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# **INTERNATIONAL CONGRESS ON BEE SCIENCES**

## **ABSTRACT BOOK**

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**Editor Ulaş ACARÖZ**

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## **Editor's Note**

The first 'International Congress on Bee Sciences' was organized online and free of charge. We are very happy and proud that various Bee science-related fields attended the congress. During this event, distinguished and respected scientists came together to exchange ideas, develop and implement new researches and joint projects. There were 25 invited speakers from 18 different countries. The scientific committee of the congress consisted of 211 scientists from more than 160 universities. Almost 500 participants participated in the congress. We would like to thank all participants and supporters. Hope to see you at our next congress.

Best wishes from Turkey

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# **ORAL PRESENTATIONS**

## Portuguese propolis and apple post-harvest diseases: new perspectives

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### Abstract:

The apple culture is one of the most important globally, but its production, preservation and marketing face several challenges. Postharvest rots are responsible for serious economic losses, besides contributing to food waste. Control of postharvest diseases is done mainly by the application of synthetic fungicides that guarantee good results in a short period of time. However, the persistent use of such products entails risks to the environment and living beings, also pushing the emergence of resistant pathogen strains. So, it is urgent to develop equally effective control products, but safer and more sustainable. Propolis, a natural bee product, is a resinous mixture manufactured by bees from plants and used as a building and defense material in the hives. This product has been used since ancient times for therapeutic purposes due to its multiple properties and bioactivities, namely antioxidant and antimicrobial. Although currently used in areas other than medicine, studies about its application in agrifood sector are still scarce.

The aim of this work was to evaluate the antifungal potential of ethanolic and hydroalcoholic extracts of Portuguese propolis - Cr18.EE and Cr18.EE<sub>70</sub>, respectively - in the control of apple postharvest fungi, namely *Botrytis cinerea* and *Penicillium expansum*. The propolis sample used was rejected for commercialization in pharmaceutical industry, becoming a residue, but results proved its quality. *In vitro*, the extracts proved to be active against both fungal species, but mainly *B. cinerea*, which mycelium growth was inhibited by 45, 60 and 61 % after four days, with concentrations of 500, 1000 and 2000 µg/ml of Cr18.EE, respectively, and in 36, 49 and 63 % with the same concentrations of Cr18.EE<sub>70</sub>. The *in vivo* results showed a reduction in lesion size caused by *P. expansum* up to 67 % in Golden Delicious apples treated with 5 % Cr18.EE<sub>70</sub>, after 14 days, without relevant phytotoxic effects on fruit tissues. These results suggest that Portuguese propolis extracts have potential as bio-fungicides in apple culture postharvest, being an environmentally friendly and safer alternative to reduce food waste, and making farms more profitable, while also valuing the beekeeping sector in Portugal.

**Keywords:** Agriculture, antifungal, apple, propolis, sustainability





## Ability Of Probiotic Candidates For Honeybees (*Apis mellifera* L.) To Detoxify Coumaphos – An *In Vitro* Study #

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### Abstract:

One of the probable factors contributing to Colony Collapse Disorder of honeybees is industrialised agriculture and the use of insecticides, which directly affects the health and condition of honeybees. Some of the most common pesticides found in dead honeybee colonies are chlorpyrifos, imidacloprid, clothianidin or coumaphos. They contribute to acute poisoning, weakening honeybee immunity and increase in the susceptibility to infection with pathogens. Due to the above, increasing interest in searching for new, natural and ecological methods to improve the condition and immunity of honeybees is observed worldwide. One of the alternatives can be lactic acid bacteria (LAB), which are natural inhabitants of the gastrointestinal tract (GIT) of honeybees, fulfilling a number of beneficial functions in their body. An example of desired LAB activity is detoxification of pesticides. Searching for potentially probiotic bacterial strains with pesticide detoxification abilities is a big challenge and is highly desirable. The aim of the study was to determine the ability to detoxify an organophosphorus insecticide - coumaphos by 25 strains of LAB of various origins, selected on the basis of previous screening tests. Methods. Living LAB cells were suspended in pure water ( $10^9$  CFU/mL) and incubated with 100  $\mu$ g/mL of coumaphos (24h, 30°C, 200 rpm, anoxic conditions). Positive control was devoid of LAB. Concentration of coumaphos was determined with HPLC. Detoxification ability was assessed with the use of cell line Sf-9 (ovaries of *Spodoptera frugiperda* insect larvae). Cytotoxicity was evaluated with MTT, while genotoxicity with the single cell gel electrophoresis (comet) assays. Results. LAB decreased coumaphos concentration in the range of 2.2% $\pm$ 0.5 (*Pediococcus pentosaceus* 10/2 isolated from *Sambucus nigra* flowers) to 57% $\pm$ 0.3 (*Pediococcus pentosaceus* 14/1 isolated from *Lavandula angustifolia* flowers). Also *Pediococcus pentosaceus* 14/1 decreased the cyto- and genotoxicity of coumaphos by 69.8% $\pm$ 0.6 and 86.9% $\pm$ 0.5, respectively, and this strain was the most effective in detoxifying the insecticide. Conclusions. The degree of insecticide detoxification depended on the LAB genus/species/strain and isolate origin. Strains isolated from the honeybee environment (*e.g.* flowers, honey) demonstrated the most effective activity in detoxifying coumaphos compared to collection strains and *Apilactobacillus kunkeei* DSM 12363 from GIT of *A. mellifera* L.

