NKFI K 132044

Active compounds and bioactivity of nectar and honey in invasive medicinal plants

2019.12.01-2024.05.31.

Final Report

The main objectives of our project were to clarify the following issues:

- To what extent do **ecological conditions** (soil, climate) and year influence the **nectar production** and the honey quality of ***Asclepias*** and ***Solidago*** species?
- Does the nectar and honey of milkweed contain the potentially toxic cardiac glycosides characteristic for *Asclepias* species?
- Does the floral nectar of milkweed and goldenrod species exert antimicrobial effect?
- Are the **honeys marketed as milkweed and goldenrod honeys** in fact unifloral honeys of *Asclepias* and *Solidago*? Do botanical analyses confirm the origin of each unifloral honey?
- Does geographical origin affect the **quality of *Asclepias* and *Solidago* honeys**? To what extent do honeys of the same plant species differ if collected at various sites? Does geographical origin influence the presence of bioactive compounds, e.g. polyphenolic substances in honeys?
- Does botanical and geographical origin affect the **biological activity** (e.g. antioxidant capacity, antimicrobial effect) of honey samples?

NECTAR STUDIES

Due to the **pandemic**, the field work in **our project had to be modified**. Due to travelling restrictions, nectar measurements were done only in **3 *Asclepias* (milkweed) and 3 *Solidago* (goldenrod) populations in Hungary**, while field work in the originally planned 2 Slovakian sites could not be realized. Since this was the situation in the first two years of the project, we kept the 3 study sites for both *Asclepias* and *Solidago* in the 3rd year of the project, too.

Methods: In each of the *Asclepias* study sites, 24 h nectar production of 20 flowers from 15 inflorescences (altogether 300 flowers per site) was measured in the field. Nectar volumes were measured with microcapillaries, and nectar sugar concentration was determined with hand-held refractometer. In the case of *Solidago*, flowering plants had to be carried into the laboratory, where nectar measurements were performed using a stereo microscope and analytical balance, due to the small size of capitulum inflorescences and very low volumes of nectar. Because of these technical difficulties, the pooled nectar from 10 *Solidago* flowers was measured together, from 20 individuals per each site, measuring two pooled samples from each individual (altogether 400 flowers per site). Nectar measurement data of both species were processed and analyzed statistically. Based on the first year's field experience, nectar sampling for microbiological tests and chemical analysis was not realistic, due to low volumes of nectar, so these originally planned steps were not performed. In each study site, coenological relevés (2×2 m) were recorded, listing all occurring plant species and their cover in the area. At each study site, air temperature and relative humidity values were recorded, and soil moisture was measured. Soil samples were collected from each study site and analyzed at the Research Institute for Viticulture and Oenology of University of Pécs.

Results:

Common milkweed (*Asclepias syriaca* L.)

In the first year of studies, 2020, the mean **nectar volumes** per *Asclepias* flower ranged between 0.9-2.7 microliter, while mean **nectar sugar concentrations** were in the range of 27 to 34%. In the summer of 2021, mean nectar volumes were in general lower (0.5-1.5 μL/flower), while mean nectar sugar concentrations were in general higher (29 to 40%), compared to 2020. In the first two years, **both nectar volumes and sugar concentrations differed significantly at the 3 study sites**, and within each site statistically relevant differences were found between inflorescences. In 2020, **soil moisture content** was significantly lower at Site 3 (Bócsa) compared to Site 1 (Balotaszállás) and 2 (Kelebia), correlating with the lowest nectar volumes measured at Site 3. At the same time, **relative humidity** values were the highest at Site 3 on the day of nectar measurement, causing the dilution of nectar, and consequently the lowest sugar concentration values were measured at this site. In 2021, soil moisture content was similar at each study site, while relative humidity values were the highest in Site 3 on the day of nectar measurement, causing the dilution of nectar, with the lowest sugar concentration values.

