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APIS

Editorial

Preface to the second volume

When the materials for the first conference posters arrived for publication, we realized that we had accidentally opened the door to an exceptional publishing opportunity. One of the speakers at the IV. International Propolis Conference, held in Ribeirão Preto, Brazil, included in their article the most frequently asked questions from visitors along with the answers provided. This created a level of closeness between the researcher and those interested in the research that is rarely achieved through traditional publishing opportunities. The questions raised addressed the core of the subject, revealing details that were not even included in the original presentation.

This issue comprises scholarly articles spanning a range of disciplines, including technological and quality-related advancements, pollination biology and melliferous flora, as well as historical perspectives and developments in apitherapy.

Pollen collection constitutes the principal trophic activity in the foraging behaviour of honeybees. The systematic study of these ethological patterns constitutes a domain of considerable scientific relevance and intellectual interest.

Hungarian beekeepers also participated in the EU-INSIGNIA project. Over nine sampling cycles, 15 beekeepers collected pollen samples with a 24-hour sampling duration. This data collection was unique, and all participating citizen scientist beekeepers received the results. The datasets sent to a well-known Hungarian palynologist, analyzed using their specialized methodology and meticulous work, provided an exceptionally detailed picture of the plants collected by bees on specific days. Naturally, the beekeepers provided the necessary GDPR declarations.

The investigation of the volatile organic constituents of Uruguayan propolis, originally presented at a conference, is also featured in this volume.

What is still part of the present will soon become history as apitherapy continues to evolve. One of the key milestones in the development of apitherapy is its progress in China. By reviewing this development, we can gain insight not only into the past, but also into the present.

Spermidine, a polyamine compound, has emerged as a biomolecule of potential significance in health maintenance and disease prevention. In addition to its previously recognised natural sources, a novel origin has recently been identified: the extract derived from drone (male bee) larvae.

What effect does being near bees have on us?

If a bed is placed directly above the hives in a bee house, we find ourselves in very close proximity to the bees. Studying these effects scientifically is an important field of research. It forms a bridge between naturopathic observations and evidence-based medical possibilities.

We trust that the articles in this issue will not only inform but also inspire—inviting readers to reflect on the rich potential of beekeeping and apitherapy in both scientific and practical contexts.

As interest in nature-based health solutions and sustainable practices grows, we believe this field holds many untapped opportunities for exploration.

We warmly encourage researchers, practitioners, and enthusiasts alike to contribute their insights to future issues and to join us in advancing the dialogue between tradition and science.

With kind regards,

Dr. János Körmendy-Rácz
Editor-in-Chief

APIS

Original Research

Analysis of Pollen Samples from Hungary Based on the INSIGNIA 2023 Programme

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ABSTRACT

In 2023, under the INSIGNIA-EU project, pollen samples were collected across 315 locations in 27 European Union countries over nine sampling rounds. In Hungary, 15 apiaries from different geographic regions participated in the nine sampling rounds. However, two apiaries were unable to collect samples during certain rounds, resulting in a total of 133 domestic samples being analysed and evaluated. The Hungarian baseline data allows for further and more detailed analyses at both the national level and by individual apiary, sampling period, and plant species. Apiaries from more than half of Hungary's 19 counties—specifically, from 10 counties and the capital—contributed to the INSIGNIA 2023 programme.

Across the 15 apiaries and nine sampling rounds, 173 plant species were identified in the samples collected by bees. The number of species ranged from 36–60 per apiary and 22–69 per sampling round. A smaller proportion of these species were common across multiple apiaries. The maximum number of shared species per apiary was 13–16, and the minimum was 3–7. Similarly, during sampling periods, the maximum number of shared species was 10–16, and the minimum was 3–11. Of the 173 plant species identified, 133 provided nectar and 40 were nectarless species producing only pollen. Bees predominantly collected from nectariferous plants. In total, 31 species were common to all the apiaries, of which 19 provided nectar and 12 were nectarless.

The most frequently occurring species across all 15 apiaries included nectariferous cultivated agricultural plants such as *Brassica napus*, *Brassica rapa*, *Helianthus annuus*, and the nectarless herbaceous wild plant *Plantago lanceolata* (NN). These plant species included both short- and long-blooming varieties. By sampling period, the most frequently visited species were *Chelidonium majus* (NN), *Plantago lanceolata* (NN), *Trifolium repens*, and *Papaver rhoeas* (NN). Within specific sampling periods, the highest quantities were observed for *Brassica napus*, *Anthriscus cerefolium*, *Helianthus annuus*, *Papaver rhoeas* (NN), *Plantago lanceolata* (NN), *Robinia pseudoacacia*, and *Clematis vitalba* (NN).

The plant species collected in the largest quantities varied by apiary:

- *Raphanus raphanistrum* (Békéscsaba)
- *Phacelia tanacetifolia* (Nagykovácsi)
- *Plantago lanceolata* (NN) (Budafok, Újszentmargita, Drávafok, Nemesvita)
- *Papaver rhoeas* (NN) (Zámoly, Pannonhalma, Páhi, Fertőendréd)
- *Papaver somniferum* (NN) (Mezőfalva)
- *Brassica napus* (NN) (Vizsoly, Murakeresztúr)
- *Helianthus annuus* (Szalánta)
- *Ononis spinosa* (Kiskunmajsa).

Significant plant species included *Brassica napus*, *Plantago lanceolata* (NN), *Helianthus annuus*, *Papaver rhoeas* (NN), *Clematis vitalba* (NN), *Anthriscus cerefolium*, *Ononis spinosa*, *Phacelia tanacetifolia*, *Solidago gigantea*, and *Trifolium repens*. Among species occurring in only one apiary, some were specific indicators of local regions, while others were cultivated agricultural plants potentially grown elsewhere, such as *Amorpha fruticosa*, *Ballota nigra*, *Castanea sativa*, *Centaurea cyanus*, *Chelidonium majus* (NN), *Crepis setosa*, *Hypericum perforatum* (NN), *Lythrum salicaria*, *Melilotus albus*, *Papaver rhoeas* (NN), *Pisum sativum*, *Raphanus sativus var. oleifera*, *Sanguisorba officinalis* (NN),

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Sinapis alba, and *Tripleurospermum maritimum* (likely *Matricaria chamomilla*).

Regionally, in 2023, the Southern Great Plain (Dél-Alföld) region's three apiaries recorded the lowest number of plant species, while the highest diversity was observed in the apiaries of the Northern Hungary (Észak-Magyarország) region.

INTRODUCTION

As part of the INSIGNIA-EU project, pollen samples were collected in 2023 across 315 locations in 27 European Union countries during nine sampling rounds. A total of 2,525 viable samples were sent to the designated laboratory in Portugal for evaluation. In Hungary, 15 apiaries from various geographic regions participated in the nine sampling rounds. However, two apiaries were unable to collect samples during certain periods, resulting in a total of 133 samples being submitted for analysis. Each pollen sample contained different coloured pollen pellets, which were brushed off the bees' legs using a pollen trap.

Samples were collected by volunteer beekeepers (citizen scientists, referred to as "CS") according to the detailed guidelines of the monitoring plan, under the leadership of the national coordinator, Dr. János Körmendy Rácz. The samples were collected using pollen traps attached to two hives per apiary. After combining the pollen from the two hives, approximately two teaspoons of the mixture were placed in a sample container with silica gel and an identification label before being sent for analysis.

The botanical identification of the pollen samples submitted by each apiary was carried out using ITS2 metabarcoding at the EU-designated laboratory. According to the INSIGNIA-2023 summary report, the molecular ITS2 metabarcoding method—developed in previous years—was used to identify the botanical composition of the pollen samples. The key steps of this procedure included homogenising a specific portion of the sample, extracting DNA, and amplifying the ITS2 region of nuclear DNA using polymerase chain reaction (PCR). The PCR product was sequenced using the high-throughput Illumina MiSeq sequencing platform. The sequences obtained were compared to a reference ITS2 sequence database developed by INSIGNIA-EU for use by researchers in different countries [1].

For each mixed pollen sample, the laboratory determined the number of plant species present and estimated their relative abundance, which was converted into percentages for ease of interpretation. A purpose-built automated computational analysis system was used to evaluate the sequence data, compare it to the reference database, and calculate the relative abundance of each species.

The number of samples submitted from Hungary was 133, from which the relative abundance values for 1,259 plant taxa were determined. The data were grouped and aggregated at both the national and apiary levels. Tables provided the relative abundance of plant species by apiary and sampling round, while charts illustrated trends, occurrences, and temporal changes in abundance at the plant genus level.

For each sampling round, the relative abundance values did not exceed 1,00. A threshold of 1% (0.01) was applied to filter out genera that appeared only rarely in the samples.

The baseline data for Hungary allow for further and more detailed analysis at the national level, as well as by apiary, sampling period, and plant species. The following section presents the evaluation of the Hungarian data.

SAMPLING LOCATIONS IN HUNGARY

In 2023, 15 apiaries from 11 shires and the capital participated in the INSIGNIA program, representing more than half of Hungary's 19 shires. The pollen samples were collected from apiaries located in the following regions:

- Southern Great Plain (Dél-Alföld): 3 samples
- Northern Great Plain (Észak-Alföld): 1 sample
- Northern Hungary (Észak-Magyarország): 1 sample
- Central Hungary (Közép-Magyarország), including Pest shire: 1 sample
- Central Transdanubia (Közép-Dunántúl): 3 samples
- Western Transdanubia (Nyugat-Dunántúl): 3 samples
- Southern Transdanubia (Dél-Dunántúl): 2 samples
- Budapest (the capital): 1 sample

The distribution of sampling locations is summarised in Table 1, and their geographic locations are visualised in Figure 1 (map created using resources from www.terkepek.net).

SAMPLING PERIODS

In 2023, pollen samples were collected biweekly from early May to the end of August. The collection occurred on a designated day within each specified period, and if the pollen sample was insufficient, additional days were utilised for collection.

SR01	SR02	SR03	SR04	SR05	SR06	SR07	SR08	SR09
may 4-8	may 18-22	june 1-4	june 15-18	june 29-july 2	july 13-16	july 27-30	august 10-13	august 24-27

Table 2: Sampling periods / Sampling rounds

Sampling location	Region	Shire	Geographical area
HU 13 Kiskunmajsa	Southern Great Plain	Bács-Kiskun	Great Plain (Alföld)
HU 14 Páhi	Southern Great Plain	Bács-Kiskun	Great Plain (Alföld)
HU 01 Békéscsaba	Southern Great Plain	Békés	Great Plain (Alföld)
HU 04 Újszentmargita	Northern Great Plain	Hajdu-Bihar	Great Plain (Alföld)
HU 07 Vizsoly	Northern Hungary	Borsod-Abaúj-Zemplén	Great Plain (Alföld)
HU 09 Drávafok	Southern Transdanubia	Baranya	Transdanubia (Dunántúl)
HU 10 Szalánta	Southern Transdanubia	Baranya	Transdanubia (Dunántúl)
HU 05 Zámoly	Central Transdanubia	Fejér	Transdanubia (Dunántúl)
HU 06 Mezőfalva	Central Transdanubia	Fejér	Transdanubia (Dunántúl)
HU 11 Nemesvita	Central Transdanubia	Veszprém	Transdanubia (Dunántúl)
HU 02 Nagykovácsi	Central Hungary	Pest	Transdanubia (Dunántúl)
HU 12 Pannonhalma	Western Transdanubia	Győr-Moson-Sopron	Transdanubia (Dunántúl)
HU 15 Fertőendréd	Western Transdanubia	Győr-Moson-Sopron	Transdanubia (Dunántúl)
HU 08 Murakeresztúr	Western Transdanubia	Zala	Transdanubia (Dunántúl)
HU 03 Budafok	Central Hungary	Pest	Capital/Budapest
All apiaries	7 regions	11 shires	2 geographical areas and the capital city

Table 1: Sampling locations in Hungary

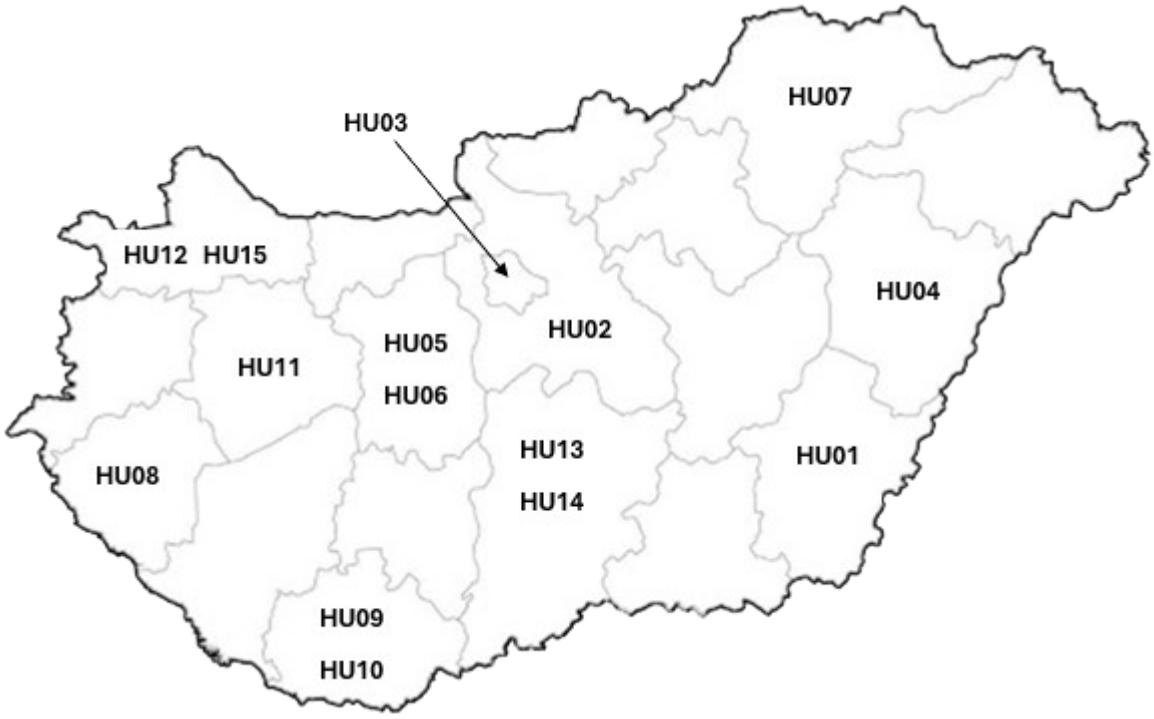

Figure 1: Geographic locations of the apieries in the Shires

HU01: Békéscsaba/Békés Shire
HU02: Nagykovácsi/Pest Shire
HU03: Budafok/Budapest XXII. district
HU04: Újszentmargita/Hajdú-Bihar Shire
HU05: Zámoly/Fejér Shire
HU06: Mezőfalva/Fejér Shire
HU07: Vizsoly/Borsod-Abaúj-Zemplén Shire
HU08: Murakeresztúr/Zala Shire
HU09: Drávafok/Baranya Shire
HU10: Szalánta/Baranya Shire
HU11: Nemesvita/Veszprém Shire
HU12: Pannonhalma/Győr-Moson-Sopron Shire
HU13: Kiskunmajsa/Bács-Kiskun Shire
HU14: Páhi/Bács-Kiskun Shire
HU15: Fertőendréd/Győr-Moson-Sopron Shire

OCCURRENCE OF PLANT SPECIES BY APIARY AND SAMPLING PERIOD

A total of 133 pollen samples from Hungary were analysed for plant origin. The study provided relative frequency values for the plant species found in each apiary during each sampling period. Altogether, 1,259 data points were generated. The analysis below presents the results organised by apiary and sampling period.

Number of species in apiaries by period										Plants identified	SR01-09
Apiary/Period	SR01	SR02	SR03	SR04	SR05	SR06	SR07	SR08	SR09	Per apiary	Species
HU 01 Békéscsaba	7	9	7	4	8	13	11	13	0	72	**43**
HU 02 Nagykovácsi	10	9	4	11	12	10	13	12	11	92	**56**
HU 03 Budafok	6	6	8	6	9	8	13	11	11	78	**46**
HU 04 Újszentmargita	5	8	9	0	12	9	15	15	14	87	**47**
HU 05 Zámoly	7	10	6	6	16	7	9	13	15	89	**52**
HU 06 Mezőfalva	5	4	4	5	10	12	10	16	12	78	**41**
HU 07 Vizsoly	7	10	10	10	10	10	15	16	16	104	**60**
HU 08 Murak-eresztúr	7	7	11	7	13	9	10	8	13	85	**45**
HU 09 Drávafok	9	6	7	12	14	11	9	14	13	95	**57**
HU 10 Szalánta	9	5	9	7	9	9	11	11	13	83	**45**
HU 11 Nemes-vita	4	14	11	9	11	12	10	12	14	97	**51**
HU 12 Pannonhalma	7	6	12	7	8	12	11	15	12	90	**46**
HU 13 Kiskunmajsa	6	7	5	5	4	10	13	9	11	70	**38**
HU 14 Páhi	4	9	5	5	4	3	6	8	13	57	**36**
HU 15 Fertőendréd	10	8	6	6	8	11	6	11	16	82	**45**
Identified plants	103	118	114	100	148	146	162	184	184	1259	
species	**22**	**44**	**38**	**42**	**48**	**46**	**62**	**69**	**69**		

Table 3: Occurrence of plant species in apiaries and periods;
Number of apiaries: 15; number of samples: 133; Total species count: 173
During each sampling period, we marked the apiary showcasing the highest number of species by highlighting its background.

IDENTIFIED PLANT SPECIES AND VARIATIONS BY APIARY AND SAMPLING PERIOD

The number of identified plant species varied by apiary and sampling period, as summarised in Table 3, and visual representation Figure 2 and Figure 3.

Figure 2 shows the number of identified plants and species per apiary. Both values are the lowest in the Páhi apiary and the highest in the Vizsoly apiary.

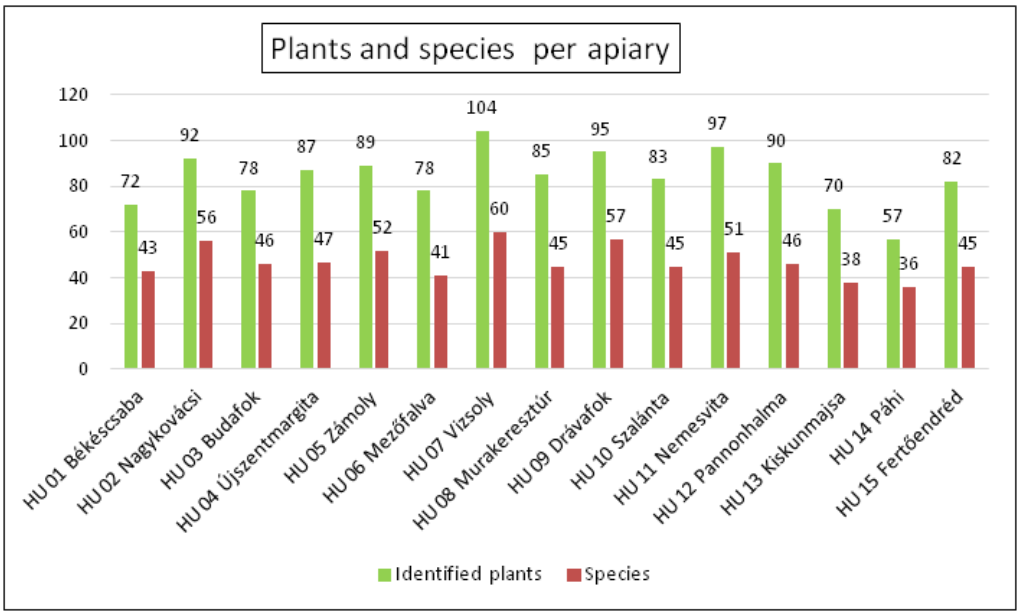

Figure 2: Number of identified plants and species per period (a visual representation of the last two columns of Table 3).

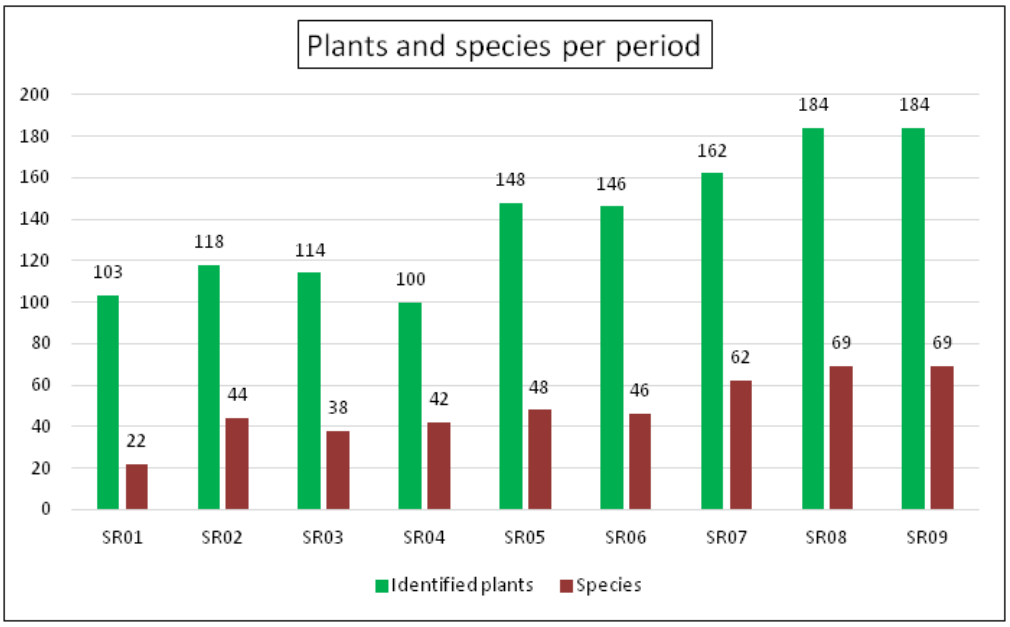

Figure 3: The number of plants and species identified per period (A visual representation of the last two rows of Table 3)

The number of identified plants per apiary is the simple sum of the plants identified during each sampling period for that particular apiary. However, the same plant species may appear in multiple sampling periods for a given apiary. Therefore, the number of species represents the total number of different plant species from which bees collected pollen during sampling periods 1–9.