**Keywords:** probiotics, honeybees, coumaphos, insecticides, detoxification.

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## Addressing Modern Challenges For Bees Through A Novel Mobile App<sup>#</sup>

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### Abstract:

The importance of bees as pollinators for biodiversity and food security is elementary for mankind. Despite their significance, bees are increasingly exposed to unfavorable environmental influences caused by humans, and domesticated honeybees can also be a dangerous foraging competitor for wild bee species. To address these challenges, the project HIVEOPOLIS aims to augment honeybee colonies with technologies - internet, databases, satellite data and robotic actuators - that would otherwise not be available to them. As part of this project, we developed a mobile application to support the interaction of the hive with the surrounding ecosystem, i.e., to influence the bees in their interaction with the outside world through technology by means of an extended environment map. The application could also help connecting and engaging different social groups. By displaying various values - such as environmental and weather factors - beekeepers can plan an optimal placement of the hive or change it if necessary. Environmentalists can provide beekeepers with information, such as where to find plants of interest to beekeepers' bees or where wild bees are located. Managed hives should not be placed in protected areas where there is a risk of wild bees being displaced or attacked. The prototype was tested and evaluated by beekeepers and non beekeepers in two surveys: first was an online survey with a video of the app and a subsequent questionnaire; the second was conducted hands-on with a group of beekeepers. Results of both questionnaires show that the application can help beekeepers and illuminate most desirable use cases, like weather forecast, plant recognition and a beekeeping journal. We will present the application, discuss possible scenarios for usage and discuss the result of the user studies.

**Keywords:** hiveopolis, beekeeping, mobile app

<sup>#</sup>This project is funded by the Horizon 2020 FET Programme of the European Union under grant agreement No. 824069.

## Isolation of Extremophilic Yeasts Associated With Native Bees For Application In Biotechnology And Space Exploration

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- <sup>4</sup> Departamento de Química Fundamental - Instituto de Química (IQ-USP).
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### Abstract:

Native bees are the most efficient pollinators of tropical and subtropical habitats on the planet, being responsible for the maintenance and preservation of the various biomes that they have been found. As important as these bees are the microorganisms that are related to the preservation of food in the hive, microbiological protection against pathogenic organisms and even the maturation and life cycle of these pollinators. Among these microorganisms, fermentative yeasts stand out, which during the fermentation process increase the nutritive value of pollen, in addition to enabling the preservation of food stored by bees. Since honey and the internal environment of the colonies are considered extreme, due to the presence of high osmolarity, low humidity, microbicidal agents and low pH, it is assumed the possibility of finding extremophilic yeasts between the microbiome associated with native bees. Extremophiles, especially yeasts, have impacted biotechnology, becoming an inevitable instrument for modern science, in addition to being fundamental for the development of new drugs, improvement of food, beverages and fuel. This amplifies the potential of these organisms as a biotechnological tool on Earth and space exploration. In this context, yeasts were isolated from samples of different components of native bee hives, through specific protocols. Among the samples with the highest number of yeasts, propolis stands out, known for its antimicrobial potential. Yeasts showed resistance to high doses of UV-C radiation, characterizing these organisms as possible extremophiles, demonstrating the biotechnological potential of these yeasts for application in space exploration.

**Keywords:** yeasts, native bees, astrobiology.

# This project is financially supported by the Office of Provost of Culture and Extension of University of São Paulo.



## Anticancer and antiviral potential of propolis originating from Canada and Georgia

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### Abstract:

To evaluate the anticancer potential of extracts obtained from propolis originating from Canada (PC) and Georgia (PG), their cytotoxicity towards two selected cancer cell lines, namely cervical adenocarcinoma (HeLa) and colon cancer (RKO), was tested and compared with the results obtained for normal kidney fibroblasts (VERO). The antiviral activity was tested against the Human Herpesvirus type 1 (Herpes Simplex Virus 1, HHV-1, HSV-1) cultured in VERO cells, using cytopathic effect (CPE) inhibition assay, virus titer reduction and viral load decrease.