In the summer of **2022**, mean nectar volumes of *A. syriaca* (0.5 to 1.1 μL/flower) were the lowest from the 3 years of study. Mean nectar sugar concentrations (30 to 38%) were similar to those measured in 2021, and higher compared to 2020. Lower volumes and higher concentrations of nectar can be explained by the extremely hot temperatures at the time of field studies (up to 35-40°C). In addition, a dry period, with no rainfall, preceded the blooming period of milkweed, due to which soil moisture contents were low at the end of June 2022 (mean values 11.08% and 3.33% at study site 1 and 2, respectively). In 2022, *Asclepias* nectar volumes and sugar concentrations measured in the same period of the day were not statistically different ($P \leq 0.05$) at the two study sites. However, both **volume and sugar concentration of nectar was different measured at the same study site during different time periods of the day** (morning vs. afternoon hours).

From the 15 measured **soil parameters** [pH (H_2O), pH (KCl), plasticity according to Arany (K_A), total water soluble salts, $CaCO_3$, humus, (NO_3+NO_2)-N, P_2O_5, K_2O, Mg, Na, SO_4-S, Cu, Mn, Zn], the most pronounced differences between the sites were measured in nitrogen and phosphor content in 2020; in calcium carbonate and phosphor content in 2021; calcium carbonate, humus, nitrogen, phosphor, copper and zinc content in 2022, which factors may contribute to differences in nectar production.

Conclusions: Our **3-year field studies** indicated that ***A. syriaca* more successfully occupied abandoned agricultural fields than sandy dry grasslands**, and **nectar production was higher on abandoned fields**. In two years of the study the **amount and sugar content of nectar was significantly different in each study site and correlated with the cover of milkweed**. *Asclepias* plants were taller and covered larger parts of sample plots on abandoned vineyards than on dry sandy grasslands. **Nectar production was positively affected by higher levels of humidity and soil moisture**, while no correlation was found between other soil characteristics and nectar traits.

Giant goldenrod (*Solidago gigantea* Ait.)

The **nectar production of *S. gigantea*** was investigated at 3 study sites: 1) Vejti – near Drava river, 2) Homokmégy – vicinity of Danube river, 3) Hévíz – vicinity of Lake Balaton**. In two study years neither nectar volumes, nor concentrations were different at the various study sites** at $P<0.05$: mean nectar volumes were 0.05-0.06±0.03 mg/disc floret at each site, with mean sugar concentrations of 33 to 36% and 29 to 31% in 2020 and 2022, respectively. In 2021, nectar volumes, ranging from 0.04±0.02 to 0.08±0.05 mg/flower, were characterized by high standard deviation. Nectar production in Site 3 was significantly lower compared to Site 1 and 2 at $P<0.05$. Mean sugar concentrations varied

between 34 and 44%, the values measured at Site 3 being significantly higher than at Site 1 and 2 at $P<0.05$.
In addition to our initial project objectives, our research team conducted **anatomical studies on *A. syriaca* and *S. gigantea***, providing detailed histological analysis of each plant organ (root, stem, leaf, flower), with special attention to the nectar producing glands within the flowers. These results were published in diploma theses.

Dissemination of results:

Our results regarding the **nectar production of *Asclepias syriaca*** were **presented at** the **international conference** "14th SFSES Symposium on the Flora of Southeastern Serbia and Neighboring Regions" (26-29 June 2022, Kladovo, Serbia):

- Purger D. et al. (2022): A noxious weed vs. useful honey plant: nectar production of common milkweed (*Asclepias syriaca* L.) depending on environmental conditions

During the project, **3 MSc theses** were defended **related to the above topics**:

- Al-Shawawreh, Marah Saleem (2021): Anatomical studies on giant goldenrod (*Solidago gigantea* Ait.)
- Morvai, Dalma (2022): Nectar production of common milkweed (*Asclepias syriaca* L.) stands as a function of environmental factors (in Hungarian)
- Pálfi, Lili (2022): Histological evaluation of common milkweed (*Asclepias syriaca* L.) (in Hungarian)

Manuscript in preparation:

This manuscript will **summarize the results of our 3-year studies on the nectar production of the two invasive species**, milkweed and goldenrod. The statistical analysis of nectar volume and concentration data is ready, while the correlation analyses regarding relationships between nectar traits, ecological factors and co-occurring species are in progress. Nectar studies will be discussed together with **honey quality issues**: the sensory evaluation, pollen analysis and measurement of physico-chemical characters was carried out on milkweed and goldenrod honey samples from 3 years.