In each sampling column (SR01–SR09), we highlighted the apiary where the highest number of species was identified during that specific sampling period. It is noteworthy that Vizsoly ranked at the top in three samplings, with 15 and 16 identified species, respectively.

Certain plant species were present in one or more periods within the individual apiaries, resulting in a total of 173 species identified in the Hungarian samples. The number of plant species per apiary varied, ranging between 36 and 60 /Figure 4/.

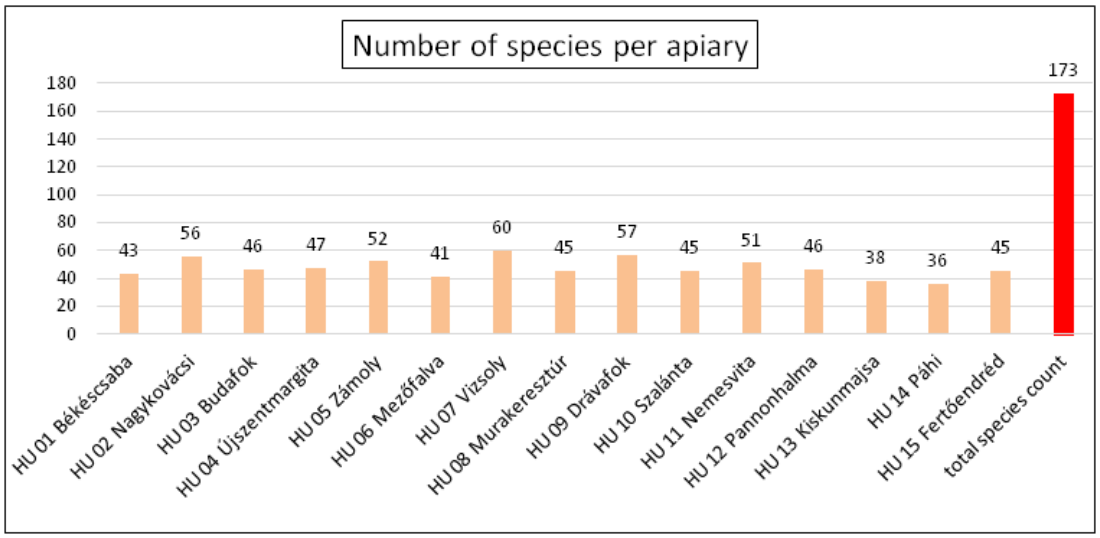

Figure 4: Number of plant species per apiary and the total species count.

The number of plant species during the sampling periods was lowest in the first period of May, with 22 species, and highest in the late summer–early autumn periods, exceeding 60 species /Figure 5/.

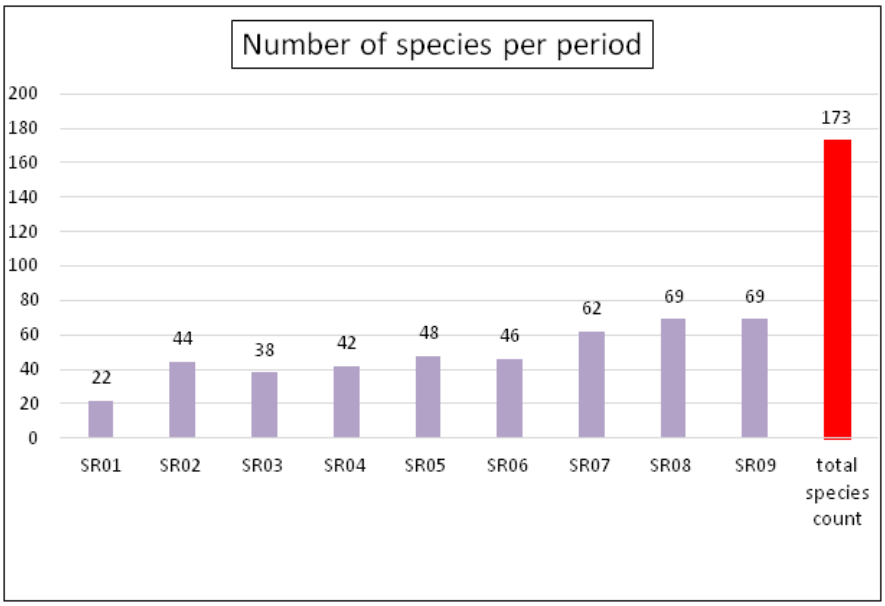

Figure 5: Number of plant species per period and the total species count.

The variability in the number of identified plant species across apiaries and sampling periods is illustrated in the graph below. In 2023, the pollen collection showed the least variability in Páhi, located in Bács-Kiskun Shire, while the highest variability was observed in Vizsoly, located in Borsod-Abaúj-Zemplén Shire. Additionally, pollen collection was highly variable in nine other apiary areas, while it showed lower variability in four locations /Figure 6/.

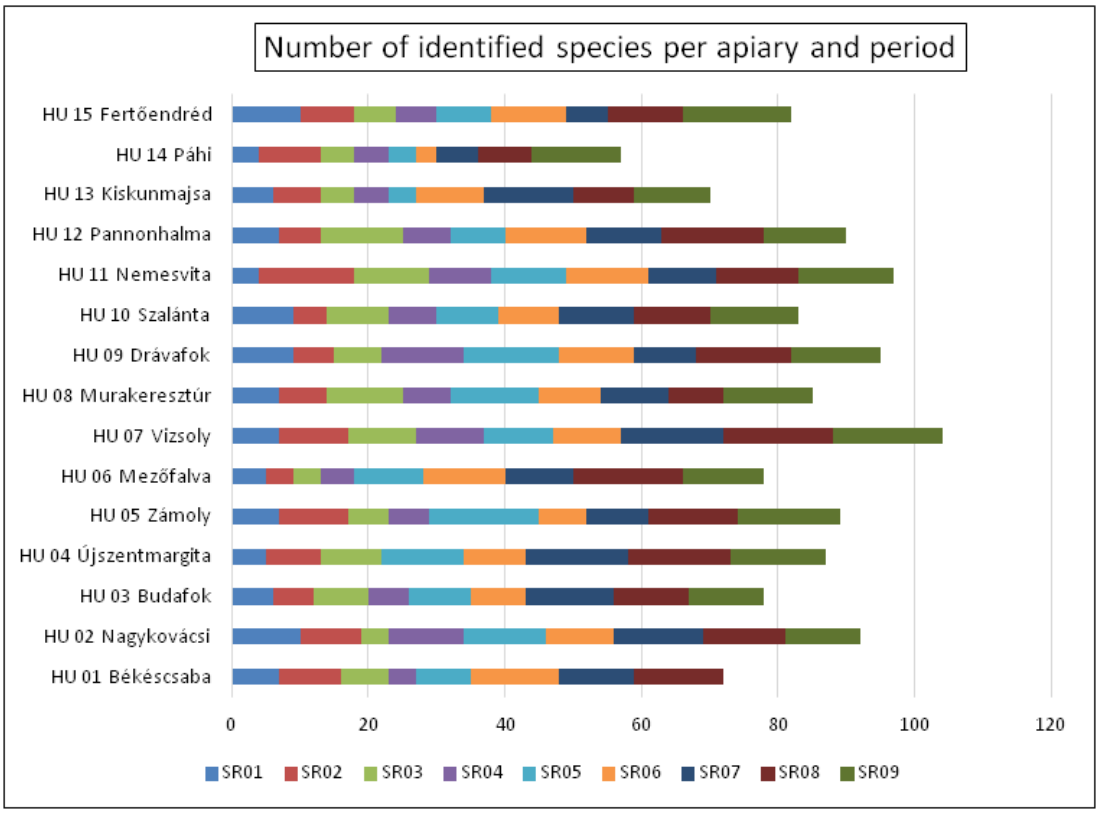

Figure 6: Number of identified species per apiary and sampling period

FREQUENCY OF PLANT SPECIES OCCURRENCE

The 1,259 identified plants from all sampling periods and apiaries represent 173 species. The number of species is inversely proportional to their frequency of occurrence. As the number of species increases, the occurrences within the apiaries decrease. The most frequently occurring species was *Plantago lanceolata* (NN), recorded 68 times, while 35 species were recorded only once. The graph illustrates the frequency of plant occurrences across all samples /Figure 7/.

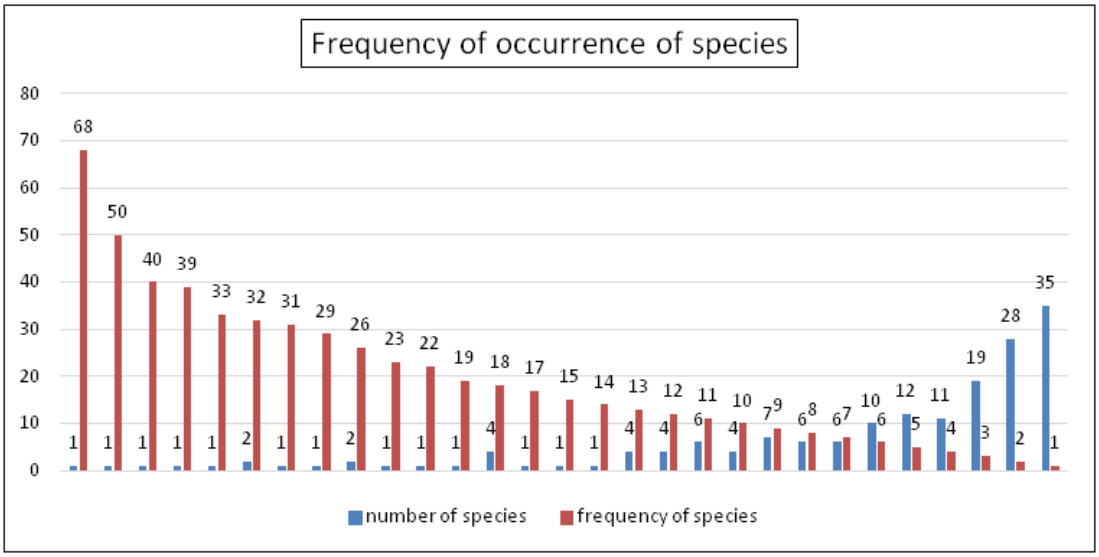

Figure 7: Frequency of species occurrence.

OCCURRENCE OF PLANT SPECIES BY APIARY

In the apiaries, bees collected pollen from a minimum of 3–7 and a maximum of 13–16 plant species /Figure 8/.

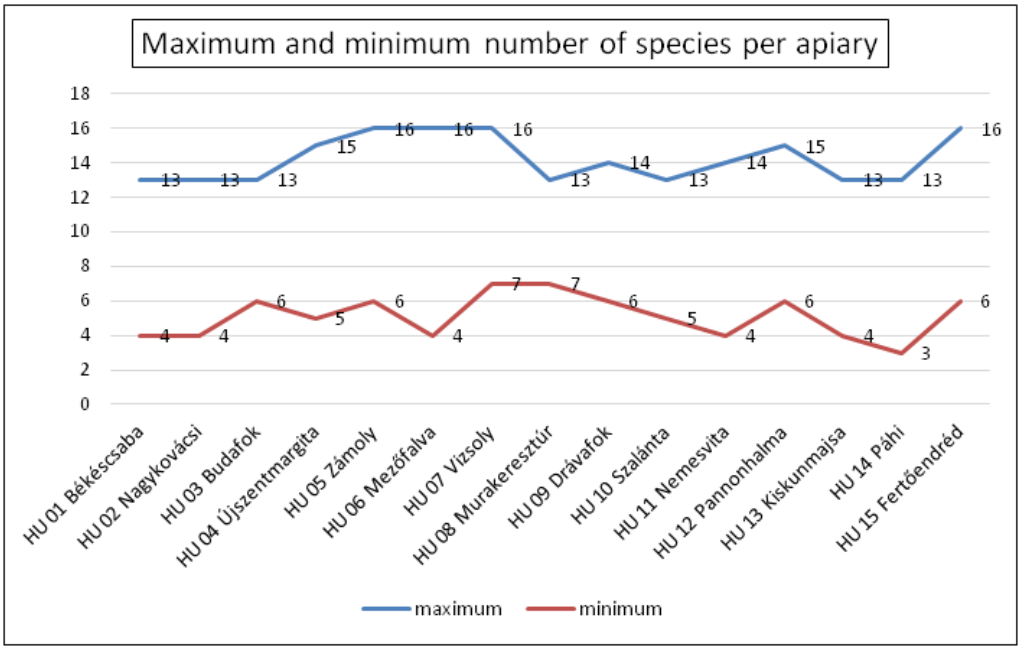

Figure 8: Maximum and minimum number of species per apiary

NUMBER OF PLANT SPECIES OCCURRING ACROSS APIARIES

The distribution of the 173 plant species found in the apiaries is illustrated in the following figure. The number of species shared by multiple apiaries is relatively low, while the number of species found in fewer apiaries increases. Seven shared species were identified across seven apiaries, and as the number of apiaries decreases, the number of shared plant species increases. Notably, the number of species occurring in only one or two apiaries is particularly high /Figure 9/.

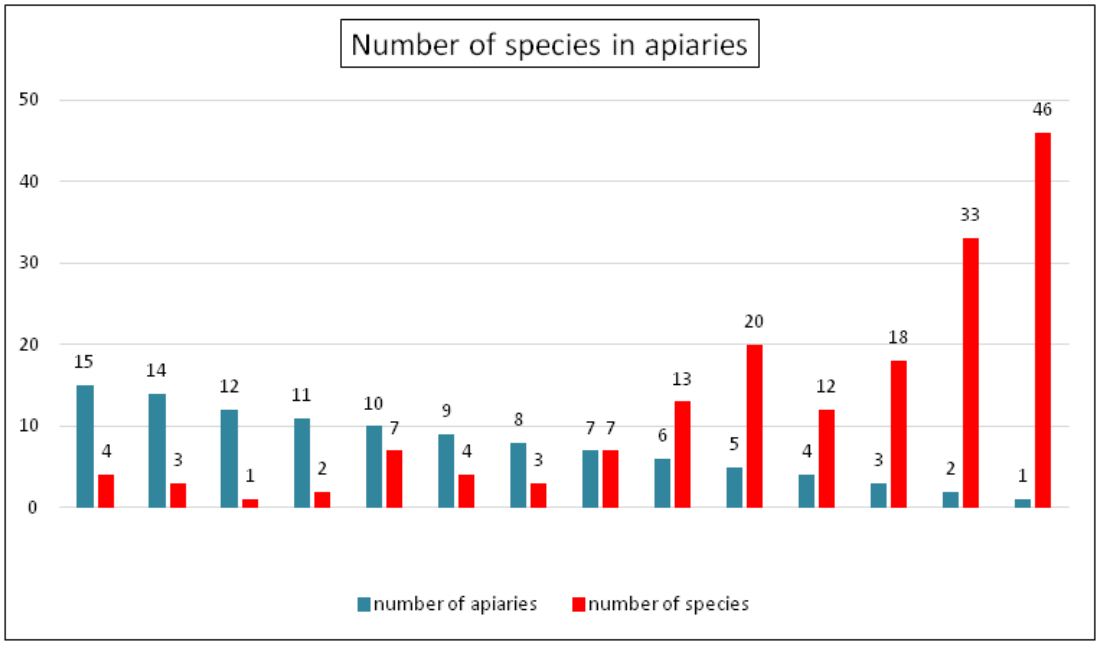

Figure 9: Number of species occurring in apiaries

During pollen collection, bees gathered plant species that provided either both nectar and pollen (nectariferous) or only pollen (nectarless). Among the 173 plant species, 133 were nectariferous, while 40 were nectarless (Figure 10)

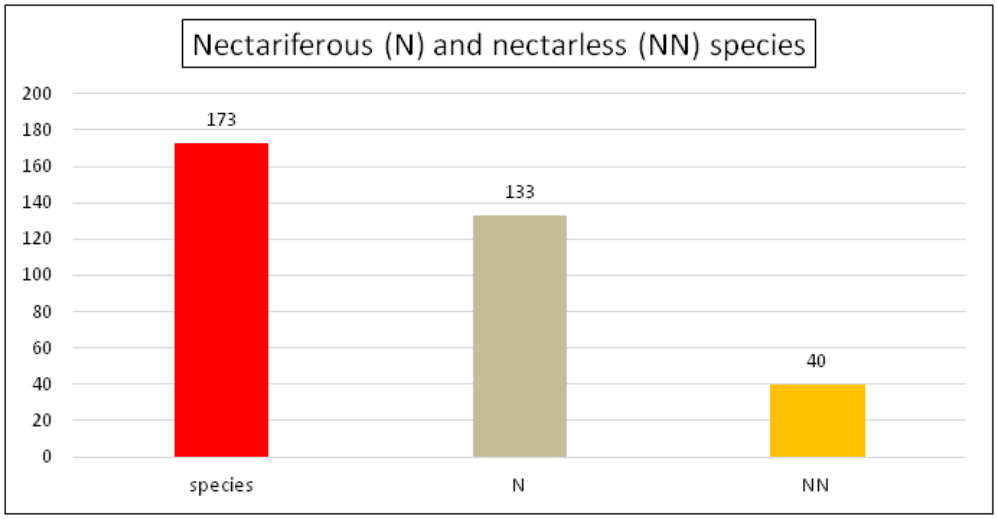

Figure 10: Number of nectariferous and nectarless species

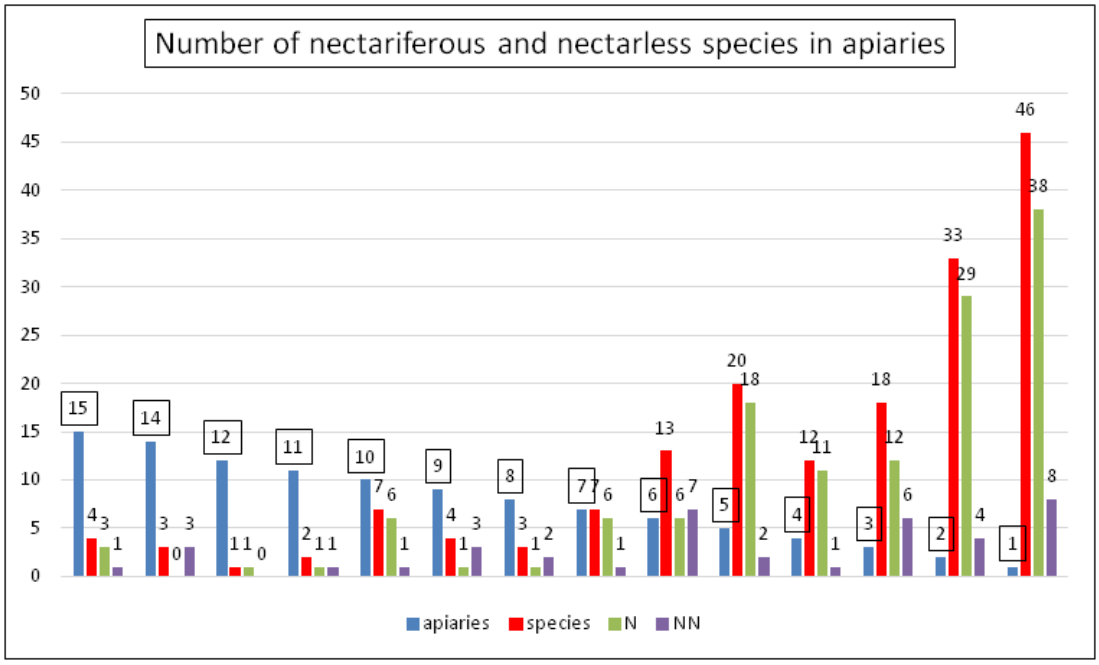

Figure 11: Number of nectariferous and nectarless species occurring in apiaries

The data reflect that bees typically collect pollen to a greater extent from plants that also provide nectar. The occurrence frequency of plant species in more than seven apiaries is still low. The number of species occurring in fewer than six apiaries has increased sharply, and among the identical plant species found in fewer than six apiaries, the number of nectariferous plant species is significantly higher than that of nectarless ones (Figure 11).

OCCURRENCE OF PLANT SPECIES BY PERIOD

Bees collected pollen from a minimum of 3-11 and a maximum of 10-16 plant species per period. The spring period is characterized by a low number of plant species visited, while from mid-summer to autumn, the number of plant species visited gradually increases (Figure 12).