The cytotoxicity was assessed using a microculture tetrazolium assay (MTT) and revealed that both propolis extracts showed significant anticancer selectivity. However, the selectivity indexes (SI) towards HeLa and RKO were markedly higher in the case of PG (7.78 and 5.04, respectively) than PC (2.11 and 2.03, respectively). The CC<sub>50</sub> values obtained for PG towards cancer cell lines were below 20 µg/mL, which points to significant cytotoxicity.

Propolis extracts didn't inhibit the HHV-1 induced CPE formation in VERO cells. However, CPE appeared to be less advanced in the samples treated with the highest tested concentrations of propolis extracts. Subsequently, the infectious titer and viral load of HHV-1 in collected samples were evaluated. It was found that PC (80 µg/mL) and PG (30 µg/mL) managed to decrease the virus infectious titer by 1.35 and 1.09 log (mean ΔlogCCID<sub>50</sub>), respectively. However, since the tested sample should reduce the infectious titer by at least 3 log to show significant antiviral potential, propolis extracts cannot be regarded as such. The Real-Time PCR analysis was used to evaluate the influence of tested extracts on the HHV-1 viral load and revealed that the highest inhibition of viral load (1.02 log) was observed for the PC in the concentration of 80 µg/mL.

To conclude, propolis collected in Georgia exerts significant anticancer activity and selectivity towards colon and cervical cancers. Propolis extracts didn't significantly inhibit the HHV-1 replication in the virus-infected VERO cells.

**Keywords:** anticancer, antiviral, propolis, human herpesvirus

## The Biological Value of Honey from Natural Park of Montesinho

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### Abstract:

The Natural Park of Montesinho (NPM) is a big mountain/rural area located in the far northeast of Portugal. Due to the great geologic and climatic variability, an enormous and rich diversity of vegetation can be found, making the NPM a high economic and social important resource in the region, with apiculture activity playing an essential role. Honey produced in the NPM is considered as Protected Denominated Origin (PDO) honey that contributes for a strategic activity for integrated and economically sustainable use of the NPM. The attributes related with an PDO honey are widely looked for consumer due to its relation with high quality and specific biological properties. However, several external factors, such as climatic changes allied to the producer's efforts to combat pests and reduced crop yields, could contribute to the decrease of these properties, without affecting the organoleptic properties. This study was performed aiming to answer the question “How to add value to the NPM's honey and valorize the bioeconomy of NPM region?”.

For that, quality parameters and biological properties were assessed in a set of honey samples collected in different beekeeping zones of the NPM. Apiaries were selected in order to cover different environmental situations. Firstly, samples were characterized regarding total polyphenol content (TPC), total flavonoid content (TFC) and antioxidant/antiradical activities (FRAP, ABTS and DPPH•). The qualitative evaluation was performed by diastase activity(DA), hydroxymethylfurfural(HMF), pH and free acidity, moisture, conductivity and ash content. The results revealed that the TPC ranged between 1.26 and 2.30 mg(GAE)/g honey, while the TFC was between 0.17 and 0.33 mg(EE)/g honey. The antioxidant activity was also high, varying between 0.26 and 1.19% for the FRAP assay. Regarding the DA and HMF content, the results agree with legislation. The pH ranged from 3.92 and 5.21, while the free acidity minimum and maximum were 30.0 and 49.0 mmol acid/kg honey. Regarding conductivity, the minimum result was 3.89mS and the maximum 15.09mS. The results obtained are in agreement with literature results of Portuguese honeys. However, further studies are needed to monitor the studied properties in the selected apiaries and relate them with external factors modifications.

**Keywords:** Honey, Honey quality parameters, Biological properties, Natural Park of Montesinho.

**Acknowledgments:** This work was funded by the project MTS/SAS/0077/2020 - Honey+ - New reasons to care honey from the Natural Park of Montesinho: A bioindicator of environmental quality & its therapeutic potential. Manuela M. Moreira (CEECIND/02702/2017) and Francisca Rodrigues (CEECIND/01886/2020) are thankful for their contracts financed by FCT/MCTES—CEEC Individual Program Contract.