The manuscript intends to answer the following questions:

- is there a difference between different locations regarding the nectar and honey quality of these invasive species?
- which factors can be in the background of the above differences?
- can we count on milkweed and goldenrod as reliable bee pastures under the current climatic conditions in Hungary?
- can the study sites provide a good quality monofloral honey?

Proposed Q1 journal for publication: Heliyon (Elsevier) / Apidologie (Springer) / Plants (MDPI).

HONEY STUDIES

General comment:

Due to the pandemic situation, in 2020 some laboratories were closed for a while, delaying analytical studies directed at bioactive compounds (phenolic substances, minerals), but in turn, biological activity studies were started earlier than planned in the project.

Honey samples – pollen analysis:

In the fall of 2020, 2021 and 2022 we received **honey samples from beekeepers** who kept their beehives in the vicinity of our study sites during the nectar measurements of *Asclepias* and *Solidago*. However, all the beekeepers claimed that they could not obtain pure milkweed or goldenrod honey these years, due to unfavorable climatic conditions (prolonged dry periods before and during bloom). The **pollen analysis** of honey samples was performed with light microscopic examination, which supported the beekeepers' statement, i.e. none of the honey samples originating from our research areas could be considered a unifloral honey, based on their pollen composition. Therefore, we decided to purchase further goldenrod and milkweed honey samples, some of which proved to be unifloral honeys. These samples were also included in further analytical measurements and biological activity studies. In 2023, milkweed (*Asclepias*) and goldenrod (*Solidago*) **honey samples** were obtained from beekeepers in Hungary and Slovakia. Some beekeepers stated that they could not obtain a pure goldenrod honey this year. Further analysis included only honey samples, which could be evaluated as unifloral honeys according to the **melissopalynological analysis**.

Bioactive compounds of honey samples:

The **phenolic profile of honey samples** was analyzed with high performance liquid chromatography, coupled with diode array and mass spectrometry detection (**HPLC-DAD-MS**), following a fluid-fluid extraction. From the 27 examined substances, each honey sample contained abscisic acid, apigenin, benzoic acid, caffeic acid, chrysin, ferulic acid, galangin, hesperetin, kaempferol, naringenin, p-coumaric acid, p-hydrobenzoic acid, pinobanksin, protocatechualdehyde, syringic acid and vanillic acid; whereas eriodictyol, gallic acid, isorhamnetin, luteolin and naringin was not detected in any of the samples. Chlorogenic acid, gentisic acid, p-syringaldehyde, quercetin, quercitrin and taxifolin were present only in some of the honey samples. The unique combination of phenolic substances is suitable for authenticating honey types of diverse floral origin, and may contribute to differences existing between various honey types regarding their health benefits.

These results were published in:

- Farkas Ágnes, Horváth Györgyi, Kuzma Mónika, Mayer Mátyás, Kocsis Marianna: ***Phenolic compounds in Hungarian acacia, linden, milkweed and goldenrod honeys***, CURRENT RESEARCH IN FOOD SCIENCE 6: 100526, 2023

