*means monofloral honey, **means wrong statement of botanical origin

identification was carried out by referencing previously published studies and internationally recognized palynological databases, including PalDat (<https://www.paldat.org/>) and The Global Pollen Project (<https://globalpollenproject.org/>).

Morphological characterization of pollen was conducted using a light microscope (BX53, OLYMPUS, Tokyo, Japan), and images were captured with the IMT i-Solution Lite software (IMT i-Solution Inc., Burnaby, British Columbia, Canada).

4. Data analysis

All analyses were conducted using R Studio. To ex-

plore the similarity among honey samples based on their pollen composition, hierarchical cluster analysis was performed using the identified pollen taxa. Additionally, to assess the diversity, dominance, richness, and evenness of pollen taxa in Uzbek honey samples, the Kruskal-Wallis test was applied. Subsequently, Dunn's test was conducted as a post hoc analysis to identify significant pairwise differences between groups.

RESULTS

1. Melissopalynological analysis

17 types of pollen were identified at the genus level in

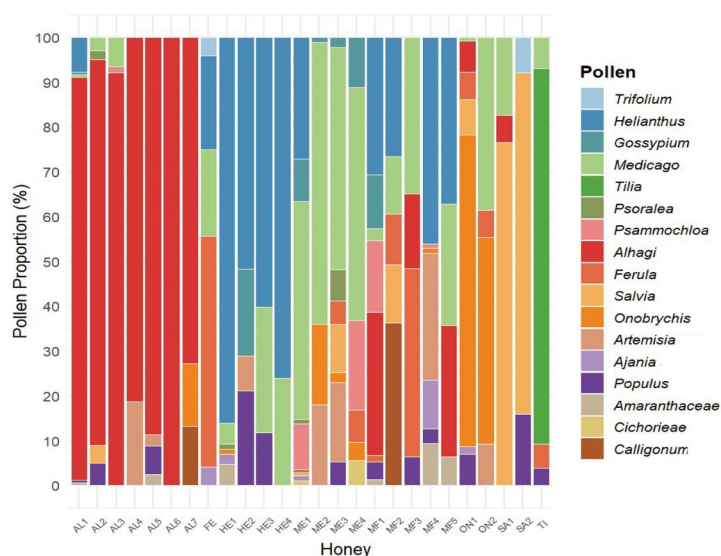


Fig. 1. Relative pollen composition of honey samples produced from Uzbekistan based on melissopalynological analysis, Al: *Alhagi*, FE: *Ferula*, HE: *Helianthus*, ME: *Medicago*, MF: Multifloral, ON: *Onobrychis*, SA: *Salvia*, TI: *Tilia*.

the 26 honey samples analyzed. The types and proportions of pollen varied among the samples. The lowest proportion was observed for Amaranthaceae (0.56%), while the highest was for *Tilia* (95.61%), indicating considerable variation in pollen content among the honey samples. Based on the melissopalynological analysis, 20 honey samples were classified as monofloral, each containing more than 45% of a single dominant pollen type. The remaining 6 samples were classified as multifloral, with the dominant pollen type ranging from 16% to 45%, which was still sufficient for them to be categorized separately. Finally, 7 types of mono floral honey were identified: *Alhagi* (n=7), *Helianthus* (n=4), *Medicago* (n=4), *Salvia* (n=2), *Ferula* (n=1), *Onobrychis* (n=1), *Tilia* (n=1), as well as five types of multi flower honey with dominant species: Multifloral (*Helianthus*) (n=2), Multifloral (*Alhagi*) (n=1), Multifloral (*Calligonum*) (n=1), Multifloral (*Ferula*) (n=1), and Multifloral (*Onobrychis*) (n=1). The content of dominant and subordinate pollen is detailed in Table 1.

The diverse plant origins of honey produced in Uzbekistan are illustrated in Fig. 1. The pollen content of the 11 honey samples classified by nectar source was analyzed. *Medicago* was commonly found in all samples, followed by *Populus*, *Helianthus*, and *Ferula*, which were present in 9, 8, and 8 samples, respectively. Additionally, *Tilia* and Cichorieae were found in only one sample each.