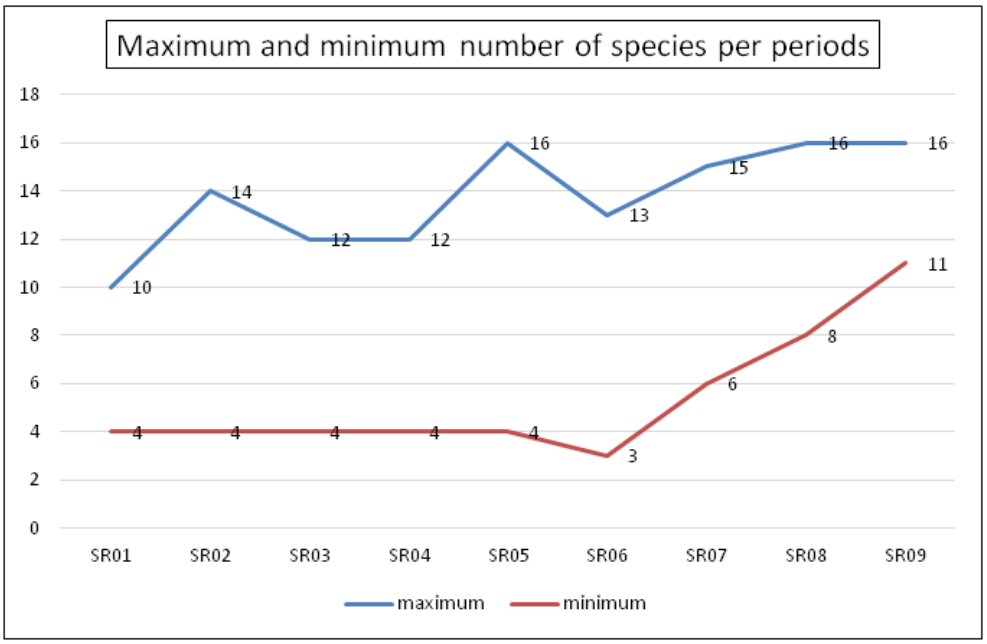

Figure 12: Maximum and minimum number of species per period

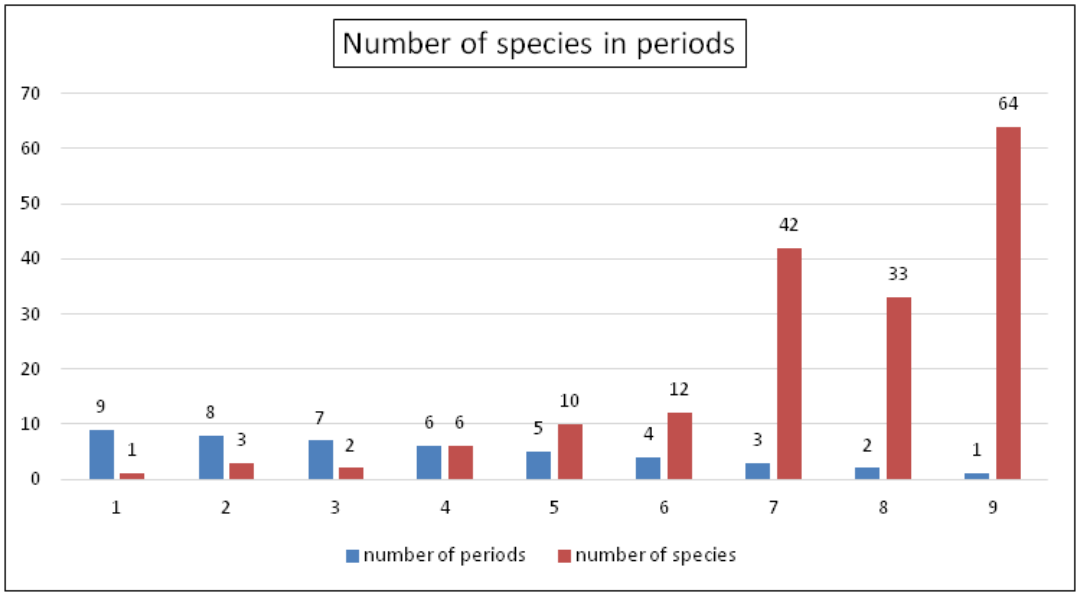

Figure 13: Number of species occurring in different periods

Only one plant species was collected in all nine periods, the nectarless *Chelidonium majus*. Regarding the pollen-collecting period, the nectarless *Papaver rhoeas* and *Plantago lanceolata*, along with the nectariferous T*rifolium repens*, were collected in eight periods. In seven periods, bees collected pollen from the nectariferous *Brassica napus* and *Melilotus albus*. In six periods, pollen was collected from the nectarless *Cannabis sativa*, *Clematis vitalba*, and *Hypericum perforatum*, as well as the nectariferous *Raphanus sativus var. oleiferus*, *Reseda lutea*, and *Verbascum thapsus*. The number of plant species increases significantly when considering the other periods, with most species (64) occurring in only one period (Figure 13).

In the various periods, pollen was typically collected in greater quantities from nectariferous plant species (Figure 14).

The most characteristic species of the spring pollen-collecting period, *Chelidonium majus* (NN), was not present in all apiaries.

Among plants flowering for several months, *Plantago lanceolata* (NN) occurred in the highest quantity (abundance 1066) across eight periods, from May to the end of August, though in May it was present in only one apiary.

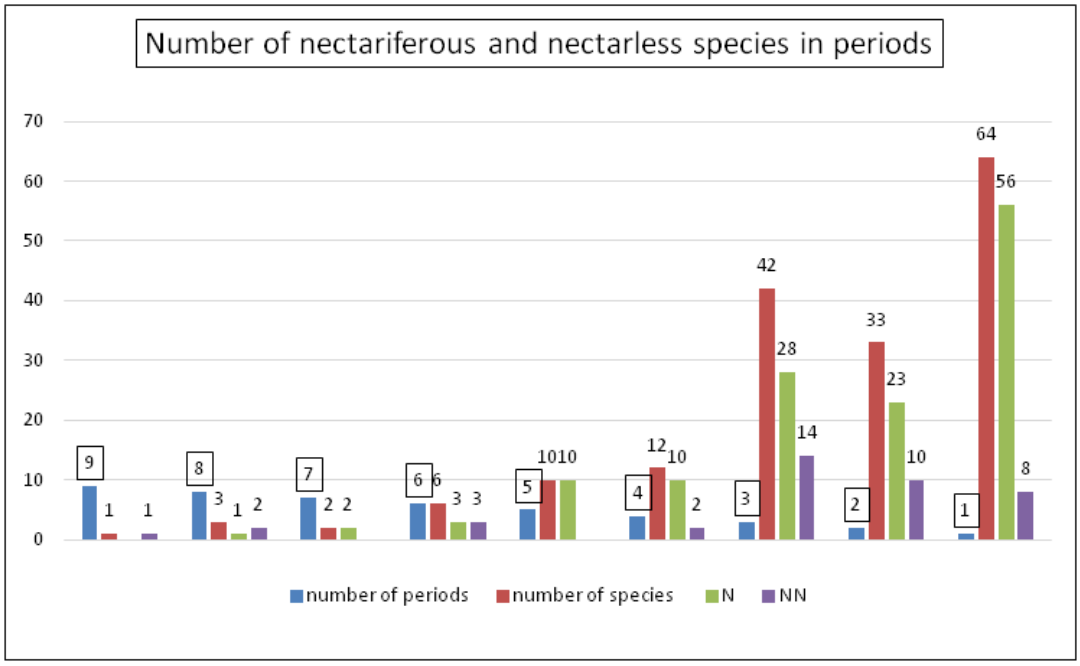

Figure 14: Number of nectariferous and nectarless species occurring in different periods

SPECTRUM CLASSIFICATION

Bees were able to collect pollen from certain plant species across varying numbers of periods.

- Basic spectrum: Includes species occurring in 75-100% of the periods.
- Supplementary I spectrum: Includes species present in 50-74% of the periods.
- Supplementary II spectrum: Includes species present in 25-49% of the periods.
- Remainder spectrum: Includes species present in fewer than 25% of the periods.

During the 2023 study, six species were classified into the Basic spectrum, 16 species into the Supplementary I spectrum, 54 species into the Supplementary II spectrum, and 97 species into the Remainder spectrum.

The species occurring in 5-9 periods indicate plants with longer pollen-collecting periods. Among these, cultivated plants were present alongside a significant number of wild plants. It was also observed that, during these periods, nectarless plants that provide only pollen were present in considerable numbers.

Spectrum	Periods number	Species Names
Basic spectrum	9	*Chelidonium majus* (NN)
	8	*Papaver rhoeas* (NN); *Plantago lanceolata* (NN); *Trifolium repens*
	7	*Brassica napus*; *Melilotus albus*
Supplementary I spectrum	6	*Cannabis sativa* (NN); *Clematis vitalba* (NN); *Hypericum perforatum* (NN); *Raphanus sativus var. oleiferus*; *Reseda lutea*; *Verbascum thapsus*
	5	*Ballota nigra*; *Brassica rapa*; *Carduus acanthoides*; *Convolvulus arvensis*; *Delosperma cooperi*; *Helianthus annuus*; *Lythrum salicaria*; *Ononis spinosa*; *Rubus ulmifolius x Rubus caesius*; *Sinapis alba*
Supplementary II spectrum	4	.....; *Phacelia tanacetifolia*; *Sorghum bicolor* (NN); *Zea mays* (NN)
	3	.....; *Ambrosia artemisifolia* (NN); *Ambrosia coronopifolia* (NN); *Artemisia vulgaris* (NN); *Papaver somniferum* (NN); *Rosa canina* (NN)
Remainder spectrum	2	.....; *Amorpha fruticosa*; *Anthriscus cerefolium*; *Castanea sativa*; *Crataegus monogyna x. C. punctata*
	1	.....; *Elaeagnus angustifolia*; *Fagopyrum esculentum*; *Hedera helix*; *Juglans regia* (NN); ***Malus sylvestris; Prunus serotina***; *Quercus frainetto* (NN); *Quercus pubescens* (NN); *Quercus rubra* (NN); *Rumex patientia* (NN); ***Salix triandra***; *Robinia pseudoacacia*; *Solidago canadensis*; ***Taraxacum officinale; Tilia cordata, Tilia tomentosa***; *Trifolium alexandrinum*; *Urtica dioica* (NN)

Table 4: Spectrum classification of key plant species

In the Supplementary II and Remainder spectra, certain species—such as nectarless plants *Zea mays*, *Sorghum bicolor*, *Ambrosia artemisiifolia*, and nectariferous plant *Solidago gigantea*—were particularly important to bees during specific periods.

It is noteworthy that among the species found in only one period (bolded in the table), several provided abundant pollen-collecting opportunities for bees even before the first May period in certain regions during the 2023 study in Hungary (Table 4).

OCCURRENCE OF PLANT SPECIES IN APIARIES AND PERIODS

FREQUENCY OF PLANT SPECIES BY APIARY

The number of plant species occurring varies across the different apiaries (Figure 15).

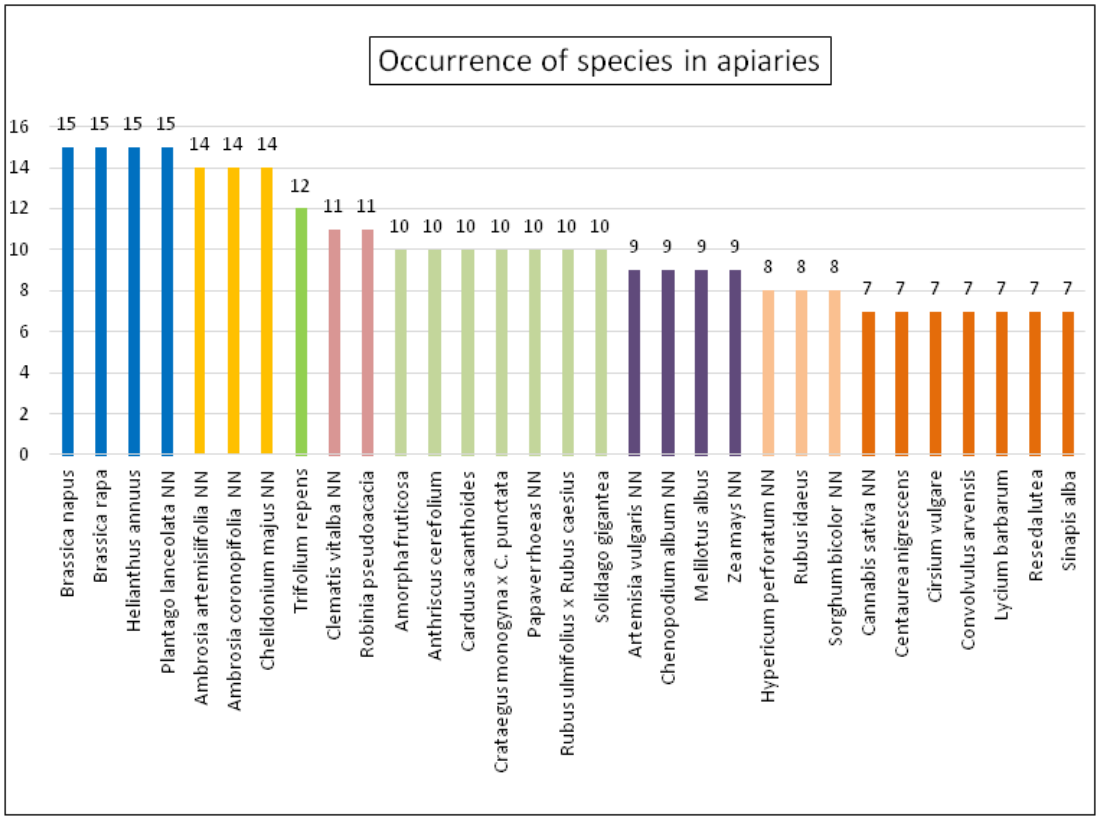

Figure 15: Occurrence of species in the apiaries

Among the same species, four species were found in all fifteen apiaries: the nectariferous *Brassica napus*, *Brassica rapa*, *Helianthus annuus*, and the nectarless *Plantago lanceolata*. In fourteen apiaries, three nectarless species, *Ambrosia artemisiifolia*, *Ambrosia coronopifolia*, and *Chelidonium majus*, were present. Additionally, in twelve apiaries, one nectariferous species, *Trifolium repens*, was recorded. Eleven apiaries contained two species: the nectarless *Clematis vitalba* and the nectariferous *Robinia pseudoacacia*.

In ten apiaries, seven species occurred: the nectariferous *Amorpha fruticosa*, *Anthriscus cerefolium*, *Carduus acanthoides*, *Crataegus monogyna x C. punctata*, *Rubus ulmifolius x Rubus caesius*, *Solidago gigantea*, and the nectarless *Papaver rhoeas*.

In nine apiaries, four species were found: the nectarless *Artemisia vulgaris*, *Chenopodium album*, *Zea mays*, and the nectariferous *Melilotus albus*. Eight apiaries recorded three species: the nectariferous *Rubus idaeus* and the nectarless *Hypericum perforatum* and *Sorghum bicolor*.

Seven apiaries contained seven species: the nectarless *Cannabis sativa* and the nectariferous *Centaurea nigrescens*, *Cirsium vulgare*, *Convolvulus arvensis*, *Lycium barbarum*, *Reseda lutea*, and *Sinapis alba*.

Pollen collection occurred most frequently from cultivated agricultural plants, wild plants, and less often from trees and shrubs. The species occurring in 15–7 apiaries that are more significant in terms of frequency, along with the number of occurrence periods, are summarized in the table below (Table 5).

Number of Apiaries	Number of Sampling rounds	Species
15	8	*Plantago lanceolata (NN)*
	7	*Brassica napus*
	5	*Helianthus annuus; Brassica rapa*
14	9	*Chelidonium majus (NN)*
	3	*Ambrosia coronopifolia (NN); Ambrosia artemisiifolia (NN)*
12	8	*Trifolium repens*
11	6	*Clematis vitalba (NN)*
	1	*Robinia pseudoacacia*
10	8	*Papaver rhoeas (NN)*
	5	*Rubus ulmifolius x Rubus caesius; Carduus acanthoides*
	3	*Solidago gigantea*
	2	*Anthriscus cerefolium; Amorpha fruticosa; Crataegus monogyna x C. punctata*
9	7	*Melilotus albus*
	4	*Zea mays (NN)*
	3	*Artemisia vulgaris (NN); Chenopodium album(NN)*
8	6	*Hypericum perforatum(NN)*
	4	*Sorghum bicolor (NN)*
	3	*Rubus idaeus*
7	6	*Reseda lutea; Cannabis sativa (NN)*
	5	*Sinapis alba; Convolvulus arvensis*
	3	*Centaurea nigrescens; Lycium barbarum; Cirsium vulgare*

Table 5: Occurrence of plant species in apiaries and periods

THE OCCURRENCE FREQUENCY OF PLANT SPECIES BY PERIOD

Only one species, the nectarless *Chelidonium majus*, appeared in every period. Three species occurred in eight periods (Figure 16), but in different numbers of apiaries: the nectarless *Plantago lanceolata* was found in 15 apiaries, *Papaver rhoeas* in 10 apiaries, and the nectariferous cultivated agricultural plant *Trifolium repens* in 12 apiaries (Table 5).

Species that occur in multiple periods and simultaneously in several apiaries, as well as those found in more than 10 apiaries—regardless of their presence in only a few periods—represent important pollen sources (Table 6).

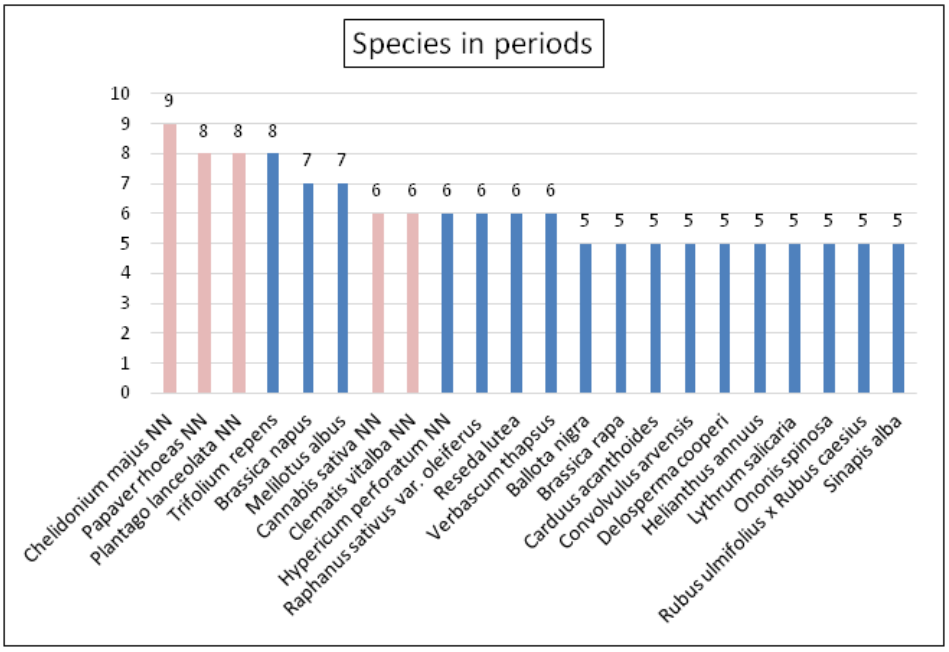

Figure 16: Occurrence of species across periods

Latin name	Number of Periods	Number of Apiaries
Chelidonium majus (NN)	9	14
Plantago lanceolata (NN)	8	15
Trifolium repens	8	12
Papaver rhoeas (NN)	8	10
Brassica napus	7	15
Clematis vitalba (NN)	6	11
Brassica rapa	5	15
Helianthus annuus	5	15
Carduus acanthoides	5	10
Rubus ulmifolius x Rubus caesius	5	10
Ambrosia artemisiifolia (NN)	3	14
Ambrosia coronopifolia (NN)	3	14
Solidago gigantea	3	10
Anthriscus cerefolium	2	11
Amorpha fruticosa	2	10
Crataegus monogyna x C. punctata	2	10
Robinia pseudoacacia	1	11

Table 6: Important pollen sources across periods and apiaries

SUM OF THE RELATIVE FREQUENCY BY PLANT SPECIES

The maximum abundance of plant species indicates the period in which bees collected the largest amount of pollen (Table 7).

Species name	Sum of abundance	Sampling Round / Period	Number of apiaries
Brassica napus	486	SR01	15
Anthriscus cerefolium	288	SR01	9
Helianthus annuus	288	SR06	14
Papaver rhoeas (NN)	260	SR04	8
Plantago lanceolata (NN)	246	SR08	10
Robinia pseudoacacia	233	SR02	11
Clematis vitalba (NN)	211	SR05	9
Brassica rapa	164	SR01	15
Chelidonium majus (NN)	164	SR01	12
Amorpha fruticosa	148	SR03	10
Solidago gigantea	141	SR08	9
Ononis spinosa	115	SR05	5
Phacelia tanacetifolia	106	SR03	3
Sorghum bicolor	100	SR07	7
20 species	50-99	...	...
27 species	30-49	...	...
60 species	10-29	...	...
52 species	2-9	...	...

Table 7: Sum of the relative frequencies by period in the apiaries

The bees collected the most pollen from Brassica napus across seven periods, with the highest amount observed in the first period and a significant amount in the second period. In the remaining periods, its presence was minimal (Figure 17).

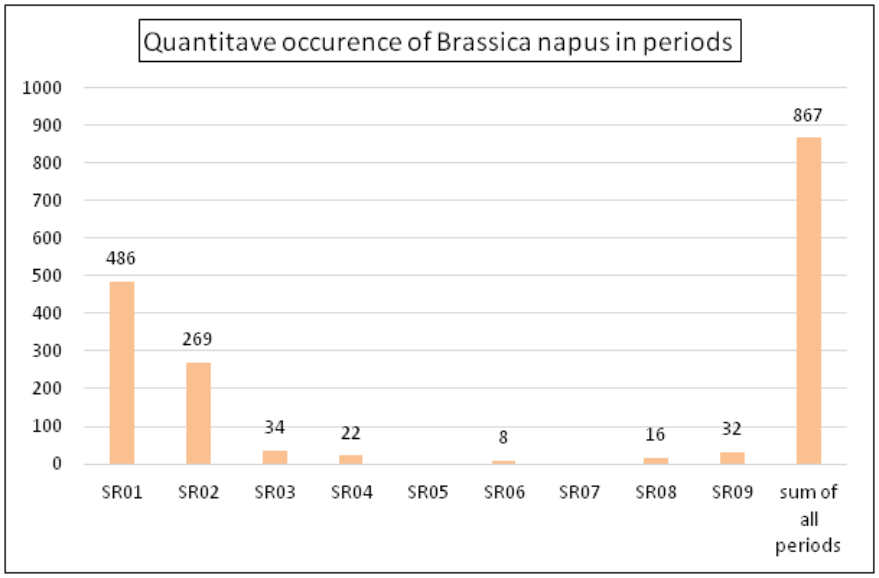

Figure 17: Quantitative occurrence of Brassica napus across periods

MAXIMUM OCCURRENCE OF PLANT SPECIES

The relative frequency percentages of plant species across various periods for each apiary are presented in Table 8. Two samples, HU01 SR09 and HU04 SR04, could not be evaluated.