The **mineral content of honey samples** was measured with ICP-AES (inductively coupled plasma atomic emission spectroscopy). Both milkweed and goldenrod honey contained the measured macroelements, namely K, Ca, P, S, Mg and Na, but goldenrod honey contained significantly higher amounts of these nutrients than milkweed honey. Expectedly, the total amount of macroelements was about 3 times higher in goldenrod (515 mg/kg) compared to milkweed honey (152 mg/kg). The most abundant macroelement was K in both honey types (82 and 235 mg/kg in milkweed and goldenrod honey, respectively). The decreasing quantity order of minerals gave some differences; e.g. the second most abundant macroelement was P (35 mg/kg) in milkweed, and Ca (126 mg/kg) in goldenrod honey. Regarding the microelement content, in milkweed honey Fe and Mn, while in goldenrod honey Fe and Cu were under detection limit. The concentration of the most abundant micromineral, B was 1.5 times higher in goldenrod honey (7.7 mg/kg) than in milkweed honey (4.8 mg/kg). Zn was found in both honey types in similar amounts (0.7 and 0.6 mg/kg in milkweed and goldenrod honey, respectively). Our honey samples fit well into the generally accepted concept that the color and mineral content of honey are in strict correlation. In accordance with our expectations, the darker-colored goldenrod honey was found to be rich in minerals, while the light-colored milkweed honey was rather poor in these elements.

Antioxidant effect of honey samples:

In one of our experimental series in 2020, the antioxidant capacity of **goldenrod (*Solidago*) honeys from three countries (Hungary, Slovakia, Poland)** was compared. Since the combination of non-enzymatic antioxidant assays provides the most reliable results, three different assays were performed: DPPH (1,1-diphenyl-2-picrylhydrazyl), ABTS/TEAC (Trolox equivalent antioxidant capacity), FRAP (ferric reducing antioxidant power assay). Based on each assay, the antioxidant capacity of honey samples differed significantly between different sampling sites within the same country, and also between different countries. With each method, Hungarian goldenrod honeys were found to have the strongest antioxidant activity, whereas Polish honey samples were characterized by the lowest values.

These results were published in:

- Czigle Szilvia, Filep Rita, Balažová Ema, Szentgyörgyi Hajnalka, Balázs Viktória Lilla, Kocsis Marianna, Purger Dragica, Papp Nóra, Farkas Ágnes: ***Antioxidant Capacity Determination of Hungarian-, Slovak-, and Polish-Origin Goldenrod Honeys***, PLANTS-BASEL 11: (6) 792, 2022

The other experimental set clarified differences regarding the total reducing capacity (TRC) / total polyphenol content (TPC) of different Hungarian honey samples, performing the Folin-Ciocalteu assay. According to these studies, both goldenrod and milkweed honeys were characterized by higher TRC values, compared to other honey types, including black locust, lime and sunflower honeys.

In 2021 and 2022, the total antioxidant capacity of honey samples was measured performing three different assays: ORAC (oxygen radical absorbance capacity), DPPH and TEAC tests. **In 2021** our samples included **goldenrod (*Solidago*) and milkweed (*Asclepias*) honeys**, obtained **from 3 and 5 different geographical regions of Hungary**, respectively. **In 2022** we extended our sample set to **33 Hungarian honey samples** from 3 different years. **In 2023** we carried out the antioxidant capacity measurements of **30 Hungarian honey samples**, and for comparative purposes, **6 foreign honeys** were included as well, from Italy, Slovakia and Slovenia. The total phenolic content, as well as flavonoid and phenolic acid content was measured for each honey sample. Four different antioxidant assays, DPPH, FRAP, ORAC and TEAC were performed.

In each year, based on each assay, **the antioxidant capacity of honey samples differed significantly**. The total antioxidant capacities were evaluated between 7.80-19.48 μmol/g, 5.19 to 88.26 μmol/g and 7.26 to 103.08 μmol/g in case of ORAC test; while the antioxidant values of honey samples ranged from 117.81 to 239.26 mg/ml, 65.64 to 1104.64 mg/ml and 43.94 to 1082.28 mg/ml in DPPH assay; and from 41.75 to 83.21 mg/ml, 33.44 to 363.82 mg/ml and 22.25 to 262.14 mg/ml in TEAC method in 2021, 2022 and 2023, respectively. Based on the results of each assay, **milkweed (*Asclepias*) honeys** were among the honeys with **lower antioxidant activity**, with similar values as measured for acacia (*Robinia pseudoacacia*) honey samples; whereas ***Solidago*-containing honeys** belonged to the samples with **higher antioxidant capacity**, with similar values to sunflower (*Helianthus*) honeys. Regarding honey samples harvested around our nectar study sites, goldenrod honeys from Homokmégy were found to have the strongest antioxidant activity, whereas milkweed honey samples from Bócsa were characterized by the lowest values.