2. Ecological index analysis of pollen in honey produced in Uzbekistan

We analyzed four ecological indices—diversity, dominance, evenness, and abundance—based on the botanical origins of pollen identified in 26 honey samples produced in Uzbekistan. The indices were grouped according to nectar source, and samples without biological replicates were represented as individual dots in the visualizations (Fig. 2). Kruskal-Wallis test results indicated significant differences in dominance ($p=0.02007$) and diversity ($p=0.02046$), whereas abundance and evenness did not show statistically significant differences ($p=0.268$ and $p=0.1542$, respectively). Post-hoc analysis using Dunn's test revealed that, in terms of dominance, *Alhagi* honey significantly differed from *Medicago*, Multifloral (*Alhagi*), Multifloral (*Calligonum*), and Multifloral (*Helianthus*) honeys. Similarly, for diversity, *Alhagi* honey was significantly different from the same four groups.

DISCUSSION

Melissopalynology is a valuable method for determining the authenticity, potential adulteration, and botanical distinctiveness of honey (Shakoori *et al.*, 2023). In this study, we analyzed honey samples produced in Uz-

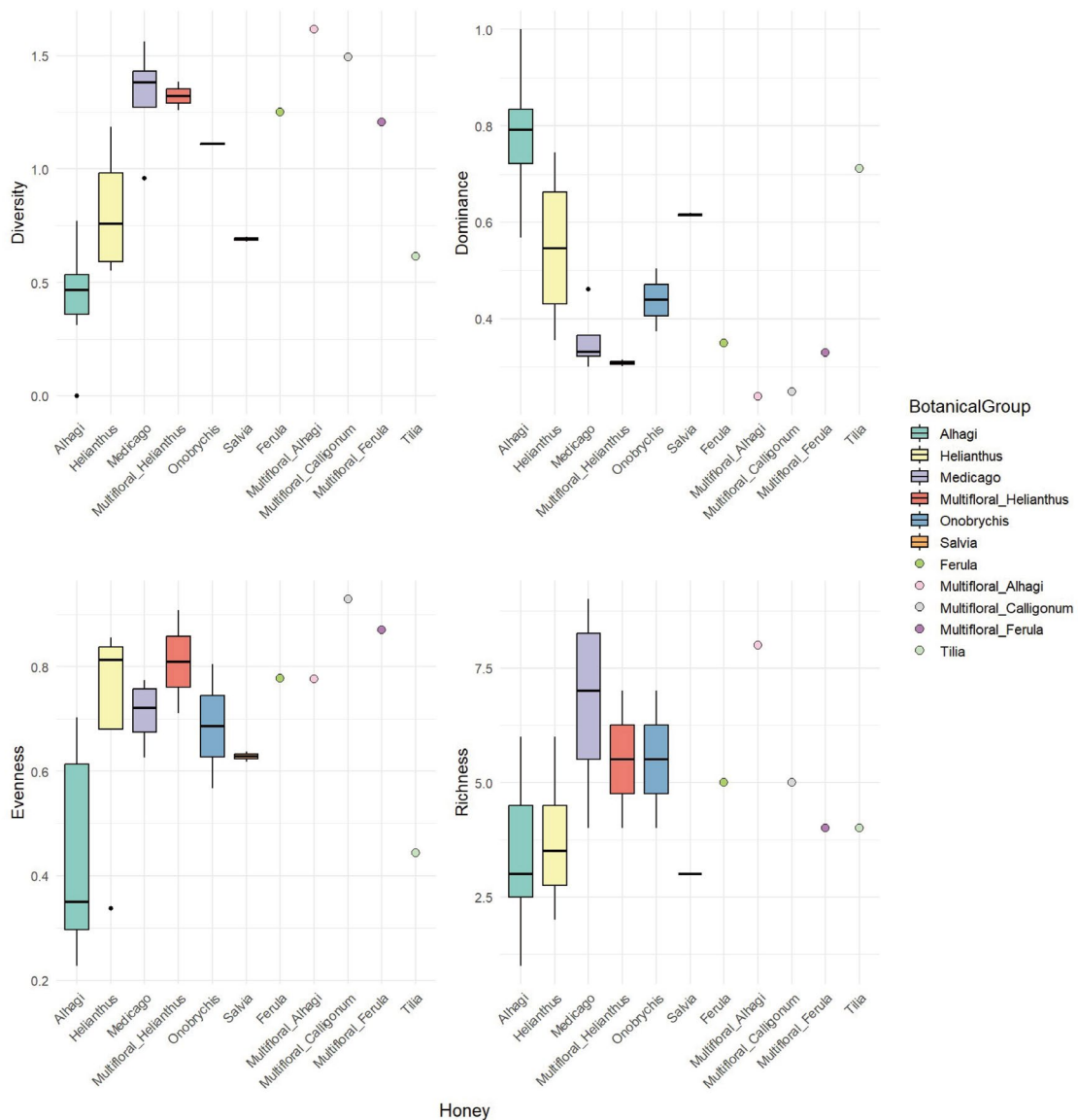