Species name	SR01	SR02	SR03	SR04	SR05	SR06	SR07	SR08	SR09
HU01 BÉKÉSCSABA /BÉKÉS Shire									
Anthriscus cerefolium	40								
Brassica napus		30							
Chenopodium album (NN)								20	
Helianthus annuus						15	29		
Raphanus sativus var. oleiferus			40	63	30				
HU02 NAGYKOVÁCSI /PEST Shire									
Brassica napus	27								
Castanea sativa					26				
Clematis vitalba (NN)							25		
Fraxinus ornus (NN)		35							
Helianthus annuus						25			
Phacelia tanacetifolia			61	27					
Picris hieracioides								16	
Plantago media (NN)									23
HU03 BUDAFOK /BUDAPEST XXII.ker.									
Brassica napus	29								
Clematis vitalba (NN)				32			27		
Melilotus albus			40						
Plantago lanceolata (NN)					41	43		29	51
Robinia pseudoacacia		39							
HU04 ÚJSZENTMARGITA /HAJDÚ-BIHAR Shire									
Amorpha fruticosa			22						
Brassica napus	52								
Helianthus annuus					16				
Ononis spinosa						40			
Plantago lanceolata (NN)								26	

Species name	SR01	SR02	SR03	SR04	SR05	SR06	SR07	SR08	SR09
Robinia pseudoacacia		24							
Sinapis alba							27		30
HU05 ZÁMOLY /FEJÉR Shire									
Ballota nigra								21	
Brassica napus	32	37							
Papaver rhoeas (NN)				40	30		20		
Papaver somniferum (NN)			32						
Trifolium alexandrinum						42			
Zea mays (NN)									15
HU06 MEZŐFALVA /FEJÉR Shire									
Anthriscus cerefolium	53								
Artemisia vulgaris (NN)								25	
Hedera helix									22
Helianthus annuus						27			
Papaver rhoeas (NN)					25				
Papaver somniferum (NN)			40	46					
Plantago lanceolata (NN)							23		
Robinia pseudoacacia		32							
HU07 VIZSOLY /BORSOD-ABAÚJ-ZEMPLÉN Shire									
Brassica napus	38	44	34						
Clematis vitalba (NN)					32	26			
Helianthus annuus							26		
Papaver rhoeas (NN)				25					
Solidago gigantea								39	26
HU08 MURAKERESZTÚR /ZALA Shire									
Brassica napus		25							24
Chelidonium majus (NN)	34								
Helianthus annuus						26			
Ranunculus sardous			29						
Solidago gigantea								33	
Trifolium repens							24		
Tripleurospermum maritimum				36	26				
HU09 DRÁVAFOK /BARANYA Shire									
Amorpha fruticosa			35						
Chelidonium majus (NN)	23								
Crepis setosa					23				
Erigeron annuus (NN)				33					
Plantago lanceolata (NN)						18		29	18
Raphanus sativus var. oleiferus							26		
Robinia pseudoacacia		42							
HU10 SZALÁNTA /BARANYA Shire									
Anethum graveolens						22			
Anthriscus cerefolium	25								
Chenopodium album (NN)							24		
Clematis vitalba (NN)					29				

Species name	SR01	SR02	SR03	SR04	SR05	SR06	SR07	SR08	SR09
Papaver rhoeas (NN)			31						
Plantago lanceolata (NN)								25	
Raphanus sativus var. oleiferus									28
Robinia pseudoacacia		41							
Tripleurospermum maritimum				34					
HU11-NEMESVITA /VESZPRÉM Shire									
Amorpha fruticosa			27						
Brassica napus	53	26							
Castanea sativa				23	25				
Hedera helix									18
Hypericum perforatum (NN)						19			
Plantago lanceolata (NN)							26	25	
HU12-PANNONHALMA /GYŐR-MOSON-SOPRON Shire									
Brassica napus	41	35							
Onobrychis viciifolia						30			
Papaver rhoeas (NN)			30	43	32				
Plantago lanceolata (NN)							26	25	
Sinapis alba									17
HU13 KISKUNMAJSA /BÁCS-KISKUN Shire									
Anthriscus cerefolium	55								
Chenopodium album (NN)							19		
Helianthus annuus						38			
Ononis spinosa				37	43			25	35
Papaver rhoeas (NN)		38	40						
HU14 PÁHI /BÁCS-KISKUN Shire									
Anthriscus cerefolium	62								
Helianthus annuus						55			
Lythrum salicaria								30	
Ononis spinosa					42				
Papaver rhoeas (NN)		28	55	45					
Sanguisorba officinalis (NN)							42		22
HU15 FERTŐENDRÉD /GYŐR-MOSON-SOPRON Shire									
Brassica napus	42	35							
Helianthus annuus							34		
Hypericum perforatum (NN)									21
Papaver rhoeas (NN)			38	44	33				
Plantago lanceolata (NN)						40		35	

Table 8: Maximum relative frequency values of plant species per apiary and period.

The plant species with the highest maximum value is *Raphanus sativus var. oleiferus*, with 63% in the June 15-18 (SR04) period, which was found in the sample from the HU01 Békéscsaba/Békés Shire apiary. The species with the lowest maximum value is *Zea mays* (NN), with 15%, occurring in the August 24-27 (SR09) period from the HU05 Zámoly/Fejér Shire apiary (Figure 18)

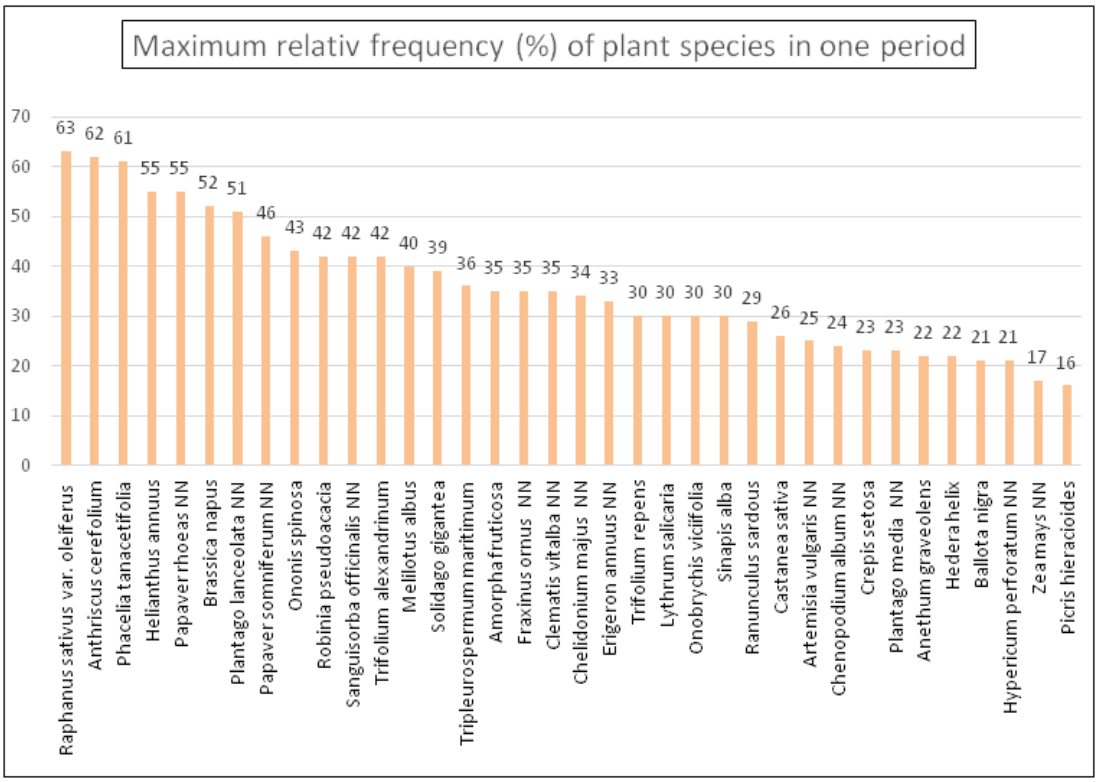

Figure 18: Maximum relative frequency values of plant species across periods.

KEY SPECIES ACROSS PERIODS

Among the key species (with a relative frequency abundance greater than 50) characteristic of each period, there are both plants that flowered for a longer and shorter duration (Table 9).

Primarily, the number of foraging periods increased with the length of the flowering period of the plants.

Species	Number of periods	SR01	SR02	SR03	SR04	SR05	SR06	SR07	SR08	SR09
Brassica napus	2	x	x							
Brassica rapa	2	x	x							
Chelidonium majus (NN)	2	x	x							
Anthriscus cerefolium	1	x								
Crataegus monogyna x C. punctata	1	x								
Papaver rhoeas (NN)	4		x	x	x	x				
Robinia pseudoacacia	1		x							
Papaver somniferum (NN)	2			x	x					
Phacelia tanacetifolia	2			x	x					
Amorpha fruticosa	1			x						
Melilotus albus	1			x						
Clematis vitalba (NN)	4				x	x	x		x	
Castanea sativa	1				x					
Raphanus sativus var. oleiferus	1				x					
Tripleurospermum maritimum	1				x					
Plantago lanceolata (NN)	5					x	x	x	x	x
Helianthus annuus	3					x	x	x		
Ononis spinosa	2					x				x
Sorghum bicolor (NN)	2						x	x		
Trifolium repens	2						x	x		
Chenopodium album (NN)	1							x		

Species	Number of periods	SR01	SR02	SR03	SR04	SR05	SR06	SR07	SR08	SR09
Rubus ulmifolius x R. caesius	1							x		
Solidago gigantea	2								x	x
Artemisia vulgaris (NN)	1								x	
Carduus acanthoides	1								x	
Chenopodium album (NN)	1								x	
Ambrosia artemisiifolia (NN)	1									x
Ambrosia coronopifolia (NN)	1									x
Sinapis alba	1									x

Table 9: Key species across periods

KEY SPECIES CHARACTERISTIC OF EACH APIARY

Among the key species (with a relative frequency abundance greater than 50) in each apiary, there are both nectariferous species and nectarless species, which provide only pollen. The most common nectariferous species in the apiaries were *Brassica napus* (found in nine apiaries), *Helianthus annuus* (found in six apiaries), as well as nectarless, pollen-only species such as *Plantago lanceolata* (found in eight apiaries), *Papaver rhoeas* (found in six apiaries), and *Clematis vitalba* (found in five apiaries) during the 2023 study year. Among the nectariferous species, *Anthriscus cerefolium* and *Ononis spinosa* were found in three apiaries, while *Phacelia tanacetifolia*, *Solidago gigantea*, and *Trifolium repens* were found in two apiaries. The other species listed in the table appeared in only one apiary (Table 10).

Species	Number of apiaries	HU01	HU02	HU03	HU04	HU05	HU06	HU07	HU08	HU09	HU10	HU11	HU12	HU13	HU14	HU15
Brassica napus	9	x			x	x		x	x		x	x	x			x
Plantago lanceolata (NN)	8			x	x		x			x	x	x	x			x
Helianthus annuus	6	x					x				x			x	x	x
Papaver rhoeas (NN)	6					x					x		x	x	x	x
Clematis vitalba (NN)	5		x	x				x			x	x				
Anthriscus cerefolium	3						x							x	x	
Ononis spinosa	3				x									x	x	
Phacelia tanacetifolia	2		x													x
Solidago gigantea	2							x	x							
Trifolium repens	2								x			x				
Amorpha fruticosa	1									x						
Ballota nigra	1					x										
Castanea sativa	1		x													
Centaurea cyanus	1						x									
Chelidonium majus (NN)	1								x							
Crepis setosa	1									x						
Hypericum perforatum (NN)	1															x
Lythrum salicaria	1														x	
Melilotus albus	1			x												
Papaver somniferum (NN)	1						x									
Pisum sativum	1	x														
Raphanus sativus var. oleiferus	1	x														
Sanguisorba officinalis (NN)	1														x	
Sinapis alba	1				x											
Tripleurospermum maritimum	1								x							

Table 10: Key species in the apiaries

KEY PLANT SPECIES IN THE APIARIES

KEY CHARACTERISTICS BY PLANT SPECIES

Based on the plant composition observed in the apiary samples, it is possible to identify which species are dominant in the area during a given period, i.e., the key species. Common key species include agricultural plants, as well as wildflowers. At maximum percentage values, alongside the mentioned plant groups, an invasive tree species, *Robinia pseudoacacia*, also appears. At minimum percentage values, in addition to agricultural plants and herbaceous wild plants, species such as the herb *Anethum graveolens* and the climbing ornamental plant *Hedera helix* were also found (Table 8).

KEY SPECIES (>45%) IN THE APIARIES (HUXX) AND THEIR PERIODIC OCCURRENCE (SR XX):

- HU01: *Raphanus raphanistrum* 63% (SR04) – Agricultural crop plant
- HU02: *Phacelia tanacetifolia* 61% (SR03) – Agricultural crop plant
- HU03: *Plantago lanceolata* (NN) 51% (SR09) – Herbaceous wild plant
- HU04: *Brassica napus* 52% (SR01) – Agricultural crop plant
- HU06: *Anthriscus cerefolium* 53% (SR01) – Herbaceous wild plant
- HU06: *Papaver somniferum* (NN) 46% (SR04) – Agricultural crop plant
- HU11: *Brassica napus* 53% (SR01) – Agricultural crop plant
- HU13: *Anthriscus cerefolium* 55% (SR01) – Herbaceous wild plant
- HU14: *Anthriscus cerefolium* 62% (SR01) – Herbaceous wild plant

MAXIMUM % IN THE APIARIES:

- HU01: *Raphanus raphanistrum* 63% (SR04) – Agricultural crop plant
- HU02: *Phacelia tanacetifolia* 61% (SR03) – Agricultural crop plant
- HU03: *Plantago lanceolata* (NN) 51% (SR09) – Herbaceous wild plant
- HU04: *Brassica napus* 52% (SR01) – Agricultural crop plant
- HU05: *Trifolium alexandrinum* 42% (SR06) – Agricultural crop plant
- HU06: *Anthriscus cerefolium* 53% (SR01) – Herbaceous wild plant
- HU07: *Brassica napus* 44% (SR02) – Agricultural crop plant
- HU08: *Tripleurospermum maritimum* 36% (SR04) – Matricaria chamomilla (herb)
- HU09: *Robinia pseudoacacia* 42% (SR02) – Forest tree species
- HU10: *Robinia pseudoacacia* 41% (SR02) – Forest tree species
- HU11: *Brassica napus* 53% (SR01) – Agricultural crop plant
- HU12: *Papaver rhoeas* (NN) 43% (SR04) – Herbaceous wild plant
- HU13: *Anthriscus cerefolium* 55% (SR01) – Herbaceous wild plant
- HU14: *Anthriscus cerefolium* 62% (SR01) – Herbaceous wild plant
- HU15: *Papaver rhoeas NN 44% (SR 04)* – Herbaceous wild plant

RESULTS

1. In the fifteen apiaries and nine periods, the number of plant species recorded in the bee-collected samples was 173, with 36-60 species per apiary and 22-69 species per period (Figures 4-5).
2. The occurrence frequency of species shows a notable trend reversal for species appearing fewer than 10 times, with a sharp increase in occurrences. Among the 173 plant species found in the apiaries, only a smaller number were common across multiple apiaries. From the sixth apiary onwards, as the number of apiaries decreased, the number of recurring species increased sharply (Figure 7).
3. In the apiaries, bees collected from a maximum of 13-16 and a minimum of 3-7 plant species (Figure 8).
4. In the periods, bees collected from a maximum of 10-16 and a minimum of 3-11 plant species (Figure 12).
5. Of the 173 plant species, 133 produced nectar and 40 were pollen-only species (Figure 10).
6. Bees collected significantly larger quantities from nectariferous species (Figures 11 and 14).
7. In the first period, the highest percentage of 62% was collected from *Anthriscus cerefolium* in Bács-Kiskun Shire/Páhi. In the second period, 44% was collected from *Brassica napus* in Borsod-Abaúj-Zemplén Shire/Vizsoly. In the third period, 61% was collected from *Phacelia tanacetifolia* in Pest Shire/Nagykovácsi. In the fourth period, 63% was collected from *Raphanus raphanistrum var. oleifera* in Békés Shire/Békéscsaba. In the fifth period, 43% was collected from *Ononis spinosa* in Bács-Kiskun Shire/Kiskunmajsa. In the sixth period, 55% was collected from *Helianthus annuus* in Bács-Kiskun Shire/Páhi. In the seventh period, 42% was collected from S*anguisorba officinalis* (NN) in Bács-Kiskun Shire/Páhi. In the eighth period, 39% was collected from *Solidago gigantea* in Borsod-Abaúj-Zemplén Shire/Vizsoly. In the ninth period, 51% was collected from *Plantago lanceolata* (NN) in Pest Shire/Budafok (Tables 8).
8. Common species typically appeared in more than seven apiaries. Of the 31 species, 19 were nectariferous, and 12 were pollen-only species. The most common species, appearing in fifteen apiaries, were the nectariferous agricultural plants *Brassica napus*, *Brassica rapa*, *Helianthus annuus*, and the pollen-only herbaceous wild plant *Plantago lanceolata* (Figure 15).

9. Among the plant species, there are both short-lived and longer-flowering species (Table 10).
10. The most frequently collected plant species by period:
    - o *Chelidonium majus* (NN) in nine periods/fourteen apiaries
    - o *Plantago lanceolata* (NN) in eight periods/fifteen apiaries
    - o *Trifolium repens* in eight periods/twelve apiaries
    - o *Papaver rhoeas* (NN) in eight periods/ten apiaries (Table 6).
11. The species collected in the largest quantities per period:
    - o *Brassica napus* 486/15 apiaries in SR01
    - o *Anthriscus cerefolium* 288/9 apiaries in SR01
    - o *Helianthus annuus* 288/14 apiaries in SR06
    - o *Papaver rhoeas* (NN) 260/8 apiaries in SR04
    - o *Plantago lanceolata* (NN) 248/10 apiaries in SR08
    - o *Robinia pseudoacacia* 233/11 apiaries in SR02
    - o *Clematis vitalba* (NN) 211/9 apiaries in SR05

    Further data can be found in Table 7.
12. The most abundant species collected from the apiaries:
    - o *Raphanus raphanistrum* /Békéscsaba
    - o *Phacelia tanacetifolia* /Nagykovácsi
    - o *Plantago lanceolata* (NN) /Budafok, Újszentmargita, Drávafok, Nemesvita
    - o *Papaver rhoeas* (NN) /Zámoly, Pannonhalma, Páhi, Fertőendréd
    - o *Papaver somniferum* (NN) /Mezőfalva
    - o *Brassica napus* (NN) /Vizsoly, Murakeresztúr
    - o *Helianthus annuus* /Szalánta
    - o *Ononis spinosa* /Kiskunmajsa (Table 8).
13. The most important plant species with greater than 50% relative frequency in the most apiaries:
    - o *Brassica napus* /9 apiaries
    - o *Plantago lanceolata* (NN) /8 apiaries
    - o *Helianthus annuus* and *Papaver rhoeas* (NN) /6 apiaries
    - o *Clematis vitalba* (NN) /5 apiaries
    - o *Anthriscus cerefolium* and Ononis spinosa /3 apiaries
    - o *Phacelia tanacetifolia*, *Solidago gigantea*, and *Trifolium repens* /2 apiaries. Some species were recorded in only one apiary (Table 10).
14. Among the important species recorded in only one apiary, some are specific to certain areas, while others are cultivated agricultural plants that could theoretically be grown elsewhere. These include *Amorpha fruticosa*, *Ballota nigra*, *Castanea sativa*, *Centaurea cyanus*, *Chelidonium majus* (NN), *Crepis setosa*, *Hypericum perforatum* (NN), *Lythrum salicaria*, *Melilotus albus*, *Papaver rhoeas* (NN), *Pisum sativum*, *Raphanus sativus var. oleifera*, *Sanguisorba officinalis* (NN), *Sinapis alba*, and *Tripleurospermum maritimum* (Table 10).

    Tripleurospermum maritimum, known as sea mayweed, was kept as such in the evaluation, but it is likely that *Matricaria chamomilla*, also known as medicinal chamomile, is meant, which occurs in several places in Hungary.
15. Regarding the Hungarian regions, the lowest number of plant species collected was found in three apiaries in the Southern Great Plain region (HU01/43 species in Békés Shire; HU13/38 species and HU14/56 species in Bács-Kiskun Shire). Similarly, the Western Transdanubia region, with three apiaries (HU08/45 species in Zala Shire; HU12/46 species and HU15/45 species in Győr-Moson-Sopron Shire), had lower species counts. The highest number of species was recorded in the Northern Hungary region (HU07/60 species in Borsod-Abaúj-Zemplén Shire) (Table 3).
16. It is important to note that due to Hungary's natural conditions, intensive pollen collection begins as early as April, with the most frequently occurring species being *Salix caprea*, *Salix alba*, *Taraxacum officinale*, and *Prunus spp.* species.
17. The study results are from the year 2023, and the occurrence and quantity of species depend primarily on the ecological, local, and weather conditions as well as the sampling protocols. Species occurrence and abundance are primarily area- and weather-dependent, but can also be significantly influenced by changing agricultural activities.

COMMENTS:

1. For the nectarless, pollen-only plant species, the 'NN' designation was only included when confirmed by multiple sources. For species such as *Verbascum sp.*, there is no consistent evidence, therefore it was not marked.
2. The spelling of Hungarian plant names and the standardization of synonym names were based on [2].
3. Inconsistencies in Species Names in the INSIGNIA Basic Data

   Some species names in the INSIGNIA basic data differ from the officially recognized scientific names. This discrepancy may require correction to ensure consistency with accepted botanical nomenclature. It is important to cross-reference the data with the latest botanical references to update these species names accordingly.

INSIGNIA	**After correction**
Latin name	**Valid Latin name**
Brassica oleracea	= Brassica rapa subsp. oleifera = Brassica rapa
Brassica rapa	= Brassica rapa subsp. napus = Brassica napus subsp. napus = Brassica napus
Inula britannica (synonym name)	Pentanema britannicum
Lotus glaber (synonym name)	Lotus tenuis
Ambrosia psilostachya (synonym name)	Ambrosia coronopifolia

LEDLEGEND

Non-nectariferous plants (NN) are marked accordingly.