These results were published in:

- Farkas Ágnes, Balázs Viktória Lilla, Kőszegi Tamás, Csepregi Rita, Kerekes Erika, Horváth Györgyi, Szabó Péter, Gaál Krisztián, Kocsis Marianna: ***Antibacterial and Biofilm Degradation Effects of Hungarian Honeys Linked With Botanical Origin, Antioxidant Capacity and Mineral Content***, FRONTIERS IN NUTRITION 9: 953470, 2022
- Kocsis Marianna, Bodó Alexandra, Gaál Krisztián, Farkas Ágnes: ***Summer Gifts from the Hive: Botanical Origin, Antioxidant Capacity, and Mineral Content of Hungarian Honeys***, In:

Muhammad, Imran; Muhammad, Haseeb Ahmad; Rabia, Shabir Ahmad (szerk.) Honey, IntechOpen (2022) Chapter 4, 2022

- Kocsis Marianna, Bodó Alexandra, Kőszegi Tamás, Csepregi Rita, Filep Rita, Hoffmann Gyula, Farkas Ágnes: ***Quality Assessment of Goldenrod, Milkweed and Multifloral Honeys Based on Botanical Origin, Antioxidant Capacity and Mineral Content***, INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES 23: (2) 769, 2022

Antibacterial effect of honey samples:

The antibacterial activity of honey samples was examined with various *in vitro* methods. Minimal inhibitory concentration (MIC) was determined with broth dilution method. The ability to inhibit biofilm formation was determined in 96-well microtiter plates with crystal violet assay.

In 2020 we tested the antibacterial effect of honey samples from both 2019 and 2020 (altogether 11 types of honeys). The examined bacterial strains included both Gram-positive (*Micrococcus luteus*, *Staphylococcus aureus*) and Gram-negative bacteria (*Haemophilus influenzae*, *Pseudomonas aeruginosa*). In the agar well diffusion assay, *M. luteus* proved to be more sensitive to various types of honeys, compared to *S. aureus*, the latter being inhibited only by half of the tested honey samples. The MIC value for *P. aeruginosa* was determined as 35% m/v honey solution, while for *H. influenzae* as 40% m/v. The biofilm forming ability of both *Pseudomonas* and *Haemophilus* strains was inhibited by each type of honey with at least 70%, with some differences regarding the activity of honey samples and also the sensitivity of bacterial strains.

In 2021 we tested the antibacterial effect of honey samples from 2020: 3 of them marketed as goldenrod and 4 as milkweed honeys. The examined bacterial strains included both Gram-positive (*Streptococcus pneumoniae*) and Gram-negative bacteria (*H. influenzae*, *H. parainfluenzae*, *P. aeruginosa*). Each honey sample inhibited the growth and biofilm forming ability of the respiratory tract pathogens tested, with MIC values ranging between 40 and 55.5%. Similarly to the results of microdilution assays, *Solidago* honey samples showed higher inhibition rates: 62%, 69% and 72% compared to 49%, 54% and 55% of *Asclepias* honey samples in case of *P. aeruginosa*, *Haemophilus* spp. and *S. pneumoniae*, respectively. In each assay, *S. pneumoniae* was the most sensitive and *P. aeruginosa* the most resistant bacterium from the respiratory tract pathogens.