Fig. 2. Comparison of ecological indices (diversity, dominance, evenness, and richness) among Uzbekistan honey samples grouped by the dominant pollen type determined by melissopalynological analysis. The x-axis shows honey sample with dominant pollen type. Dots indicate samples without biological replicates (n = 1).

Uzbekistan using melissopalynological analysis to assess their pollen composition and the consistency between the identified botanical origins and the labeled claims. Prior to the evaluation, we confirmed in Fig. 3 that all identified plant taxa are native to Central Asia, based on previous studies (Lu *et al.*, 2018a, 2018b). According to Abdiniyazova *et al.* (2016), *Artemisia*, *Ferula*, and *Alhagi* are plants of commercial importance in Uzbekistan apiculture, and among these, *Ferula* and *Alhagi* were identified as sources of monofloral honeys in this study.

Fig. 1 visually contrasts typical pollen compositions

of monofloral and multifloral honeys. Monofloral honeys are typically dominated by a single pollen type, whereas multifloral honeys exhibit more diverse floral origins. As shown in Table 1, 20 samples were classified as monofloral honeys based on the criterion of dominant pollen exceeding 45%, while the remaining 6 samples were classified as multifloral. For the multifloral honeys, although the dominant pollen types did not exceed 45%, they still showed relatively high dominance values ranging from 26.23% to 38.81% and were accordingly highlighted.