APPENDIX

Latin plant name	**Hungarian plant name**
Acer pseudoplatanus	hegyi juhar
Achillea millefolium	cickafark
Aegopodium podagraria	podagrafű
Aesculus hippocastanum	vadgesztenye
Agrimonia eupatoria	párlófű
Allium angulosum	gyíkhagyma
Allium cepa	vöröshagyma
Allium scorodoprasum	kígyóhagyma
Allium sphaerocephalon	bunkós hagyma
Allium stipitatum	termetes hagyma
Althaea officinalis	orvosi ziliz
Ambrosia artemisiifolia (NN)	ürömlevelű parlagfű (NN)
Ambrosia coronopifolia (NN)	évelő parlagfű (NN)
Amorpha fruticosa	cserjés gyalogakác
Anchusa officinalis	orvosi atracél
Anethum graveolens	kerti kapor
Angelica sylvestris	erdei angyalgyökér
Anthriscus cerefolium	zamatos turbolya
Anthriscus sylvestris	erdei turbolya
Artemisia vulgaris (NN)	fekete üröm (NN)
Atriplex tatarica (NN)	tatár laboda (NN)
Ballota nigra	fekete peszterce
Berteroa incana	fehér hamuka
Berula erecta	keskenylevelű békakorsó
Brassica napus	káposztarepce
Brassica rapa	réparepce
Buddleja davidii	illatos nyáriorgona
Cannabis sativa (NN)	vetési kender (NN)
Cardaria draba	útszéli zsázsa
Carduus acanthoides	útszéli bogáncs
Castanea sativa	szelídgesztenye
Centaurea cyanus	búzavirág
Centaurea nigrescens	feketés imola

Latin plant name	**Hungarian plant name**
Cerinthe minor	szeplőlapu
Chaerophyllum aureum	aranyos baraboly
Chelidonium majus (NN)	vérehulló fecskefű (NN)
Chenopodium album (NN)	fehér libatop (NN)
Chenopodium ficifolium (NN)	fügelevelű libatop (NN)
Chenopodium murale (NN)	kőfali libatop (NN)
Cichorium intybus	mezei katáng
Cirsium arvense	mezei aszat
Cirsium vulgare	lándzsás aszat
Clematis vitalba (NN)	erdei iszalag (NN)
Convolvulus arvensis	apró szulák
Cornus sanguinea	veresgyűrűsom
Crataegus monogyna x Crataegus punctata	galagonya hibrid
Crepis capillaris	vékony zörgőfű
Crepis foetida	nehézszagú zörgőfű
Crepis setosa	sertés zörgőfű
Cytisus scoparius	seprűzanót
Datura stramonium	csattanó maszlag
Delosperma cooperi	bíborvörös délvirág
Elaeagnus angustifolia	keskenylevelű ezüstfa
Erigeron annuus (NN)	egynyári seprence (NN)
Eupatorium cannabinum	sédkender
Fagopyrum esculentum	pohánka
Falcaria vulgaris	sarlófű
Filipendula vulgaris (NN)	koloncos legyezőfű (NN)
Fragaria vesca	erdei szamóca
Fraxinus ornus (NN)	virágos kőris (NN)
Galega officinalis	orvosi kecskeruta
Galium album	sziklai galaj
Galium mollugo	közönséges galaj
Genista tinctoria	festő rekettye
Glycine max	szója
Hedera helix	borostyán
Helianthus annuus	napraforgó
Hypericum perforatum (NN)	lyukaslevelű orbáncfű (NN)
Hypochoeris radicata	kacuros véreslapu
Impatiens balfourii	Matild-nebáncsvirág
Iris pseudacorus	mocsári nőszirom
Juglans regia (NN)	nemes dió (NN)
Knautia arvensis	mezei varfű
Koelreuteria paniculata	bugás csörgőfa
Lamium maculatum	foltos árvacsalán
Limonium hungaricum	magyar sóvirág
Lolium multiflorum (NN)	olaszperje (NN)
Lolium perenne (NN)	angolperje (NN)

Latin plant name	**Hungarian plant name**
Loranthus europaeus	sárgafagyöngy
Lotus corniculatus	szarvas kerep
Lotus glaber (=tenuis)	sziki kerep
Lycium barbarum	ördögcérna
Lycium chinense	kínai ördögcérna
Lythrum salicaria	réti füzény
Maclura pomifera	narancseperfa
Malus sylvestris	nemes alma
Melilotus albus	fehér somkóró
Mercurialis annua (NN)	egynyári szélfű (NN)
Morus alba (NN)	fehér eperfa (NN)
Odontites vulgaris	vörös fogfű
Oenanthe aquatica	vízi mételykóró
Onobrychis viciifolia	takarmánybaltacím
Ononis spinosa	tövises iglice
Onopordum acanthium	közönséges szamárbogáncs
Ornithogalum refractum	csilláros madártej
Papaver rhoeas (NN)	mezei pipacs (NN)
Papaver somniferum (NN)	termesztett mák (NN)
Pentanema britannicum	réti peremizs
Peucedanum cervaria	szarvaskocsord
Phacelia tanacetifolia	varádicslevelű mézontófű
Phytolacca americana	amerikai alkörmös
Picris hieracioides	közönséges keserűgyökér
Pisum sativum	veteményborsó
Plantago arenaria (NN)	homoki útifű (NN)
Plantago lanceolata (NN)	lándzsás útifű (NN)
Plantago major (NN)	nagy útifű (NN)
Plantago media (NN)	réti útifű (NN)
Portulaca grandiflora	porcsinrózsa
Portulaca oleracea	kövér porcsin
Potentilla argentea	ezüstös pimpó
Potentilla reptans	indás pimpó
Prunus serotina	kései zelnicemeggy
Pulicaria dysenterica	réti bolhafű
Pyracantha coccinea	közönséges tűztövis
Quercus frainetto (NN)	magyar tölgy (NN)
Quercus pubescens (NN)	molyhos tölgy (NN)
Quercus rubra (NN)	vörös tölgy (NN)
Ranunculus sardous	buborcs boglárka
Raphanus sativus var. oleiferus	olajretek
Reseda lutea	vadrezeda
Rhamnus cathartica x Rhamnus saxatilis	benge hibrid
Robinia pseudoacacia	fehér akác
Rorippa palustris	mocsári kányafű
Rosa canina (NN)	gyepürózsa (NN)

Latin plant name	**Hungarian plant name**
Rubus caesius	hamvas szeder
Rubus idaeus	erdei málna
Rubus radula	ráspolyos szeder
Rubus ulmifolius x Rubus caesius	hamvas tüskétlen szeder
Rumex patientia (NN)	paréjlórom (NN)
Salix alba	fehér fűz
Salix triandra	mandulalevelű fűz
Sambucus ebulus	gyalogbodza
Sambucus nigra (NN)	fekete bodza (NN)
Sanguisorba officinalis (NN)	őszi vérfű (NN)
Scabiosa lucida	fénylő ördögszem
Scorzonera parviflora	kisvirágú pozdor
Serratula tinctoria	festő zsoltina
Sinapis alba	fehér mustár
Sisymbrium orientale	hamvas zsombor
Sisymbrium strictissimum	magas zsombor
Solanum nigrum (NN)	fekete csucsor (NN)
Solanum villosum (NN)	sárga csucsor (NN)
Solidago canadensis	kanadai aranyvessző
Solidago gigantea	magas aranyvessző
Sonchus arvensis	mezei csorbóka
Sorghum bicolor (NN)	takarmánycirok (NN)
Sparganium erectum (NN)	ágas békabuzogány (NN)
Spiraea japonica	japán gyöngyvessző
Symphytum officinale	fekete nadálytő
Syringa josikaea	Jósika-orgona
Syringa vulgaris	kerti orgona
Tanacetum vulgare	gilisztaűző varádics
Taraxacum officinale	pongyola pitypang
Tetradium daniellii	koreai mézesfa
Tilia cordata	kislevelű hárs
Tilia tomentosa	ezüst hárs
Tragopogon pratensis	réti bakszakáll
Tribulus terrestris (NN)	földi királydinnye (NN)
Trifolium alexandrinum	egyiptomi here
Trifolium hybridum	korcs here
Trifolium incarnatum	bíbor here
Trifolium pratense	vörös here
Trifolium repens	fehér here
Trinia glauca	szürke nyúlkapor
Tripleurospermum maritimum	tengerparti ebszékfű
Urtica dioica (NN)	nagy csalán (NN)
Verbascum chaixii	déli ökörfarkkóró
Verbascum thapsus	molyhos ökörfarkkóró
Vicia faba	lóbab
Vicia villosa	szöszös bükköny

Latin plant name	**Hungarian plant name**
Vitis vinifera (NN)	bortermő szőlő (NN)
Xanthium strumarium	bojtorjánszerbtövis
Zea mays (NN)	kukorica (NN)

(NN) = non-nectariferous plant/nectarless

CONFLICT OF INTEREST

The author declares no conflicts of interest related to this research.

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APIS

Conference Abstract/Poster/Slides

Organic Propolis from Uruguay: Developing a Methodology to Analyze its Volatile Components

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ABSTRACT

Propolis Volatile Components (PVCs) are key for the pleasant aroma of this bee product but also have demonstrated several biological activities. In Uruguay, only one report of PVCs has been published, without any specification of the origin and the production conditions. In the present work, we analysed samples from an organic apiary in Rocha Province (eastern Uruguay) collected according to the Uruguayan official recommendations. Headspace sampling of 0.03 g of ground propolis was conducted at 50°C for 30 minutes using two different SPME sorbents (1: DVB/PDMS/Carboxen; 2: Polyacrylate). The extracted PVCs were then directly desorbed in the injector port of a GC-MS instrument. Two stationary phases (Rxi-5MS and Stabilwax-MS) were selected for conventional analysis, using optimized oven programs. Identification of PVCs was carried out by comparing mass spectra with commercial libraries and calculating linear retention indices (LRIs), using a C_8-C_{20} alkane solution. Additionally, separation of chiral monoterpenes was achieved *via* eGC-MS (enantioselective) with an Rt-*β*DEXsm column (stationary phase composed of a modified *β*-cyclodextrin as chiral selector). Over 100 PVCs were detected, predominantly phenylpropanoids, benzenoids and terpenic compounds. Some key PVCs identified include *α*-pinene, benzyl alcohol, terpinen-4-ol, *o*-guaiacol, benzyl benzoate, spathulenol and *β*-selinene. The detection of *trans*-nerolidol suggests (at least in part) *Baccharis dracunculifolia* (Asteraceae) as the botanical origin of the samples, like Brazilian Green Propolis. Further studies on the chiral patterns of selected PVCs could enhance quality control, define potential markers and support origin certification of this product in Uruguay.

Keywords: green propolis, Uruguay, volatile components

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INTRODUCTION

Propolis Volatile Components (PVCs) not only enhance the product's pleasant aroma but also demonstrate a wide range of biological activities, including antimicrobial, immunomodulatory, and cytotoxic effects against human cancer cells. [1]. Furthermore, PVCs serve as key indicators of propolis' botanical and geographical origin, making them indispensable for quality control [1]. To date, only one previous study has reported PVCs from Uruguayan samples using static headspace sampling, without specifying the origin or production conditions [2].

This study identified 38 compounds, most of them monoterpenes and short-chain alcohols, aldehydes and acids [2]. In this ongoing project, we studied the PVCs of organic samples from Uruguay (Figure 1), using SPME/GC-MS protocols.

AIM

To outline the initial steps in developing an analytical methodology to study PVCs of organic samples from Uruguay.

METHODS

Figure 2 shows in brief the methodology followed [4].

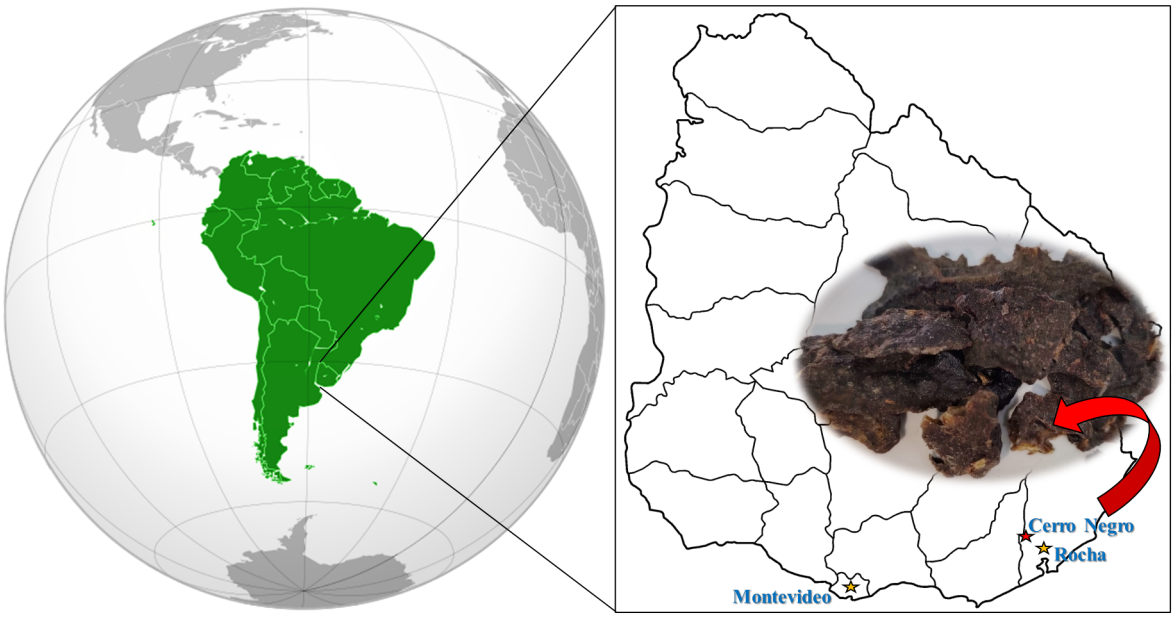

Figure 1. Area of study in Southeastern Uruguay (Cerro Negro, Rocha Department). Propolis sampling followed the official Uruguayan recommendations for best practices, including the obtention at organic conditions [3].

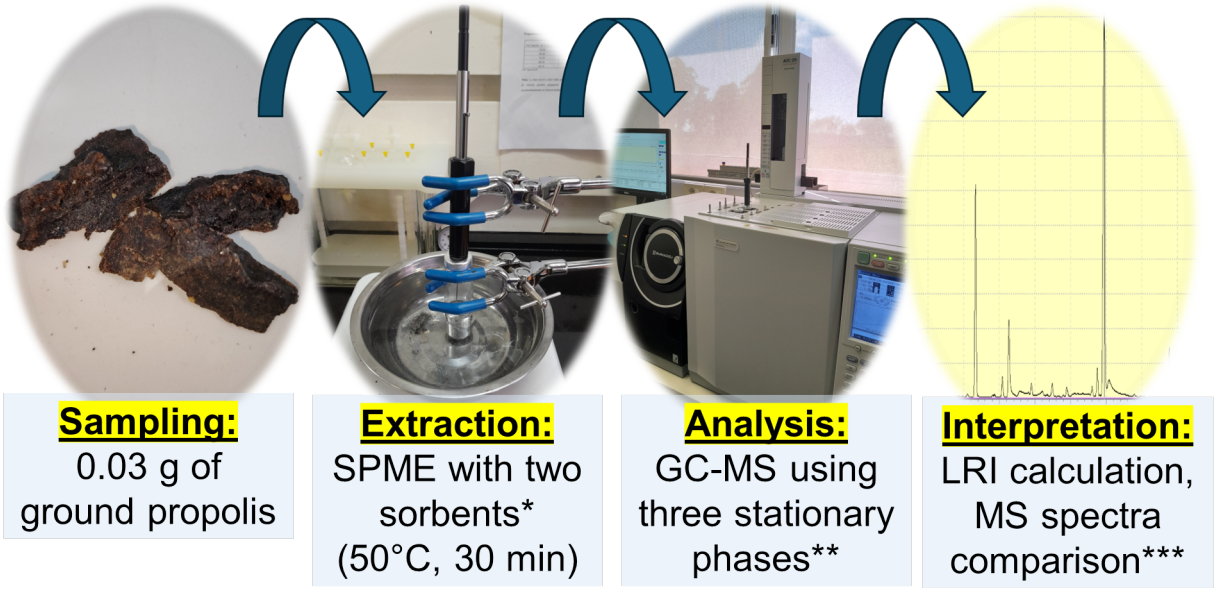

Figure 2: Methodology employed for PVCs extraction and analysis. (*) sorbents 1. SPME DVB/PDMS/Carboxen, 2. SPME Polyacrylate (PA); (**) GC stationary phases: Rxi-5MS, Stabilwax-MS, and Rt-βDEXsm (chiral selector of modified β-cyclodextrin; (***) Identification by Linear Retention Index (LRI) calculation through a C_8-C_{20} alkane solution (Sigma-Aldrich) and a Terpene Mega-Mix (Supelco).

RESULTS

Different PVC profiles were obtained using both SPME sorbents. As expected, PA extracted more polar compounds, being the hydrocarbons negligible extracted (Figure 3). The structures of the main PVCs are shown in Figure 4, while Figure 5 shows the enantiomeric separation of chiral monoterpenes.

CONCLUSIONS

The chemical analysis of organic propolis from Uruguay allowed to detect more than 100 components, including phenylpropanoids, benzenoids and terpenes, most of them not previously described; *trans*-Nerolidol suggested (at least in part) *Baccharis dracunculifolia* as the botanical origin of the samples, as described for Green Propolis [6]. Further studies including sampling for different seasons and geographical sites, as well as studies on the chiral patterns of selected PVCs could contribute to defining chemomarkers, supporting origin certification of this product.

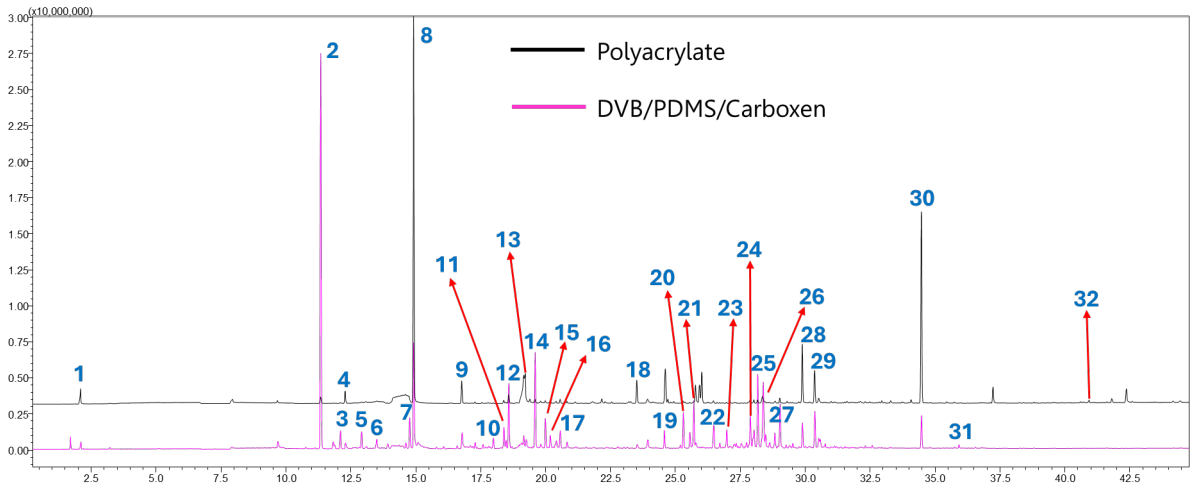

Figure 3: PVCs GC-MS profile of the organic Uruguayan propolis simples analyzed: 1. acetic acid, 2. *α*-pinene, 3. thuja-2,4(10)-diene, 4. benzaldehyde, 5. *β*-pinene, 6. myrcene, 7. limonene, 8. benzyl alcohol, 9. *o*-guaiacol, 10. *α*-campholenal, 11. *trans*-pinocarveol, 12. *trans*-verbenol, 13. benzoic acid, 14. terpinen-4-ol, 15. α-terpineol, 16. myrtenol, 17. verbenone, 18. *p*-vinyl guaiacol, 19. *α*-cubebene, 20. *α*-copaene, 21. *β*-elemene, 22. *trans*-caryophyllene, 23. aromadendrene, 24. *γ*-muurolene, 25. *β*-selinene, 26. *α*-selinene, 27. *γ*-cadinene, 28. *trans*-nerolidol, 29. spathulenol, 30. benzyl benzoate, 31. neophytadiene, 32. benzyl cinnamate. Analytical conditions: column, Rxi-5MS (30 m × 0.25 mm × 0.25 µm); oven temperature: 40°C (5 min), 40-235°C at 5°C min-1, 235°C (2 min). Gas Carrier, He (1.0 mL/min).

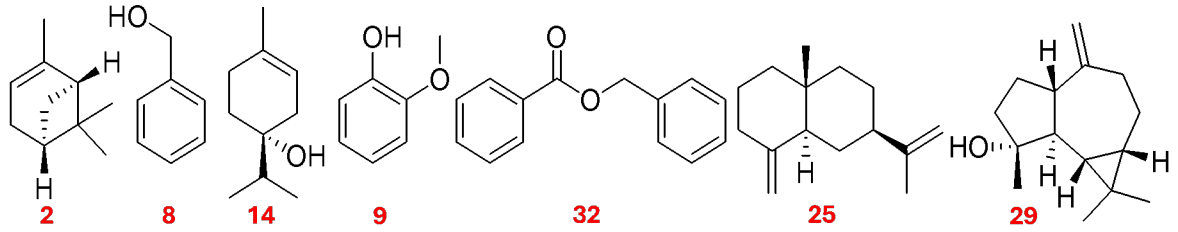

Figure 4: Main PVCs determined in this study. Numbering follows the sequence in Figure 3.