- Balázs Viktória Lilla, Nagy-Radványi Lilla, Filep Rita, Kerekes Erika, Kocsis Béla, Kocsis Marianna, Farkas Ágnes: ***In Vitro Antibacterial and Antibiofilm Activity of Hungarian Honeys against Respiratory Tract Bacteria***, FOODS 10: (7) 1632, 2021

The antibacterial effect of goldenrod and milkweed honey samples from 2021 was tested on Gram-positive *Staphylococcus aureus* and the Gram-negative bacteria *H. influenzae*, *H. parainfluenzae*, *P. aeruginosa*. Goldenrod honey was more effective against each bacterium, compared to milkweed honey. *P. aeruginosa* was the most resistant bacterium, with 62.0% and 55.5% MIC values for milkweed and goldenrod honeys, respectively. *H. parainfluenzae* was the most sensitive pathogen, with the lowest MIC values (milkweed: 45.5%; goldenrod: 40.5%). Consistent with the MIC results, **goldenrod honey inhibited biofilm formation more effectively than milkweed honey**. Honey samples had the lowest inhibitory rates against *P. aeruginosa* biofilms (milkweed: 52.7%; goldenrod: 61.9%), while higher inhibitory rates were measured for *Haemophilus* species, the most sensitive being *H. parainfluenzae*, with 70.5% inhibitory rate after treatment with goldenrod honey. **Scanning electron microscopy** (SEM) supported the results obtained with crystal violet assay. In case of each bacterium, the SEM images of control samples captured the characteristic morphological elements of a mature, three-dimensional biofilm. Treatment with MIC/2 value of goldenrod honey resulted that the cells attached to the surface, but they did not form biofilm-specific structures.

In order to explore the **mode of action of honey samples**, **bacterial membrane degradation** was studied by measuring the degree of bacteriolysis, using 20, 40, 60 and 90% concentrations of honeys. Loss of integrity of the bacterial membrane was observed at concentrations of 40% and above, but no membrane degradation was found at 20% honey concentration. *Haemophilus* species responded more sensitively to the treatment than *S. aureus* and *P. aeruginosa*. In accordance with the results of biofilm degradation assay, the goldenrod honey sample showed the best activity against each bacterium. A time course lysis with 60% (w/v) honey solutions was also performed to examine the **kinetics of bacterial membrane degradation**. The released cellular material was measured from 10 to 90 min. Bacterial cell lysis was induced after 40 min incubation. Milkweed honey had lower activity compared to goldenrod honey. The highest membrane degradation was detected at 90 min, in case of goldenrod honey against *H. parainfluenzae*. The membrane degradation effect of goldenrod honey was clearly visible in **SEM** micrographs, revealing that the honey treatment caused morphological damage in the bacterial cells, disrupting the cell membrane, which in turn resulted in release of the cellular material.

- Farkas Ágnes, Balázs Viktória Lilla, Kőszegi Tamás, Csepregi Rita, Kerekes Erika, Horváth Györgyi, Szabó Péter, Gaál Krisztián, Kocsis Marianna: ***Antibacterial and Biofilm Degradation Effects of Hungarian Honeys Linked With Botanical Origin, Antioxidant Capacity and Mineral Content***, FRONTIERS IN NUTRITION 9: 953470, 2022

The antibacterial effect of milkweed and goldenrod honey samples from 2022 was determined on Gram-negative *P. aeruginosa*, and Gram-positive *S. epidermidis* and methicillin-resistant *S. aureus* (MRSA). The MIC determined by the broth microdilution test (BDT) was 20 to 25% when treating the bacterial strains with goldenrod and milkweed honey samples. The most resistant pathogen was *P. aeruginosa*, which required the highest concentrations of honey to inhibit growth (25%). Consistent with previous year's results and the MIC values above, goldenrod honey displayed higher anti-biofilm activity than milkweed honey. The biofilms of *P. aeruginosa* and MRSA were more resistant than those of *S. epidermidis*. The biofilm formation of *P. aeruginosa* was inhibited by 49.2% and 56.0% by milkweed and goldenrod honey, respectively. The honey samples were more active in case of *S. epidermidis* (64.4% and 71.1% for milkweed and goldenrod honey, respectively). According to SEM studies conducted with *S. epidermidis* and *P. aeruginosa*, the control samples, which were not treated with honey, showed the characteristic picture of a mature, three-dimensional biofilm. Treatment with milkweed and goldenrod honey resulted in dominance of planktonic bacterial cells, without forming biofilm-specific structures.