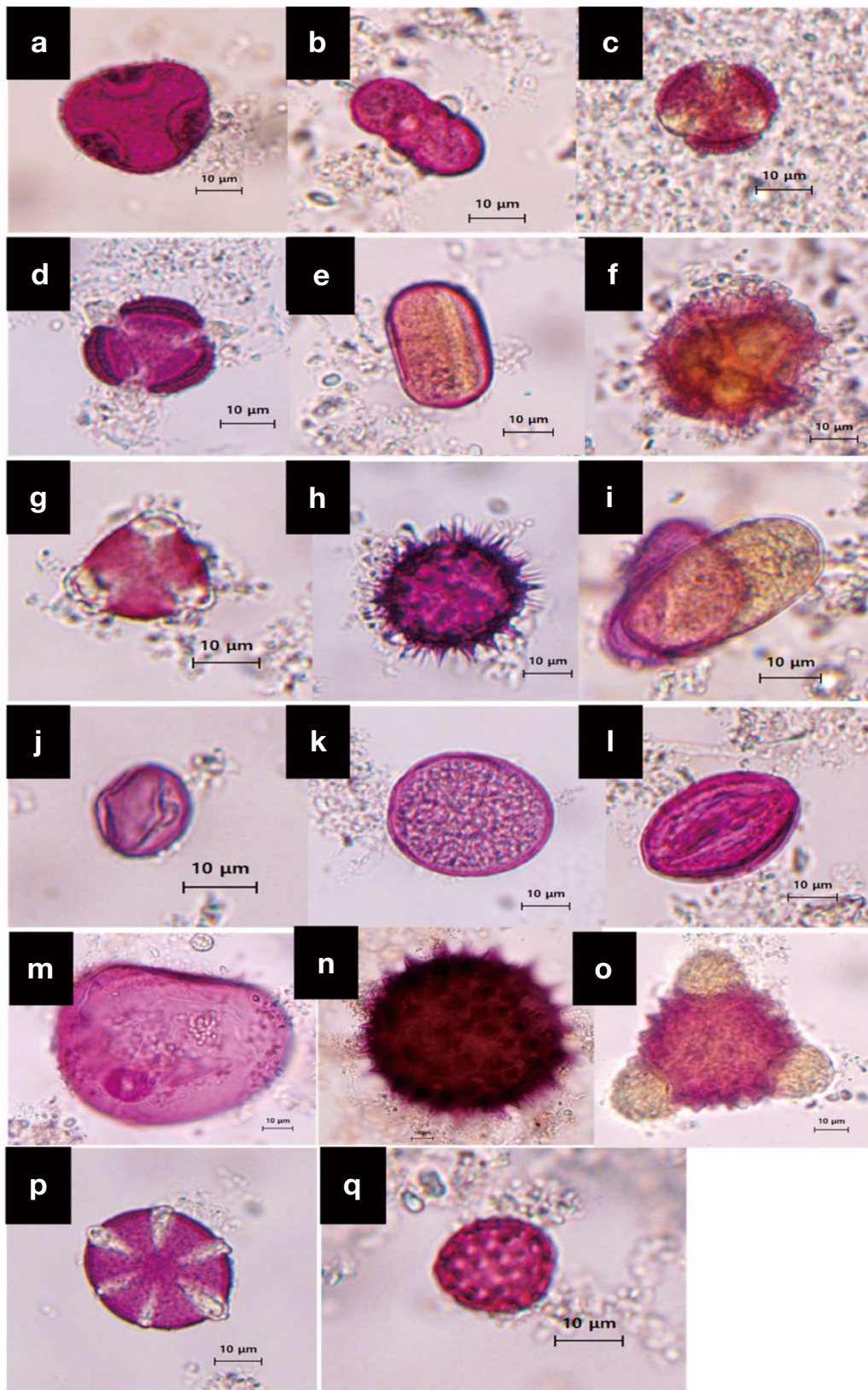


Fig. 3. Pollen morphology of major nectar plants photographed under the light microscope (400×). (a) *Tilia*, (b) *Ferula*, (c) *Ajania*, (d) *Artemisia*, (e) *Trifolium*, (f) *Cichorieae*, (g) *Medicago*, (h) *Helianthus*, (i) *Onobrychis*, (j) *Alhagi*, (k) *Populus*, (l) *Calligonum*, (m) *Psammochloa*, (n) *Gossypium*, (o) *Psoralea*, (p) *Salvia*, (q) *Amaranthaceae*.

We also examined the consistency between the labeled botanical origins and the actual pollen profiles. Among all the honey samples, only 5 had labels that matched the melissopalynological findings, while the remaining 11 were inconsistent. This discrepancy suggests that Uzbekistan's apiculture industry currently lacks a fully standardized system to ensure honey quality and accurate labeling.

One notable exception was *Medicago* honey. Although the proportion of *Medicago* pollen did not exceed the conventional 45% threshold for monofloral classification, it was still regarded as monofloral due to the clear dominance of *Medicago* pollen. This aligns with earlier studies that recognized *Medicago* as a low pollen-producing genus. As such, prior research proposed lower thresholds for certain species: $\geq 20\%$ for *Medicago* (Forcone, 2008; Ciappini *et al.*, 2016), $\geq 15\%$ for *Salvia* (Kenjerić *et al.*, 2006), and $\geq 20\%$ for *Tilia* (PN-88/A-77626, 1988). These species-specific thresholds reflect the need to consider differential pollen productivity when conducting melissopalynological evaluations.