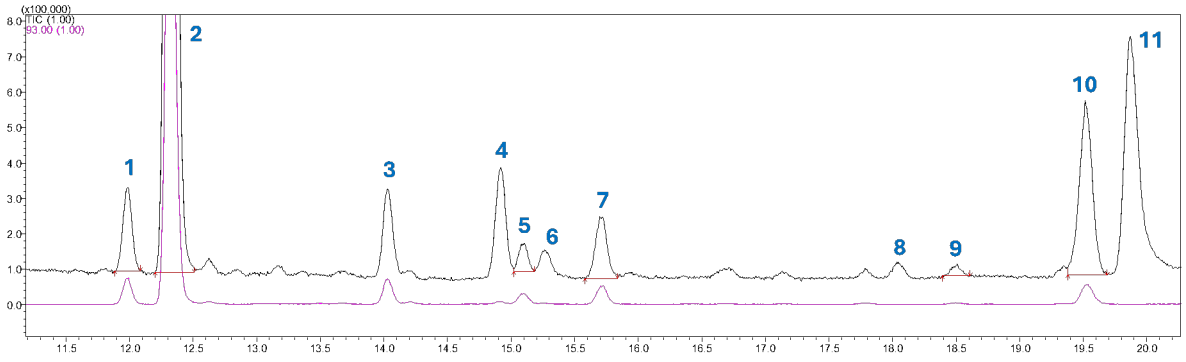

Figure 5: enantioselective GC-MS profile of the organic Uruguayan propolis samples analyzed (partial view): 1. 1*S*-(-)-*α*-pinene, 2. 1*R*-(+)-*α*-pinene, 3. myrcene, 4. thuja-2,4(10)-diene, 5. 1*R*-(+)-*β*-pinene, 6. not identified, 7. 1*S*-(-)-*β*-pinene, 8. not identified, 9. 4*R*-(+)-limonene, 10. 4*S*-(-)-limonene, 11. benzaldehyde. Enantiomeric elution order according to [5]. Analytical conditions: column, Rt-*β*DEX (30 m × 0.25 mm × 0.25 µm); oven temperature, 65°C (1 min), 65-100°C at 1°C.min-1, 100°C (1 min), 100-150°C at 2°C.min-1, 150-220°C at 10°C.min-1, 220°C (3 min). Gas Carrier, He (1.0 mL/min).

ABBREVIATIONS

DVB: divinylbenzene

eGC-MS: enantioselective gas chromatography coupled to mass spectrometry

GC-MS: gas chromatography coupled to mass spectrometry

He: helium, the GC-MS carrier gas

PDMS: polydimethylsiloxane

Rxi-5MS: commercial capillary GC-MS column, composed of 5%-phenyl-95%-polydimethylsiloxane

Rt-*β*DEXsm: commercial enantioselective capillary GC-MS column composed of 2,3-di-O-methyl-6-O-tert-butyldimethylsilyl-cyclodextrine added into 14%-cyanopropylphenyl-86% dimethyl polysiloxane

SPME: Solid phase microextraction

Stabilwax-MS: commercial capillary GC-MS column, composed of 100% polyethylene glicol

ANSWERS TO FREQUENTLY ASKED QUESTIONS DURING THE EVENT

Q1: Is there Green Propolis in Uruguay, as this research suggests?

A1: No. To date, Green Propolis has not been reported for Uruguay. Yet, the botanical origin of Uruguayan propolis was identified as *Populus alba L.* through a comparative chemical analysis of multiple samples and the bud floral resins of this species [7]. However, the botanical origin of Green Propolis, the species B*accharis dracunculifolia DC.* (Asteraceae), is widely distributed in the country [8], attracting our attention as a potential source of Uruguayan propolis, which has not been evaluated to date. Our previous publications suggest that in Uruguay, *B. dracunculifolia* has apparently two distinctive chemotypes: one from the northern region, which is rich in *trans*-nerolidol (as it is the source of the Brazilian Green Propolis) [6,9], and one from the southern region where this compound is absent all over the year [10]. Ongoing research may provide insights into the possible presence of Green Propolis in Uruguay.

Q2: Do the authors use at the same time solvent extraction to obtain the propolis volatile compounds?

A2: No. The methodology followed in this work was based on SPME, which is completely solvent-free [4]. Some interesting works published in the literature also use solvent extraction of the volatile compounds by using a Likens-Nickerson apparatus and mixtures of *n*-pentane/diethyl ether (i.e., [11]). But, in general, the use of solvents (70% ethanol as the most employed) is more frequently applied to the analyses of non-volatile components [12].

Q3: What is the contribution of enantioselective analyses in this ongoing project?

A3: The enantioselective analysis of volatile chemomarkers by gas chromatography has been pointed out as a key tool to simultaneously prevent adulteration, defining the geographical or botanical origin of a sample and, to correlate chemical and sensory information [5,13]. This is possible because the biosynthetic pathways of plants are stereochemically defined, leading to the production of final chiral products with a well defined enantiomeric excess [13]. In some cases, this enables the differentiation of closely related plant species [10]. In the advancement of this work, to establish the enantiomeric excess of monoterpene hydrocarbons (see Figure 5) in Uruguayan organic propolis could contribute not only to clearly define the botanical origin, but also to establish value ranges for certification purposes.

Q4: Why do the authors use different gas chromatographic columns to analyze the propolis volatile components?

A4: The use of capillary gas chromatography columns with different polarities is a common practice in the analysis of plant volatiles [14], optimizing components separation and enabling us to gain knowledge about the chemical composition of the propolis samples. In particular, the use of polar (polyethylene glycol; i.e., Stabilwax-MS) and nonpolar [5%-phenyl–95%-poly(dimethylsiloxane); i.e., Rxi-5MS] stationary phases are highly recommended [14], being the approach followed in this work.

Q5: Do the authors consider analyzing beehive air in their future work?

A5: Beehive air is a new and interesting research direction in Apitherapy, whose chemical determination can be conducted also through SPME [15]. In our experimental design, direct analysis of beehive air was not feasible due to the significant distance between the apiaries and the laboratory conducting the chemical analyses (over 400 kilometres). As a result, propolis samples had to be transported for analysis. A consistent beehive air analysis needs rapid sampling and immediate analysis in proximity of the apiary and the laboratory [15], which experimental design could be soon explored.

Q6: Do the authors employed just 0.03 g of ground propolis for extracting the propolis' volatiles?

A6: Yes. The headspace SPME technique has a high concentration capacity, thus it requires a minimal quantity of sample [16]. In our experimental conditions, higher quantities might eventually saturate the capillary column and the mass analyzer at the GC-MS instrument, decreasing the separation and the resolution of the chromatographic peaks. Moreover, the higher the quantity of sample the higher the number of volatile molecules in the propolis' headspace, which can eventually saturate the SPME sorbents diminishing the ab(d)sorption of those components present at low concentration (or trace level), leading to potential discrimination phenomena [16].

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APPENDIX

BEST PRACTICES FOR THE PRODUCTION AND HANDLING OF PROPOLIS NR 101 [3]

Propolis is a product made by honeybees from resins, gums, and balsams exuded by various plants. It contains phenols and flavonoids, which give it medicinal and antioxidant properties. To maintain its quality, it is necessary to minimize the risks of microbiological, physical, and chemical contamination at all stages of handling.

CLASSIFICATION OF PROPOLIS BASED ON THE EXTRACTION PROCESS**

- Scraped Propolis

Obtained by scraping propolis deposited on the wooden material inside the hive. It contains impurities and possible contaminants.

- **Mesh Propolis**

Food-grade Plastic meshes with perforations smaller than 4.5 mm or a similar weave are used.
The product obtained is of higher quality.

Sanitary Handling: Apply treatments to control Varroa with approved products.
Handling the Smoker: Avoid dripping onto the meshes or frames.

FOR MESH PROPOLIS COLLECTION

- Place the meshes over the tops of the frames, under the inner cover or lid.
- During each visit to the apiary, shift the mesh to expose unpropolized areas.
- Remove the meshes when they are approximately 80% covered in propolis.
- During hive inspections or honey harvesting, do not place the meshes on grass or soil.

TRANSPORTATION

- Place the meshes in a clean bag or container.
- Avoid direct contact of the meshes with the vehicle's cargo area, as combustion gases and dust can contaminate the propolis during transport.

PROCESSING CONDITIONS

Must be carried out in a clean, ventilated, and uncluttered area.

The working surface must be clean and free of contaminants. Preferably use a stainless steel tray, but plastic or food-grade epoxy-painted surfaces are also acceptable.

For Mesh Propolis Processing

- Roll the meshes "diploma style" inside a clean/new plastic bag.
- Place them in the freezer (-20°C) for one to two hours.
- Remove from the freezer and unroll them over a clean tray; small fragments will easily break off.
- Collect the fragments using a clean brush designated exclusively for this purpose.

For Scraped Propolis Processing

- Use stainless steel spatulas with little sharpness, cleaned with water and detergent, and dried with absorbent paper.
- To avoid contamination, do not scrape painted surfaces. Avoid collecting wax, as it encourages moth development.
- Remove all bee remains, wood debris, or other foreign materials.

STORAGE RECOMMENDATIONS

Store in closed, clean containers.
Store in a cool, dry, clean place, away from contamination risks such as dust, pests, domestic animals, and chemicals.
Avoid exposure to sunlight.

MESH PROPOLIS:

- Pack in new, food-grade plastic bags or containers.
- Traceability: Label the containers with the *National Hive Owners Registry Number (RNPC)*, name, and collection date.

AVOID THE FOLLOWING PRACTICES

- Making "propolis balls."
- Detaching wood and paint from boxes during scraping/harvesting.
- Scraping floors and brood chamber frames that have undergone synthetic acaricide treatments.
- Using metal containers, except for stainless steel or epoxy-coated ones.
- Using polyethylene bags with inscriptions, as the ink may transfer contaminants.
- Collecting dust or swept-up propolis fragments from the floor.

APIS

Original Article

The Modern Development of Apitherapy: The Integration of Traditional Wisdom and Modern Technology — Taking the Global Promotion of 39 Apitherapy Network as an Example

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Abstract: Modern apitherapy has undergone a transformation from a traditional empirical therapy to a scientific and standardized medical practice. This article combs through the key breakthroughs in the research of pharmacological mechanisms, the expansion of clinical applications, and the industrial development of apitherapy since the end of the 19th century. Taking the Chinese 39 Apitherapy Network as the core case, it analyzes its strategic role in platform construction, the formulation of international standards, and talent cultivation. The research shows that through integrating global resources and promoting cross-border cooperation, the 39 Apitherapy Network has significantly enhanced the academic authority and industrial competitiveness of apitherapy, providing a paradigmatic reference for the modernization of traditional medicine.

Keywords: China, apitherapy, cross-disciplinary collaboration, clinical applications, modernization of traditional medicine.

1. INTRODUCTION

Apitherapy, as a traditional method of medical treatment using bee products, has a history that can be traced back several centuries and is deeply rooted in traditional medical systems. The ancient Chinese medical classic Shennong Ben Cao Jing1 provides detailed records of the remarkable curative effects of honey, royal jelly, and bee venom in treating various diseases. In addition, from the papyrus documents of ancient Egypt to the folk medicine in Europe, similar apitherapy traditions can be found in different cultures.

Despite its long historical origin and significant medical value, apitherapy has long relied on empirical knowledge and lacked strict scientific verification. However, since the 20th century, with the increasing interest in natural medicines and comprehensive healthcare, apitherapy has gradually come into the field of modern scientific research. Thanks to the continuous in-depth research in biochemistry and clinical studies, the key bioactive compounds in bee products have been identified, thus promoting the application of apitherapy in evidence-based medicine.

During this transformation process, the 39 Apitherapy Network, as an important collaborative platform, has played a crucial role in the standardization, professionalization, and globalization of apitherapy. By combining the wisdom of traditional medicine with modern technology, this network has successfully promoted the recognition of apitherapy as a legitimate medical discipline.

This study takes the 39 Apitherapy Network as the research object to systematically explore the historical evolution, scientific progress, and global promotion of apitherapy. It focuses on analyzing the contributions of this network in promoting clinical standardization, driving interdisciplinary cooperation, and achieving digital integration, and then looks ahead to the future development trends of apitherapy in the fields of scientific research and practice.

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2. MATERIALS AND METHODS

2.1. RESEARCH DESIGN AND METHODOLOGY

This study adopts a historical analysis approach to examine the modernization and global expansion of apitherapy. By taking the 39 Apitherapy Network as a case study, it explores how traditional apitherapy practices are systematically integrated into the modern healthcare framework.

2.2. THE STUDY COMPREHENSIVELY APPLIES BOTH QUALITATIVE AND QUANTITATIVE METHODS

Literature Review: Conduct a comprehensive review of historical documents, modern research papers, and regulatory documents related to apitherapy.

Case Study: Study the 39 Apitherapy Network and its role in international cooperation, digital platform development, and clinical standardization.

Data Collection on Apitherapy Applications: Organize published clinical studies, industry reports, and policy documents on the therapeutic applications of bee products.

2.3. DATA SOURCES

Primary Data: Ancient Chinese medical classics ("Shennong Ben Cao Jing", "Compendium of Materia Medica"), modern pharmacological research, and clinical trial data. Secondary Data: Peer-reviewed journal articles, reports from the World Health Organization (WHO), the National Administration of Traditional Chinese Medicine (NATCM), and proceedings of apitherapy conferences. Industry Reports: Data from the 39 Apitherapy Network, including its role in standardization, industry-university-research cooperation, and the development of the digital apitherapy platform.

2.4. ANALYTICAL METHODS

Historical Analysis: Trace the evolution of apitherapy from an empirical practice to a structured medical discipline.

Comparative Analysis: Evaluate the differences between the regulatory framework of apitherapy in China and global medical standards.

Impact Assessment: Assess the impact of the 39 Apitherapy Network on the professionalization and global promotion of apitherapy.

3. THE DEVELOPMENT HISTORY OF APITHERAPY

3.1. THE FOUNDATION OF ANCIENT APITHERAPY

The history of apitherapy in China can be traced back to the Pre-Qin period. As early as in ancient books such as *The Book of Songs* and *Shennong Ben Cao Jing*, there were records of bee products being used as medicine. For example, in Shennong Ben Cao Jing [1], honey and bee larvae were listed as top-grade medicinal substances, and their effects such as "treating pathogenic qi in the heart and abdomen" and "replenishing qi and nourishing the middle energizer" were recorded. The Han Dynasty was an important stage in the development of apitherapy: Zhang Zhongjing created the "Honey Decoction Guide Recipe" in Treatise on Febrile Diseases [2] to treat constipation, which was the earliest suppository in the world; in Synopsis of the Golden Chamber [3], "Licorice, Powder and Honey Decoction" was used to expel worms and relieve pain; the Fifty-two Prescriptions for Diseases [4] unearthed from the Mawangdui Han Tomb in Changsha recorded specific prescriptions for treating diseases with bee stings and honey, confirming the clinical application of apitherapy. In the Jin Dynasty, Ge Hong used bee products for beauty and health care. Yao Sengyuan elaborated in On Medicinal Properties [5] the methods of using honey to treat oral ulcers and dysentery. Sun Simiao (581-682 AD) used ginger-honey paste to relieve cough and resist aging, promoting the popularization of apitherapy. In the Ming Dynasty, Li Shizhen's Compendium of Materia Medica [6] synthesized the experience of apitherapy in previous dynasties, and included dozens of prescriptions of bee products. Sun Yikui collected the effective prescription of using adult bees to treat scrofula. Fang Yizhi's Brief Physical Knowledge [7] first created the external treatment formula of "medicinal bee sting", marking the maturity of the external treatment method with bee venom.

In addition, unearthed cultural relics such as medical slips from the Eastern Han Dynasty, medical books from the Han Dynasty, and prescriptions from various dynasties have all verified the continuous practice and theoretical deepening of apitherapy in ancient medicine. From the Eastern Zhou Dynasty to the Ming and Qing Dynasties, the bee sting therapy evolved from empirical accumulation to the systematization of documents, and gradually formed a unique system of "treating poison with poison" and "the homology of medicine and food", laying a solid historical foundation for the disciplinary development and internationalization of modern apitherapy.

3.2. THE SCIENTIFIC DEVELOPMENT OF MODERN APITHERAPY (LATE 19TH CENTURY - MID-20TH CENTURY)

3.2.1. BREAKTHROUGH PROGRESS IN WESTERN BEE VENOM RESEARCH

In 1883, the Austrian doctor Anton Kerner isolated a crystalline substance from the venom gland of bees, named it "Apamin", and observed its analgesic and antibacterial effects. The protein with the highest content in it is melittin, which can trigger an inflammatory response by damaging the cell membrane. In 1928, the German doctor Heinrich

Stöcker treated 17 patients with rheumatoid arthritis with bee venom injection, and 12 cases were significantly relieved, revealing the remarkable effect of bee venom in treating rheumatoid diseases. In 1905, the British biochemist John Hopkins discovered that bee venom contains phospholipase A2 (PLA2), providing a theoretical basis for the anti-inflammatory mechanism of bee venom [8].

3.2.2. SYSTEMATIC ARRANGEMENT OF TRADITIONAL CHINESE APITHERAPY

From the late 19th century to the period of the Republic of China, the traditional Chinese medicine community carried out the first large-scale arrangement of the practical experience of apitherapy, promoting its transformation from a folk therapy to a theoretical system. In the Qing Dynasty, Medical Orthodoxy [9] included an effective prescription for treating scalds with external application of beeswax; during the period of the Republic of China, the traditional Chinese medicine community incorporated the bee sting therapy into the system of acupuncture and moxibustion, forming a theoretical framework of "treating poison with poison". At the beginning of the 20th century, in 1910, the first beeswax production workshop appeared in Shanghai. The National Government listed the bee sting therapy as a "legitimate traditional Chinese medicine diagnosis and treatment method", allowing doctors to register and practice medicine based on it, clearly proposing to "encourage scientific research on traditional medicine, including apitherapy", and allocating special funds to support the industrialization of bee products. In 1941, the "Chinese Pharmacopoeia" included the bee sting therapy for the first time, put forward contraindications such as "forbidden for pregnant women" and "use with caution for those with allergic constitution", and standardized the disinfection process of bee stings.

It can be seen that the scientific development of modern apitherapy takes the component analysis of Western bee venom and the system reconstruction of traditional Chinese apitherapy as the two main lines: Western research lays the medical value of bee venom through the exploration of pharmacological mechanisms, while China promotes apitherapy from folk experience to a medical technology with standardized operation and theoretical support through the arrangement of ancient books, clinical standardization, and industrial practice, laying the foundation for its subsequent global development.

3.3 THE GLOBAL DEVELOPMENT OF MODERN APITHERAPY (MID-TO-LATE 20TH CENTURY - EARLY 21ST CENTURY)

3.3.1. LEAP IN BASIC RESEARCH

In the 1970s, scientists discovered that phospholipase A2 (PLA_2) in bee venom can inhibit the inflammatory response by regulating the NF-κB pathway (Peters et al., 2017, Nature Immunology). 10-hydroxy-2-decenoic acid (10-HDA) in royal jelly has been proven to have antioxidant and immunomodulatory functions, and also has a good anti-fatigue effect [10].

3.3.2. DIVERSIFICATION OF CLINICAL APPLICATIONS

In 2002, the National Administration of Traditional Chinese Medicine listed apitherapy as a "characteristic diagnostic and treatment technology of traditional Chinese medicine". In 2007, the bee sting therapy was officially included in the national diagnostic and treatment subjects. In 2011, the "bee sting therapy" was included in the medical insurance catalog of some provinces and cities. In 2015, the "Strategic Plan Outline for the Development of Traditional Chinese Medicine" clearly proposed to develop ethnic medicines such as apitherapy.

Evidence-based medicine research shows that apitherapy has demonstrated statistically significant positive therapeutic effects in multiple clinical fields, including the management of rheumatic and immunological diseases and osteoarticular lesions [11], adjuvant treatment of tumors [12], intervention in respiratory and allergic diseases [13], regulation of nervous system function [14], and prevention and treatment of cardiovascular diseases [15].

3.4. THE REVOLUTIONARY CONTRIBUTIONS OF THE 39 APITHERAPY NETWORK (SINCE 2014)

3.4.1. RECONSTRUCTING THE INDUSTRY ECOLOGY THROUGH A DIGITAL PLATFORM

In 2014, Mr. Zhang Qinglong and Ms. Cui Yun founded the "39 Apitherapy Network", which integrates an academic thesis database, a clinical case database, and an online diagnosis and treatment system. The establishment of this platform provides a systematic knowledge system construction and a multi-dimensional academic exchange mechanism for apitherapy practitioners and researchers. This measure marks the transformation and upgrading of traditional apitherapy practice from empirical application to a standardized medical system, and its academic value and clinical efficacy have been recognized by professional institutions at home and abroad. By integrating modern medical research methods with traditional Chinese medicine theories, the application scenarios of apitherapy have been expanded from folk empirical treatment to clinical fields such as rheumatic and immunological diseases and chronic pain management, and have received key attention in international academic conferences and the WHO's traditional medicine development strategy. In 2015, the 39 Apitherapy Network held the "Innovation · Exploring the Opportunities in the Internet + Era, Stepping into the Road of the Bee Medical and Health Industry - 2015 Bee Medical Science and Technology Industry Innovation and Development Summit" at the Beijing International Conference Center. This summit focused on the in-depth integration of Internet technology and the bee medical and health field,

aiming to explore the innovative development path of the apitherapy cause in the digital era, laying a solid foundation for promoting the modernization process of the apitherapy industry. In April 2016, the 39 Apitherapy Network initiated and established the "Apitherapy Branch" of the China Medical Association of Minorities, which is affiliated with Guangzhou University of Chinese Medicine, and Professor Li Wanyao served as the first president. Professor Li enjoys a high reputation in the field of apitherapy, and the branch led by him is committed to promoting the standardization and professionalization of the apitherapy discipline. This measure has significantly enhanced the influence of apitherapy in the domestic medical community, laying a solid foundation for the academic exchange and development of the apitherapy cause.