Gram-positive and Gram-negative bacteria use quorum sensing (QS) communication to regulate diverse physiological activities, including symbiosis, virulence, motility, sporulation, and biofilm formation. The **anti-QS activity of honey samples** was assessed through the inhibition of violacein synthesis in the model organism *Chromobacterium violaceum*. Based on the liquid culture assays performed, all honey samples exhibited anti-QS activity. Goldenrod and milkweed honey totally reduced bacterial cell growth between 75–25% (v/v) concentrations. QS inhibition was observed from a 10% honey concentration, where cell numbers reached log5–log7 CFU/mL, respectively, but pigment production was still inhibited, compared to the control samples.

- Balázs Viktória L., Nagy-Radványi Lilla, Bencsik-Kerekes Erika, Koloh Regina, Szabó Dina, Kocsis Béla, Kocsis Marianna, Farkas Ágnes: ***Antibacterial and Antibiofilm Effect of Unifloral Honeys against Bacteria Isolated from Chronic Wound Infections***, MICROORGANISMS 11: (2) 509, 2023
- Nagy-Radványi Lilla, Balázs Viktória L, Kocsis Béla, Csikós Eszter, Ángyán Virág D, Szabó Péter, Biró Viktória, Kocsis Marianna, Farkas Ágnes: ***Antibacterial activity of Hungarian varietal honeys against respiratory pathogens as a function of storage time***, SCIENTIFIC REPORTS 14: (1) 10200, 2024

Budget issues, Purchase of necessary equipment:

In accordance with the budget plan, in the first year of the research project,

- a Neofuge 15R benchtop **centrifuge** with swing-out rotor and cooling-heating option, and
- a Nikon Eclipse E100 type **research microscope** equipped **with** a 6MP **digital camera** were purchased, both of which have been used in the pollen analysis of honey samples.

In the first two project years, other minor equipment, such as portable balances, hand refractometers, digital hygro-thermometers for field studies, as well as a multichannel pipette and other pipettes for laboratory studies, were also accounted in the category of 4. Equipment. These costs were not included in the original budget plan, but could be covered from other categories of the budget, i.e. from 1.7 'Daily allowance' and 3.1 'Travelling abroad'. This was possible, because project members could not travel abroad or participate at international conferences in 2020 and 2021, due to the pandemic situation.

The original budget plan did not include 1.2.1, however, it became necessary to employ a researcher in order to have the antioxidant measurements done according to the time schedule of the project.

Dissemination of results:

Due to the pandemic situation, no international conferences could be attended in 2020 and 2021. Thus, in the first two years of the project our results were presented only at **national conferences**, in the form of oral and poster presentations (2020: 2 abstracts, 2021: 4 abstracts). In the next 3 years, besides attending international meetings, we also presented our results at national conferences (2022: 1 abstract, 2023: 4 abstracts, 2024: 8 abstracts). Altogether, **19** conference **presentations** were given at various national scientific meetings.

In 2022 and 2023 our results were presented at **4 international congresses** (14th SFSES Symposium, Serbia, 2022; 15th World Congress on Polyphenols Applications, Spain, 2022; Symposium "Chestnut honey for medical use", Ljubljana, Slovenia, 2023; 26th Euro-Global Summit on Food and Beverages, Vienna, Austria - Rome, Italy, 2023).

During the project, altogether **10 scientific journal papers** were published in peer-reviewed, internationally acknowledged journals (**D1, Q1**).