This species-specific variation is also evident in Fig. 2 compared to other monofloral honeys, *Medicago* honey displayed a lower dominance index and a higher diversity index, in stark contrast to *Alhagi* honey, which showed high dominance and low diversity. Another remarkable observation was the *Tilia* honey, which, despite prior assumptions that a 20% pollen threshold suffices for its monofloral status, showed an unusually high pollen proportion of 95.61%. However, it is important to note that many of these thresholds are based on outdated data, and floral resource availability can fluctuate significantly due to alternating flowering patterns (Szkłanowska and Teper, 1999; Dmitruk, 2019; Sultanova *et al.*, 2022), climate, and other environmental factors (Timberlake *et al.*, 2019; Baldock, 2020).

Such variability may account for the deviations in pollen proportions observed in the current study. Relying solely on pollen morphology has inherent limitations, especially when identifying species within morphologically similar taxa (Brodschneider *et al.*, 2019). This is particularly true for a migration beekeeping context like Uzbekistan, where accurate botanical identification requires knowledge of flowering phenology and geographic distribution (Williams and Kremen, 2007). Therefore, to more precisely determine the botanical

origin of honey, integrating genetic analysis with morphological identification is essential (Bruni *et al.*, 2012).

Melissopalynology offers valuable insights into the distinctiveness and authenticity of honey, and it can play a critical role in enhancing the credibility of honey (Escriche *et al.*, 2023; Hussein *et al.*, 2024). To comprehensively evaluate honey quality, however, it is essential to integrate melissopalynological findings with complementary physicochemical analyses. This combined approach provides a robust foundation for identifying functional honeys in Uzbekistan, comparable to antioxidant-rich chestnut honey in Korea (Kim *et al.*, 2023) and pharmacologically active manuka honey in New Zealand (Patel and Cichello, 2013).

Overall, the results confirm that melissopalynology is a powerful tool for determining the botanical origin of honey and mapping regionally dominant nectar sources. This method provides a scientific basis for establishing honey certification standards and developing regional branding strategies for the Uzbekistan honey industry.

CONCLUSION

This study examined the botanical origin of honey produced in Uzbekistan by applying melissopalynological analysis and evaluating the consistency between labeled floral claims and actual pollen composition. Among the 26 samples analyzed, 20 met the criteria for monofloral honey based on the dominant pollen content ($\geq 45\%$). However, 12 of these were mislabeled as multifloral, and only three samples exhibited complete consistency between the label and the dominant pollen type. These discrepancies highlight the lack of standardized labeling practices and underscore the urgent need for a reliable certification system to enhance product transparency and consumer confidence.

A total of 17 pollen taxa were identified, indicating that Uzbekistan honeybees forage in a diverse and florally rich environment. Multifloral honeys demonstrated higher species richness and diversity compared to monofloral honeys, reflecting broader foraging behavior and more complex floral resources. However, exceptions such as *Medicago* and *Tilia* honeys illustrate that pollen content can vary significantly due to intrinsic factors like low pollen production and external factors such as

climate. Therefore, field-based surveys of nectar plant distribution and studies on environment-dependent pollen productivity are necessary to contextualize and complement melissopalynological data.

These findings suggest that pollen profiles in honey can serve not only as indicators of floral origin but also as proxies for local biodiversity and landscape structure. While melissopalynology provides critical insight into the botanical authenticity of honey, it is insufficient on its own for comprehensive quality evaluation. Hence, a holistic approach integrating melissopalynological and physicochemical analyses is required (Jang *et al.*, 2025, in submission).

The present results reaffirm the utility of melissopalynology as both a diagnostic and ecological tool. It offers a scientific basis for developing standardized labeling protocols, supporting floral origin authentication, and informing region-specific branding strategies. These measures are essential for improving quality control, market competitiveness, and sustainable growth within Uzbekistan's beekeeping industry.

ACKNOWLEDGEMENTS

This study was supported by the National Research Foundation of Korea (NRF) on Smart AI UZ project (NRF-2022K1A3A9A05036394).

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