3.4.2. BUILDING A GLOBAL COLLABORATIVE NETWORK

In 2017, the 39 Apitherapy Network took the lead in establishing the Professional Committee of Apitherapy of the World Federation of Chinese Medicine Societies. Professor Li Wanyao served as the president, and Mr. Zhang Qinglong served as the secretary general. The establishment of this platform marked the birth of the first authoritative academic organization in the international field of apitherapy. This institution has built a professional academic exchange platform for apitherapy practitioners and research institutions around the world, and promoted the establishment of a cross-regional and interdisciplinary research collaboration network [16]. In the same year, the 39 Apitherapy Network held the "Second Academic Exchange Meeting of the First Session of the Apitherapy Branch of the China Association of Minority Traditional Medicine" in Lanzhou, further promoting academic exchanges and cooperation in the field of apitherapy. In this year, the cause of apitherapy moved towards the path of international development with a more open attitude. In 2018, the first International Conference of Apitherapy was successfully held in Shenzhen, constructing the basic framework of multilateral cooperation in the international apitherapy community. Since then, the conference has formed a regular mechanism of being held once a year, attracting experts and scholars from around the world to participate. Through forms such as special reports, clinical case discussions, and consultations on standard formulation, a series of consensual achievements have been made in the fields of research on the pharmacological mechanism of bee venom, accumulation of evidence-based medicine evidence, and standardization of diagnosis and treatment techniques, providing a long-term cooperation mechanism for the transformation of traditional apitherapy into modern medicine.

3.4.3. INNOVATIVE PRACTICES IN THE FIELD OF PUBLIC HEALTH

In 2022, in response to the global challenges of the COVID-19 pandemic prevention and control, the expert team in the field of apitherapy in China systematically compiled and released the "Expert Suggestions on the Auxiliary Prevention and Treatment of COVID-19 with Apitherapy" based on existing clinical research and analysis of the mechanism of action. Based on the principles of evidence-based medicine and referring to the framework of the WHO's traditional medicine anti-epidemic strategy, this guideline put forward the idea of enhancing immunity through nebulized inhalation of bee venom, and the relevant research was included in the alternative plan of the "Three Medicines and Three Prescriptions" of the National Administration of Traditional Chinese Medicine.

3.4.4. INNOVATIVE PRACTICES IN THE FIELD OF PUBLIC HEALTH

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3.4.5. AGGREGATION OF UPSTREAM AND DOWNSTREAM INDUSTRIES

In October 2024, the Fifth International Conference of Apitherapy and the General Meeting for the Renewal of the Professional Committee of Bee Product Health Care was grandly held in Beijing. This meeting marked that the cause of apitherapy has officially entered a new stage of high-quality development. By systematically constructing a collaborative mechanism of industry, university, and research and an international exchange platform, it has effectively integrated industry resources and optimized the path of information sharing [17].

In summary, the 39 Apitherapy Network has established a platform that brings together experts and scholars from the world's top scientific research institutions, medical institutions, and the industrial sector. Relying on a solid disciplinary foundation, cutting-edge technological innovation achievements, and the advantages of interdisciplinary collaboration, it continuously outputs high-quality research results and cultivates a professional talent echelon, providing intellectual support and talent guarantee for the sustainable development of the industry.

Under the guidance of the "Healthy China" strategy, the Professional Committee of Bee Product Health Care actively responds to the call for the development of the big health industry. With innovation-driven as the orientation, it deeply participates in the construction of the standardization system and the process of industrial upgrading in the field of apitherapy health preservation. This committee always adheres to the working principle of "science-oriented and standardization first", and focuses on promoting the evidence-based medical research of bee products, the modern transformation of traditional therapies, and the innovation of the health management service model. By estab-

lishing a collaborative innovation mechanism of industry, university, research, and application, it accelerates the transformation of scientific and technological achievements into clinical applications and creates an internationally competitive apitherapy health industry chain. The committee is committed to building a professional and vocational apitherapy health service system, promoting the transformation of apitherapy health preservation from a traditional experience-based model to a modern technology-based model, and making it gradually develop into a health management method recognized by the public and a high-end professional field respected by society.

The global development of modern apitherapy depends on the empowerment of science and technology and the deepening of international cooperation. Through building a digital platform, outputting a standard system, and cultivating a transnational talent network, the 39 Apitherapy Network in China has not only promoted the upgrading of apitherapy from a folk therapy to an internationally recognized medical technology, but also reshaped China's discourse power in the global governance of traditional medicine. In the future, apitherapy is expected to become a bridge connecting the health cultures of the East and the West, providing innovative solutions for global health governance.

4. DEFICIENCIES AND PROSPECTS

Although this study provides a comprehensive overview, it is limited by the availability of clinical trial data outside of China. In addition, differences in regulatory aspects among various countries may affect the comparability of apitherapy practices globally.

STATEMENT OF CONFLICTS OF INTEREST

Cui Yun is the Secretary General of the 39 Apitherapy Network.

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APIS

Conference Abstract/Poster/Slides

High concentrations of Spermidine in Drone Milk (Apilarnil)

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ABSTRACT

Spermidine is a biogenic amine. Spermidine induces autophagy and may extend life span, reduce dementia and other common diseases. Therefore, it gained popularity as a food additive derived e.g. from wheat germs. Drone milk or Apilarnil (ApiDrohn®) is a high value hive product extracted from the male larvae and conserved by lyophilisation. Apilarnil is used in traditional apitherapy for versatile applications.

In this project the presence of Spermidine in Apilarnil (fresh/different-larva-stages/dry-techniques/vehicle-solutions) was identified and quantified. A workflow was set up comprising an extraction procedure from the freeze-dried Apilarnil (supplied in high purity within Api-Zentrum Ruhr) and a liquid chromatography coupled with a high-resolution time-of-flight mass spectrometer (LC-ESI-QTOF).

Apilarnil in different stages of larva developmental age contains Spermidine in higher concentrations as in wheat germs. Furthermore, Apilarnil contains other bioactive amines and polyamines besides Spermidine. This is the first time that spermidine and several other biogenic amines have been definitely proven to be present in a bee product.

The spermidine content is another column for explanation the various health applications for Apilarnil in apitherapy and is most directly related to its health benefits, although further research has to be done. One of the next tasks will be to understand the synergy between the presence of different bioactive substances like Spermidine and typical hive products e.g. flavonoids.

Keywords: Apilarnil, drone milk, Spermidine, biogenic amines, ApiDrohn®

INTRODUCTION

Drone milk or Apilarnil (ApiDrohn®) is a high value bee hive product extracted from the male larvae and conserved by lyophilisation. Apilarnil is used in traditional apitherapy for versatile applications, often around fertility of men and women. Besides this amine it contains besides other bioactive molecules hormones: Testosterone, Progesterone, Estradiol and Prolactin [1]. This explains partly the regulative function on e.g. women´s menopause symptoms or men's spermatogenesis. The application of Apilarnil shows also good results with animals [2]. In this case the quality of sperm and the number of offspring for stud rams were studied. Mixtures with honey and other bee products like propolis and royal jelly had been traditionally used to strengthen very weak and ill patients. The recipes vary greatly although it seems that Apilarnil is a crucial component for the efficacy.

The research on biogenic amine like Spermidine has recently shown a number of interesting health effects. In the blood of 90 to 100-year old people high concentrations of Spermidine were found [3]. Showing that the surviving fraction of elderly people has a significant difference in metabolism. Spermidine induces autophagy, immune modulation, cardioprotection, neuroprotection, tumour suppression and may extend life span, reduce dementia and other common diseases. Therefore, it gained popularity as a food additive derived e.g. from wheat germs.

As other germ cells (as sperm or wheat germs) contain a lot of Spermidine it was obvious that Apilarnil could also contain Spermidine.

This research project focussed on the detection and quantification of Spermidine and evident biogenic amines at different lava stages.

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MATERIALS AND METHODS

Apilarnil (drone milk) in several stages of age was supplied by Api-Zentrum Ruhr.

High purity pipeline workflow was set up comprising an extraction/re-extraction procedure from the freeze-dried Apilarnil and a liquid chromatography coupled with a high-resolution matrix-assisted laser desorption ionisation time-of-flight mass spectroscopy (LC-ESI-QTOF(HR MALDI-TOF MS).

A targeted LC-ESI-QTOF method in positive mode was developed to analyse the extracts from drone samples. The mass spectra were analysed using Agilent MassHunter Qualitative and Quantitative Analysis Software and Excel.

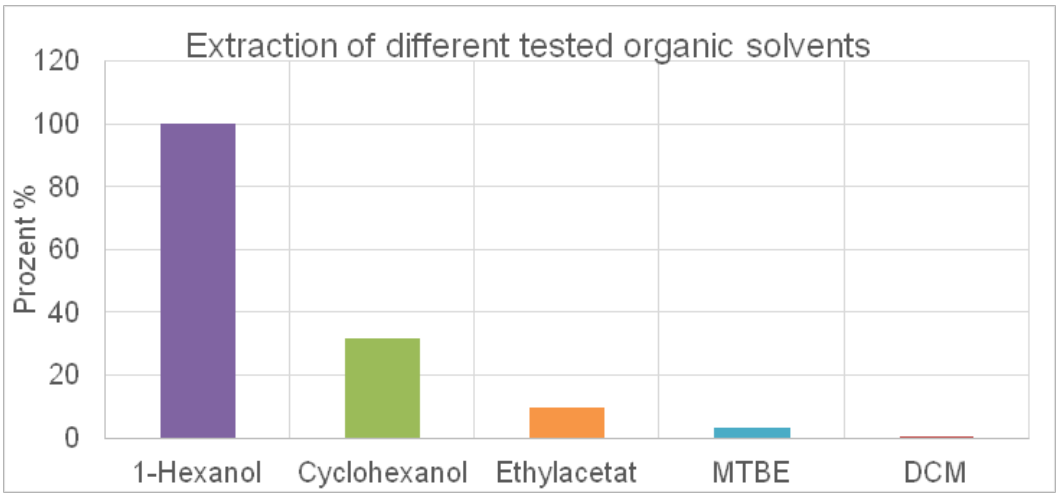

Figure1: Extraction efficiency of tested organic solvents

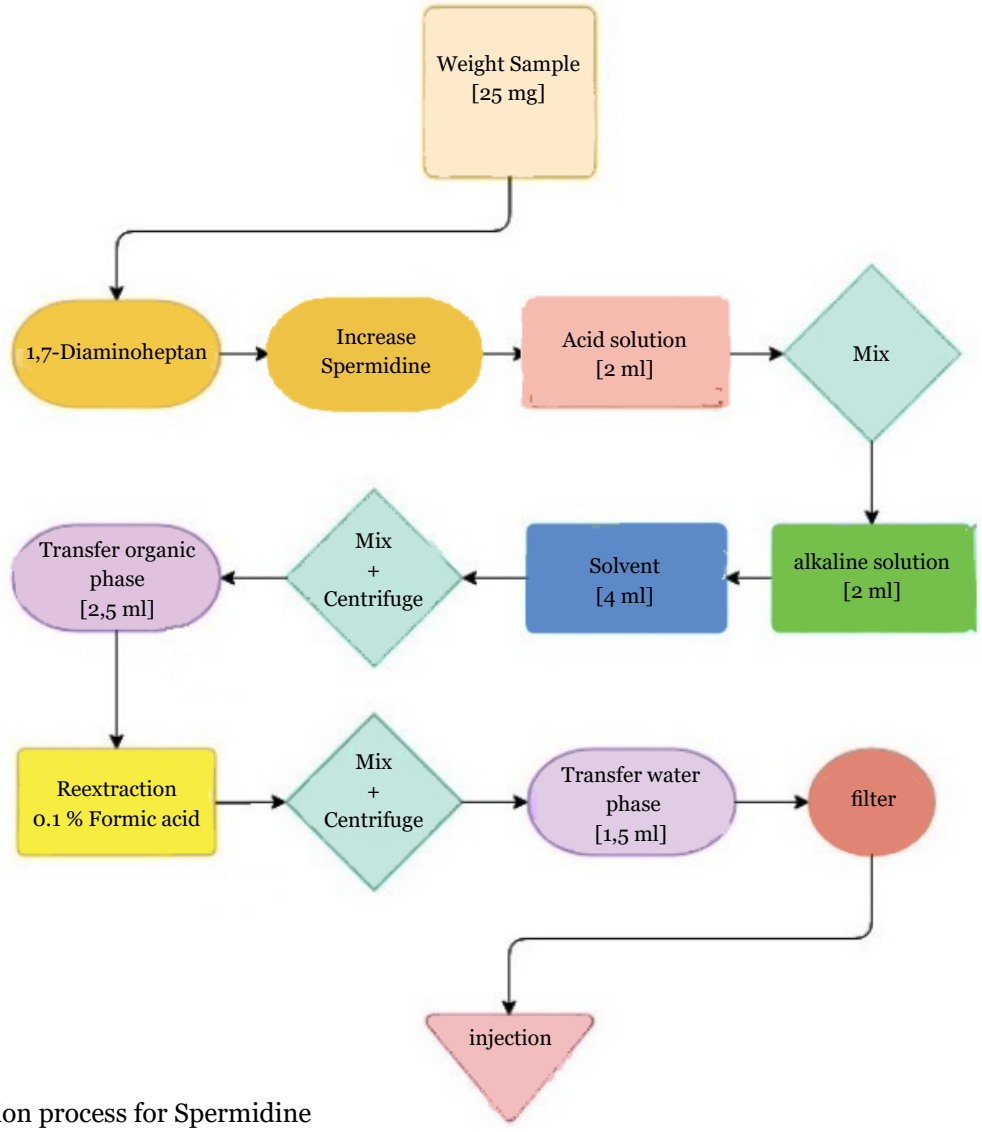

Figure 2: Extraction/re-extraction process for Spermidine

It was found that neutral water extracted less spermidine from the Apilarnil sample than acidified water. In addition to formic acid, hydrochloric acid and sulphuric acid were also tested, but showed no significant improvement in extraction yield. Good results were obtained using 1 % formic acid. In future studies, other acids and different concentrations may be tested to optimize the method.

To further optimize the sample preparation, an organic solvent for extracting the amines from the aqueous phase had to be determined. 1-hexanol, cyclohexanol, ethyl acetate, MTBE (Methyl tert-butyl ether) and DCM (Dichloromethane) were tested. The best extraction yields were achieved with 1-hexanol (see figure 1).

Principle process: Spermidine is obtained by extraction/re-extraction separating from interfering matrix (see figure 2). Optimization separated matrix components successfully and reduced Ion suppression and enhancement effects.

In a subsequent optimization of the automation process, filling speeds of the syringe, penetration depths of the needle, washing units, agitator speeds and times were tested individually for each step of the sample preparation and further optimized in several rounds

RESULTS

To determine the best time to harvest the drones, freeze-dried drone samples from different stages of development and from different locations were supplied by the Api-Zentrum Ruhr.

The stages were defined as day brackets of development of the male drone larva (see table 1).

stage	days of development	description of the development
mix	0-24	Mix as it is produced in Api-Zentrum Ruhr: main fraction is between 7-14 days
uncapped	0-9	
only larva	10-14	This fraction contains only larvae in a capped stage
lava+ pupa	12-17	This fraction contains drones in larva and partly pupped stage (“brood board”)
pupa	15-20	All larvae are already with a chitin shell.
black	21-24	Dark fraction, coloured by the black eyes of the puppa drones

Table 1: Age brackets of the samples analyzed

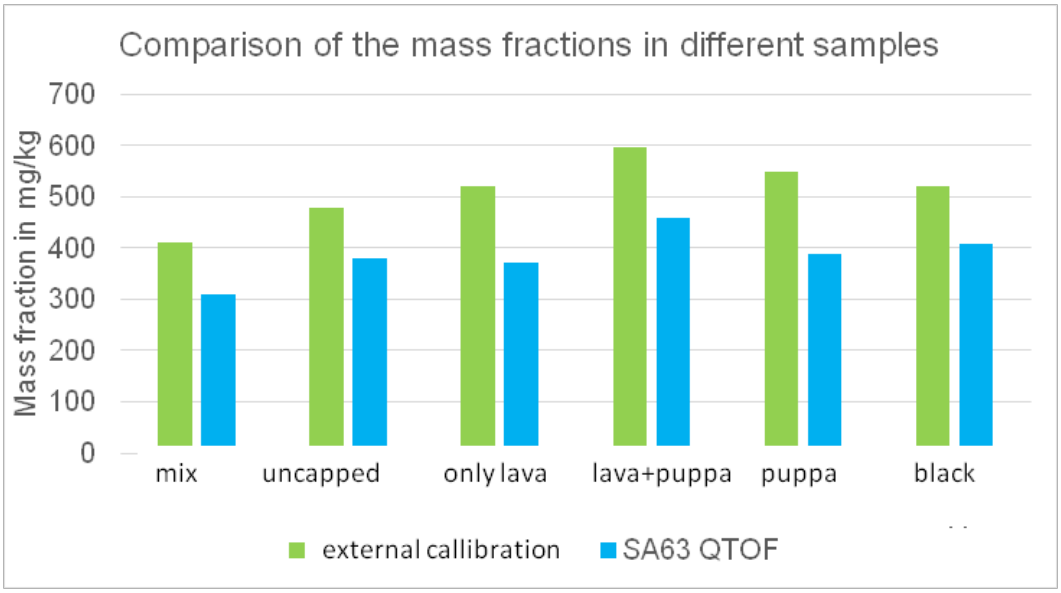

Figure 3: Comparison of the mass fractions in different samples

Combs were removed out of the hive and immediately shock frozen and then freeze dried. Samples were picked out of similar stage developed drones and a number of cells and mixed in order to get a representative sample [4].

In all three methods, the content was highest in the “larva+pupa” sample and was 459 mg/kg when quantified using the addition method (Fig. 3).

Sample	β (Spd) in mg/L	Ø w (Spd) in mg/kg	SD in mg/kg
mix	2,58	411	2
	2,56		
uncapped	2,97	478	4
	3,01		
only larvae	3,28	521	7
	3,23		
larvae+puppa	3,77	596	11
	3,68		
puppa	3,48	549	11
	3,38		
Black puppa	3,25	519	1
	3,24		

Table 2: Mass fractions of spermidine in the respective samples with internal calibration
Legend:
β (Spd) in mg/L: Beta (Spd) concentration in mg/L (Indicates a derived or calculated spermidine concentration in milligrams per liter.)
Ø w (Spd) in mg/kg: Mean spermidine content (by weight) in mg/kg (Shows the average spermidine concentration per kilogram of sample weight.)
SD in mg/kg: Standard Deviation in mg/kg (Represents the variation or dispersion of spermidine content measurements per kilogram.)

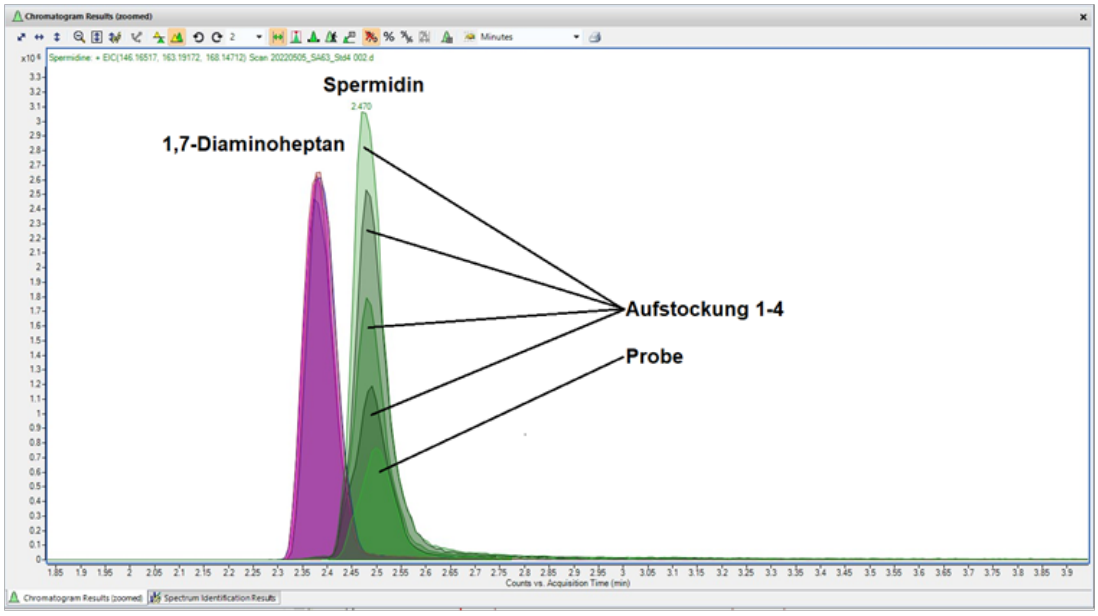

Figure 4: Overlays of the chromatograms of the standard addition method (QTOF)

The external calibration carried out here has the disadvantage that matrix components of the sample have a negative effect on the determination of the spermidine content. In order to take the matrix effects into account during the determination, the standard addition method was used in the following.

The spermidine was quantified using an external calibration with an appropriate standard and the standard addition method.

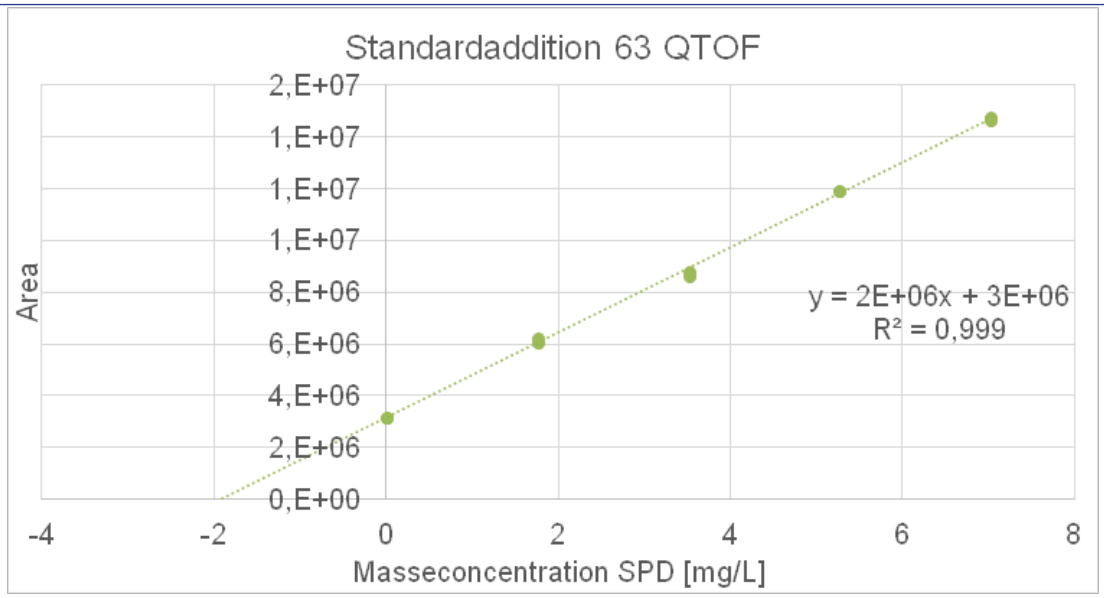

Figure 5: Regression of the standard addition of spermidine

sample	m Apilarnil in mg	Area Spd mit Masse	β (Spd) in mg/L	Ø w (Spd) in mg/kg	SD in mg/kg
mix	25,0	3,18E+06	1,95	310	2
		3,15E+06	1,92		
uncapped	25,1	3,88E+06	2,37	380	0
		3,88E+06	2,37		
only larvae	25,1	3,81E+06	2,33	371	2
		3,78E+06	2,31		
larvae+puppa	25,0	4,65E+06	2,84	**459**	6
		4,73E+06	2,89		
puppa	25,1	3,96E+06	2,42	388	1
		3,98E+06	2,43		
black puppa	25,1	4,14E+06	2,53	407	2
		4,17E+06	2,55		

Table 3: Mass fractions of Spermidine (QTOF) with external calibration

The quantification with the external calibration resulted in higher mass fractions of spermidine in the samples than the quantification with the standard addition. These differences are probably due to matrix effects, which are avoided by the standard addition method for quantification. Therefore, the lower values are considered more credible than the values obtained by external calibration (see table 3).