2020: *Food Bioscience* (Q1, IF. 4,240)

2021: *Molecules* (Q1, IF: 4,411)
Foods (Q1, IF: 4,350)

2022: *International Journal of Molecular Sciences* (D1, IF: 6,208)
Plants (Q1, IF: 4,658)
Frontiers in Nutrition (Q1, IF: 6,59)

2023: *Microorganisms* (Q1, IF:4,5)
Current Research in Food Science (Q1, IF: 6,3)

2024: *Antibiotics* (Q2, IF: 4,8)
Scientific Reports (D1, IF: 4,6)

During the project, **3 scientific-popular journal papers** were published in the leading Hungarian journal for beekeepers:

2020: Méhészet 68 (8): 18-19.; 2021: Méhészet 69 (10): 20-21.; 2022: Méhészet 70 (8): 16-17.

A **book chapter** was published:

- Kocsis Marianna, Bodó Alexandra, Gaál Krisztián, Farkas Ágnes: ***Summer Gifts from the Hive: Botanical Origin, Antioxidant Capacity, and Mineral Content of Hungarian Honeys***, In: Muhammad, Imran; Muhammad, Haseeb Ahmad; Rabia, Shabir Ahmad (szerk.) Honey, IntechOpen (2022) Chapter 4, 2022

In addition, altogether **21 diploma theses** / theses for PharmDr. degree (2020: 4; 2021: 1; 2022: 7; 2023: 4; 2024: 5) were defended in the years 2020-2024, with the supervision of various project participants (Balázs V. L., Czigle Sz., Farkas Á., Filep R., Kocsis M.).

In 2023, 2 graduate students of the honey research group presented their results at the local (Medical School and Faculty of Pharmacy, University of Pécs) **conference of the Undergraduate Research Society**, and one of them (Ángyán V.D.) was awarded **first prize** in her scientific session.

Furthermore, **2 PhD dissertations** were submitted, supervised by the principal investigator (Farkas Á.) and a senior researcher of the project (Kocsis M.):

- Alexandra Bodó: "Complex analysis of Hungarian nectar sources, multi- and unifloral honeys from different botanical and geographical origin" - defended in 2022
- Lilla Nagy-Radványi: "Changes in the antibacterial activity of Hungarian varietal honeys as a function of storage" - submitted in spring of 2024

Summary, Conclusions, Limitations:

- ✓ **Ecological conditions**: habitat, cover of milkweed, soil moisture, and relative humidity (RH) of the air were found to influence **nectar traits** and honey quality of ***Asclepias syriaca***
- ✓ Both the **year and study site** had an **effect on nectar quality** of ***A. syriaca***, due to differences in air temperatures and RH values, as well as precipitation before and during bloom → **abandoned agricultural fields** proved to provide **better bee pastures than sandy dry grasslands**
- ✓ In two out of three study years **neither nectar volumes, nor concentrations were different at the various study sites** of ***Solidago gigantea***
- ✓ The phytochemical and bioactivity studies planned with the floral nectar of milkweed and goldenrod could not be realized, due to unexpectedly low nectar volumes in each year of the study
- ✓ **In addition to the original objectives** of the project, the **anatomy of the nectar** producing **gland** (together with other plant organs) was described both in ***Asclepias*** and ***Solidago*** species
- ✓ Melissopalynological analysis revealed the botanical origin of honeys: not all **honey samples marketed as milkweed and goldenrod honeys** proved to be **unifloral honeys** of *Asclepias* and *Solidago*
- ✓ **Botanical and geographical origin** was found to affect the **quality of *Asclepias* and *Solidago* honeys**
    - the presence and amount of **bioactive compounds** (polyphenolic substances, minerals) **varied in different honey types**, and also within the same honey type harvested **in different geographical regions**
    - the **biological activities**, i.e. antioxidant capacity and antimicrobial effect of honey samples displayed **significant differences**, influenced both by the plant source and the place of harvest
- ✓ **In addition to the original objectives** of the project, our research team **revealed** some of the **mechanisms of action** that are **responsible for the antibacterial and anti-biofilm effect of honey:**
    - disrupting the bacterial membrane
    - inhibiting the formation of the 3-dimensional biofilm structure
    - inhibiting quorum-sensing of bacterial cells.