Apilarnil in different stages of larva development age contains Spermidine in higher concentrations as in wheat germs. Furthermore, Apilarnil contains other bioactive amines and polyamines besides Spermidine like the diamines cadaverine, putrescine and spermine. All could be identified by their exact mass (see fig 6).

In addition, evidence of agmatine, isopropylamine and phenethylamine was detected. However, these peaks of biogenic amines have low signal-to-noise ratios.

DISCUSSION

In this project the presence of Spermidine in Apilarnil has be proven and quantified. It is present in fresh and freeze-dried material of all larva-stages. Whereas the Spermidine content reaches its highest values in the end of the lava stage.

The highest spermidine content has been measured in wheat germ to date and is around 325 mg/kg [5]. The quantification of spermidine in the drone samples revealed a higher spermidine content of 459 mg/kg, even with the addition method.

The spermidine content is another column for explanation the various health applications for Apilarnil in apitherapy [6] and is most likely directly related to its health benefits.

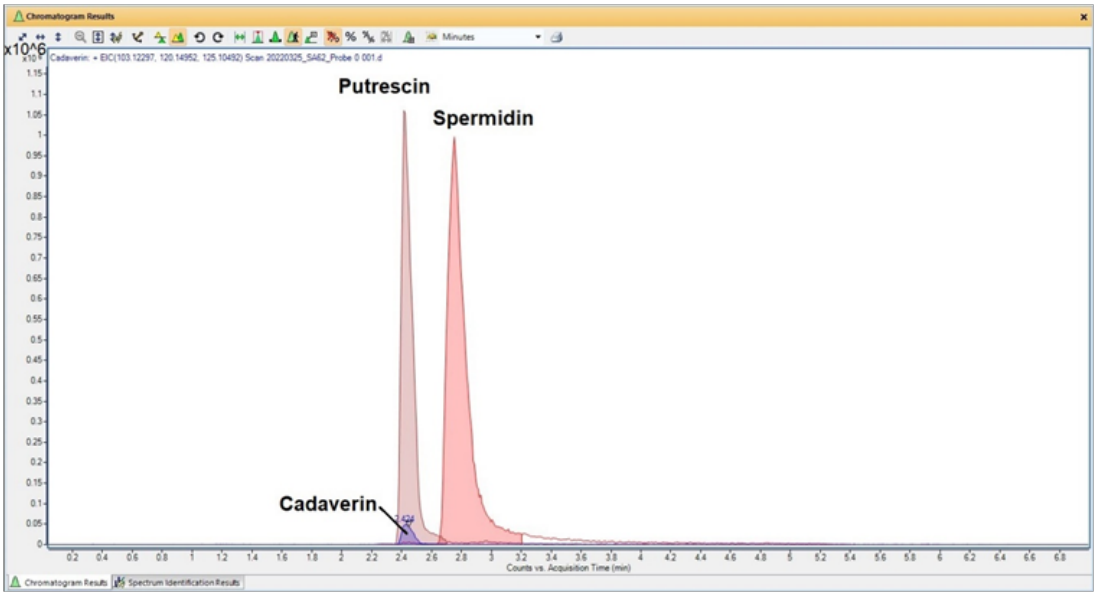

Figure 6: Chromatogram of biogenic amines detected

It will be a further challenge to reveal the molecular mechanisms especially the synergies in the presence of spermidine and the other bioactive ingredients of Apilarnil like the hormones and choline.

From traditional medicine it is known that in mixtures of Apilarnil with other hive products like Royal Jelly, propolis or pollen develop beneficial health synergies. These contain e.g. a lot of flavonoids. In the case of resveratrol, which is a known polyphenol, which is also found in red wine but also in bee products a synergy could be proven in literature: In the experimental study spermidine and resveratrol trigger [7] a certain level of autophagy if they are applied alone. Together applied only 1/10 of both triggers the same effect. This could be a good model how we can understand that low level concentrations of certain bioactive molecules can trigger reasonably sound effects, as we see them in the applications of drone milk (Apilarnil).

The sources of spermidine in human blood are the sum from own synthesis in the body, synthesis in the gut microbiome and external food like Apilarnil or wheat germs. Therefore, the effects derive from a complex matrix and are also influenced by other highly bioactive molecules like the flavonoids, or the hormones present in Apilarnil.

CONFLICT OF INTEREST

GT is producer of Apilarnil (ApiDrohn®) and member of Api-Zentrum Ruhr.

TI: no conflict of interest.

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APIS

Original Research

A Preliminary and Multidisciplinary Study: The Effect of "Sleeping on The Beehives" and Listening to Bees on Human Anxiety Levels

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Abstract: Apihouses are specialized wooden sheds designed to promote relaxation through sensory experiences associated with beehives, such as buzzing sounds and hive air, without direct interaction with bees. While anecdotal evidence suggests calming effects of this experience, scientific validation remains limited. This preliminary study evaluates the potential anxiety-reducing effects of apihouse experiences and bee buzzing sounds. 60 participants were randomly divided into two groups: one exposed to a real apihouse environment and the other to recorded bee buzzing sounds. Anxiety levels were assessed using the State-Trait Anxiety Inventory for Adults (STAI-AD) before and after exposure. Sound analysis from the apihouse revealed peak frequencies ranging from 237 Hz to 416 Hz, with a mean of 274 Hz, consistent with non-aggressive, normal bee activity. Both groups demonstrated significant reductions in state and trait anxiety levels ($p < 0.001$), indicating that both the apihouse environment and bee buzzing sounds lead to anxiety alleviation on their own. Within its limitations, this study highlights the therapeutic potential of apihouses and sets the stage for future research to uncover their underlying mechanisms and broader applications in promoting holistic well-being. The observed reduction in anxiety levels paves the way for new research opportunities and suggests further research with a larger sample size and in a more isolated environment is necessary.

Keywords: Beehive Sound Frequency, Apitherapy, Apihouse, Ecopsychology, Holistic Well-being

INTRODUCTION

Honey bees play an important role as significant pollinators of plants, help increase crop yields, and are indispensable for production of honey and other related products [12; 20]. However, in recent years, bees have also gained a reputation for their therapeutic properties under apitherapy [5; 16; 29; 31].

Apitherapy involves the use of bee products such as honey, royal jelly, propolis, and bee venom for their medicinal and health-promoting properties. Bee products have been recognized for their antioxidant, anti-inflammatory, and immune-boosting effects and are utilized as a complementary and alternative medicine for various conditions, including arthritis, allergic rhinitis and cancer [2; 14; 17; 19; 22]. While these benefits are widely recognized in traditional medicine, their scientific validation remains to be fully established. For instance, studies have demonstrated the antioxidant and anti-inflammatory effects of bee products, yet large-scale clinical trials confirming these therapeutic claims are lacking [2; 19]. Highlighting these gaps could pave the way for more robust empirical research to better understand and substantiate the efficacy of apitherapy.

The concept of apitourism has emerged alongside apitherapy, particularly in countries like Slovenia, where the practice integrates beekeeping activities with tourism and education [4; 30]. Apihouses are potentially a significant part of apitourism, offering individuals the opportunity to relax and rejuvenate by experiencing beehive air and the sounds of buzzing bees [27; 28]. These activities

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not only promote individual well-being but also support local economies and raise awareness about the ecological importance of bees [30].

Typically apitherapy involves the use of an apihouse, a specialized shed designed to provide relaxation and therapeutic benefits through proximity to beehives, without direct interaction with the bees. These structures are carefully designed to ensure a controlled environment where individuals can experience the unique stimuli associated with beehives, such as the soothing buzzing of bees, the natural micro-vibrations they generate, and the aromatic hive air. By creating this indirect yet immersive connection, apihouses aim to harness the potential relaxing properties of bee-related stimuli while ensuring the safety and comfort of users. Typically, an apihouse includes two or three beds adjacent to four beehives, which are accessed by bees through openings located outside the structure [28]. The design ensures that individuals can benefit from the natural stimuli of beehives, such as buzzing sounds, micro-vibrations, and hive air, while remaining protected from direct contact [1; 8].

These houses have been established in several European countries, including Slovenia, Hungary and Türkiye, and are gaining attention for their potential health benefits. Despite their increasing popularity, scientific research on the therapeutic effects of apihouses remains limited, necessitating further studies to validate their impact on human health and well-being [28].

Our hypothesis was that the apihouse environment, particularly the bee buzzing sound, has a relaxing effect that can decrease anxiety levels. To test this, we compared human volunteers exposed to the real apihouse experience with those exposed solely to auditory stimuli, aiming to identify the core elements responsible for the therapeutic effects. Therefore, the primary aim of this study is to evaluate the potential anxiety-reducing benefits of apihouse-specific features, such as hive air and buzzing sounds, and to provide empirical evidence supporting their claimed health benefits [27; 28]. By isolating the key components contributing to relaxation, we seek to advance the understanding of apitherapy practices and help develop apihouses as therapeutic spaces.

MATERIALS AND METHODS

The study was conducted at Ceylan Bee Farm in Karaburun, Izmir, Türkiye. The site includes three apihouses, two of which are identical wooden structures used for the experiments. These apihouses are designed to ensure controlled airflow and safety, promoting a consistent experience for participants. Each apihouse is equipped with four beehives on either side, forming “bee beds” where participants sit (Figure 1). The beehives are constructed with six frames each and the apihouses are designed to ensure safe exposure to bee-related stimuli without direct contact.

The study included 60 participants aged between 18 and 65, randomly divided into two groups of 30 each. The first group, referred to as the “apihouse group,” experienced the apihouse with beehives including bees, while the second group, the “sound group,” listened to recorded sounds of bees buzzing sitting on the beehives not including bees. Both groups were informed that bees were present. Participants were screened for bee allergies, phobias and psychiatric medication use and the ones positive for any of these were not included in the experiment. Informed consent was obtained, ensuring adherence to ethical guidelines.

To examine the effect of bee sounds, recordings were taken within the apihouse using a high-quality portable recorder (Zoom H4n Pro) in May and June 2023. These recordings captured ambient hive sounds just before the experiments and were used for the sound group to simulate the apihouse experience without live bees. The recorded sounds were processed to remove unwanted external noise and analysed by using Audacity [25] and played back to participants through earphones.

The experimental procedure included six stages: filling out the consent form, initial anxiety assessment using the STAI-AD, pre-EEG data recording, the apihouse or sound exposure, post-EEG data recording and a final anxiety assessment (EEG results are going to be reported elsewhere). Each participant spent 10 minutes in the apihouse or listening to the sound recording, and data collection occurred during the early morning to minimize environmental variations and high temperatures.

The STAI-AD was administered in Turkish to assess participants’ anxiety levels before and after both exposures [24]. This widely used inventory evaluates state and trait anxiety [21] and has been validated for its Turkish version

in Türkiye for reliability and accuracy [18]. Moreover, one of the reasons for applying STAI-AD inventory just before the experiment is to minimize the effects of different cognitive and emotional states of participants. The statistical analysis was performed by using SPSS (IBM SPSS Statistics 29.0.1.0).

RESULTS

Analysis of the bee buzzing sound recorded within the apihouse in May and June revealed dominant frequencies ranging from a minimum of 237 Hz to a maximum of 416 Hz (Figure 2), with an average peak frequency calculated at 274 Hz. In total, 30 sounds were recorded but 13 of them were eliminated from the analysis due to the problems such as bad signal quality in the EEG data of the participants. These dominant frequencies were consistently observed and were not significantly affected by weather conditions, indicating the reliability of the auditory exposure.

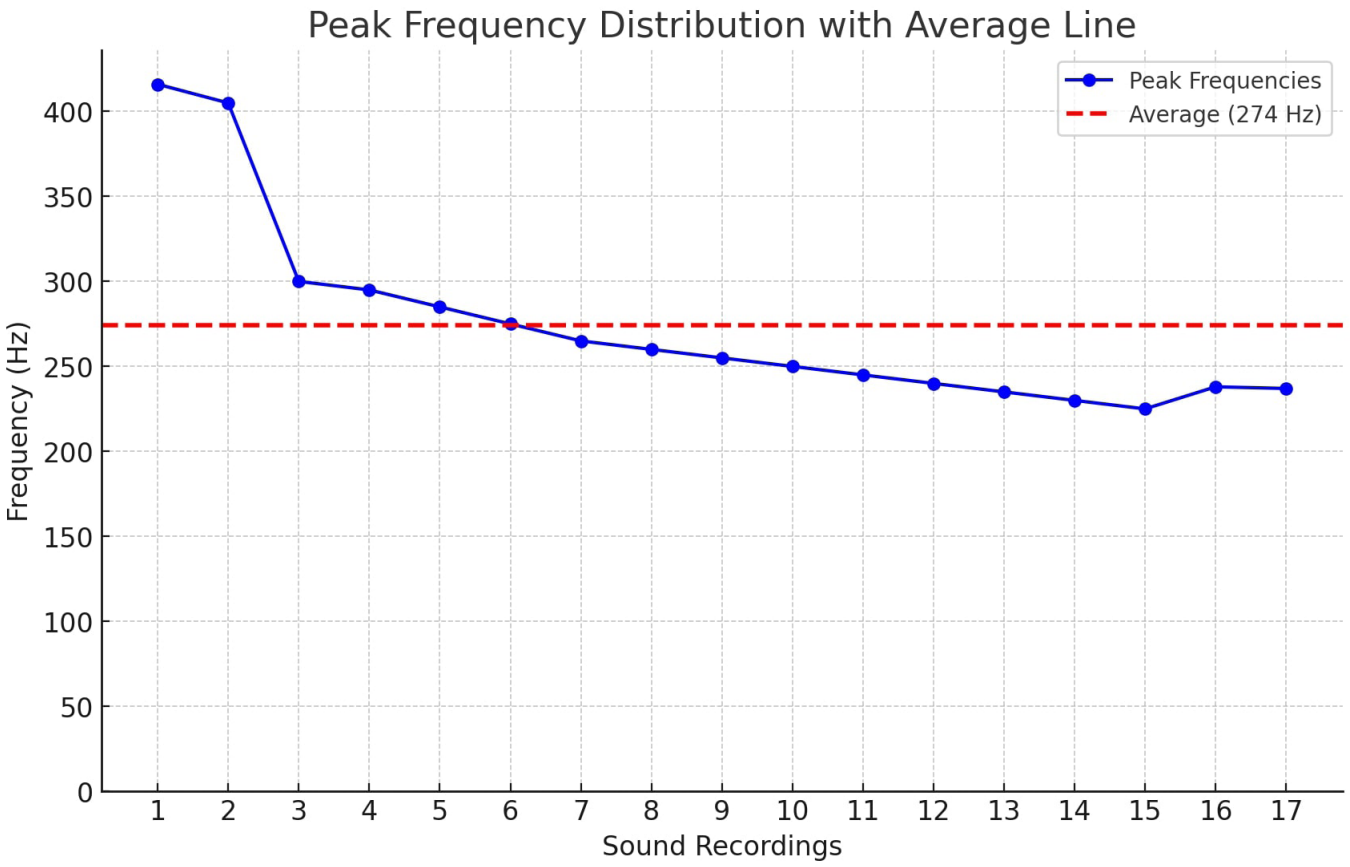

Table 1. The peak frequencies of apihouse sound recording

Participants reported significant decreases in state (n=29) and trait (n=28) anxiety levels after experiencing the real apihouse environment compared to levels before the experience. These preliminary results were statistically significant ($p < 0.001$), supporting the hypothesis that the apihouse environment may effectively reduce anxiety (Table 1). Participants exposed solely to the recorded bee buzzing sound also showed a reduction in both state (n=24) and trait (n=20) anxiety levels (Table 2). These reductions were also statistically significant ($p < 0.001$), indicating that auditory stimuli alone can also provide therapeutic benefits. Some of the participants' anxiety inventory were eliminated due to more than three unanswered questions (11).

Condition	Mean	Std. Deviation	Std. Error Mean	95% CI Lower	95% CI Upper	t	df	One-Sided p	Two-Sided p
State Before-After	5.93103	7.08586	1.31581	3.23527	8.62635	4.508	28	<.001	<.001
Trait Before-After	2.85714	4.78976	0.90518	0.99987	4.71442	3.156	27	.002	.004

Table 2. Paired samples test of the apihouse group

Condition	Mean	Std. Deviation	Std. Error Mean	95% CI Lower	95% CI Upper	t	df	One-Sided p	Two-Sided p
State Before-After	4.875	4.87507	0.99512	2.81644	6.93356	4.899	23	<.001	<.001
Trait Before-After	3.05	2.37254	0.53052	1.93962	4.16038	5.749	19	<.001	<.001

Table 3. Paired samples test of the sound group

Participants in both groups perceived the duration of the 10-minute exposure as slightly shorter than its actual length. The apihouse group perceived the elapsed time as 9.60 ± 4.57 minutes, while the sound-only group estimated it as 9.35 ± 4.66 minutes, showing no significant difference either between groups or from the actual time of the experience.

DISCUSSION

Both the real apihouse experience and bee buzzing sound exposure were effective in reducing participants' anxiety levels, as evidenced by significant reductions in both state and trait anxiety scores. The apihouse experience appeared to offer more comprehensive benefits, likely due to the multisensory nature of the environment. Elements such as the scent of the hive, the buzzing sound, and the micro-vibrations of bees may have contributed to the calming effect. While the study was unable to pinpoint which specific factor was the most influential, it suggests that the bee buzzing sound is one of the strongest candidates. These findings align with broader literature indicating that natural sounds and environments are effective in reducing stress and anxiety levels [6; 10; 23].

On the other hand, comparison between the apihouse group and the sound exposure group revealed no significant difference in the level of anxiety reduction. This suggests that the bee buzzing sound itself may be a major contributor to the therapeutic effects observed in the real apihouse experience, although variations in sound perception between the groups, likely due to the listening methods used, may have influenced the comparative results. Furthermore, comparisons with other studies indicate that the recorded peak frequencies are within the range of relaxed and normal bee activity [13; 32] and are effective in promoting relaxation, consistent with previous findings on the therapeutic potential of natural sounds [3; 7; 22].

Moreover, participants' time perception was slightly altered by both the apihouse experience and the sound exposure. On average, the perceived duration was slightly shorter than the actual exposure time of 10 minutes, although this difference was not statistically significant. Variations in individual time perception might be attributed to differences in mental states, as previous studies have shown that anxiety or stress can distort time perception [15; 26]. While these findings were not conclusive, they contribute to the understanding of the complex relationship between sensory experiences, relaxation, and cognitive processing of time.

Our study has some limitations. For instance, the cognitive and emotional states of participants before exposure might have varied due to the random selection of visitors, complicating standardization. Administering an anxiety inventory before the experiment might have mitigated some of these variables. Additionally, this study was conducted in a natural environment rather than a controlled laboratory setting, which introduced several challenges, such as external disturbances that occasionally distracted participants and compromised data quality. Importantly, the exposure duration for both groups was limited to 10 minutes, which contrasts with the longer exposure times recommended for real apihouse experiences by the owner of the bee farm. This shorter duration may have been insufficient to observe significant changes, highlighting the need for extended exposure in future research. Finally, while the apihouse group was listening to the bee buzzing sound in the natural environment, the sound group was listening to it with earphones. However, before the experiment, both groups were informed that there are live bees in the beehives to prevent any difference in the results owing to any kind of possible instinctive bee fear. To prevent the listening group realize that there are no bees in the beehives, they walked through a path that they were not able to see the near side of the apihouse because in the normal apihouse bees are flying outside of the beehives.

FURTHER RESEARCH

This study represents a first investigation into the effects of apihouses on anxiety levels, laying the groundwork for further exploration in this area. Future studies should consider implementing a fully randomized control group to isolate and better understand the specific contributions of apihouse elements to observed outcomes. Expanding the sample size and eliminating restrictions such as time constraints or posture limitations could provide further insight into mechanisms involved. Such refinements will also allow for comprehensive sleep studies and more detailed analyses of the effects of apihouses.

Additionally, extended experiments could explore other contributing factors within the apihouse environment, such as the scent of beehives, emitted chemicals, and magnetic or electrical fields. Advanced tools like functional MRI or MEG may provide a deeper understanding of neural mechanisms, especially in brain regions like the amygdala, linked to anxiety regulation. Investigating these aspects will enrich the scientific understanding of apihouse benefits.

In conclusion, this study underscores the potential of the apihouse experience and the buzzing sounds of bees within the apihouse to reduce anxiety levels in humans. While the calming effects of the apihouse environment were supported, the analysis also provided novel insights into the characteristics of the buzzing sound within the apihouse, documenting an average peak frequency of 274 Hz. The therapeutic benefits demonstrated by the apihouse experience lay a foundation for further exploration into the interplay between humans, bees, and their environment, contributing to advancements in apitherapy, ecopsychology, and holistic well-being.

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