## **Comb age significantly influences cell measurements and worker body size**

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### **Abstract:**

The study aimed to determine the changes in comb cell measurements and worker body sizes in relation to comb age. For this purpose, the cell measurements of combs at age zero (wax foundation), 1, 2, 3, 4, 5, and 6 years and the body size of workers reared in them were estimated. The weight of the comb, height of the cell base, and the weight of accumulated substances in the cell significantly increased with time. Comb age had negative effects on the cell diameter, cell depth, cell volume, cell honey or pollen capacity, and newly emerged worker body weight. Significant negative correlations were observed between the accumulated substances in a cell and the cell diameter, cell depth, and cell size, while significant positive correlations were observed among the cell volume, cell diameter, cell depth, cell honey capacity, cell pollen capacity, and worker body weight. It can be concluded that the dimensions of the comb cells and worker body size changed with the age of the comb. The obtained results recommend beekeepers to replace combs aged more than 3 years with a new comb to allow large workers to gather more nectar and pollen, rear a larger brood, and store more honey.

**Keywords:** Honey bees, comb, honey, pollen, worker,

## Influence of nosemosis and beekeeping conditions on selected parameters of the immune system in a honeybee

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### Abstract:

The immune processes in honeybees are not fully understood due to the complex social structure of the colony that honeybees form. It is assumed that the immunity of a single bee is reduced compared to other insects due to the hygienic behavior of honeybee colonies developed in the course of evolution. The aim of our research was to check how nosemosis and keeping conditions, i.e. honeybees kept in an apiary and in the laboratory, affect selected parameters of the immune system in honeybees, i.e. the level of expression of genes encoding immune peptides (defensin, hymenoptaecin) and the levels of phenoloxidase and lysozyme. The honeybees were collected in 3 time variants (1-day, 10-day and 19-day) from the laboratory and the apiary in May 2021. Hemolymph was used to determine the level of phenoloxidase activity by colorimetric method using L-DOPA, and to check lysozyme activity. The expression level of the studied genes was carried out using the Real-time PCR method. Due to the high mortality of bees, 19-day-old infected honeybees kept in the laboratory were not collected. Based on the obtained results, it was found that the level of phenoloxidase and lysozyme was higher in *Nosema ceranae* infected honeybees compared to uninfected bees. In addition, it was observed that the activity of lysozyme and phenoloxidase was higher in the 10-day-old honeybees kept in the laboratory than in the honeybees kept in the apiary. The highest level of expression of genes encoding defense peptides: defensin and hymenoptaecin was observed in 10-day-old infected honeybees kept in laboratory. The obtained results allowed for a better understanding of the mechanisms of the immune system in response to pathogen infection as well as depending on the keeping conditions. The study was carried out as part of a project financed by the Institute of Biological Sciences of the Maria Curie-Skłodowska University in Lublin, Poland. The part of results of the experiments was the basis in obtaining a grant no. UMO-2021/41/N/NZ6/00735 for the National Science Center (Cracow, Poland) in 2021.

**Keywords:** honeybee, nosemosis, gene expression, phenoloxidase, lysozyme

## Pollen Morphology of *Rhynchosorys kurdica* Nab. (Orobanchaceae) in Turkey

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### Abstract:

In this study, pollen samples belonging to *Rhynchosorys kurdica* Nab. taxon were investigated for the first time and pollen morphology was determined. The Orobanchaceae family, in which the genus *Rhynchosorys* is found, is a family of mostly parasitic plants of the Lamiales order, with about 90 genera and more than 2000 species. *Rhynchosorys* a perennial herbaceous genus, generally lives in humid climates and soils with high groundwater. 5 species (6 taxa) of the genus represented by 8 accepted species in the world show natural distribution in Turkey. The pollen morphology of the *Rhynchosorys kurdica* taxon included in the study was examined with light microscopy and Scanning Electron Microscopy. Pollens obtained from the samples in light microscopy examinations were prepared according to the method of Wodehouse. Pollens of the taxon are small pollens, tricolpat, isopolar, equatorial axis (13.02-15.15)  $\mu\text{m}$ , polar axis (10.23-16.15)  $\mu\text{m}$ . Exine is 0.92-1.23  $\mu\text{m}$  thick. Pollen shape is prolate-spheroidal. Pollen ornamentation was observed as dense granulate. The granulate ornamentation along the colpus is sparse and retipilate at the margins of the colpus. The colpus is wide at the equator and rather narrow at the poles. *Rhynchosorys kurdica* pollen yielded consistent systematic results within the genus when compared with other studies.

**Keywords:** *Rhynchosorys*, pollen, morphology, Turkey



## **DPPH Radical Scavenging Activity Of Drone Larvae (Apilarnil)**

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### **Abstract:**

Antioxidants are very effective in preventing many diseases. Antioxidants are generally divided into two classes as artificial antioxidants and natural antioxidants. For this reason, intensive studies are carried out to determine natural antioxidants. This need is increasing due to the harmful effects of artificial antioxidants. Male bees are not very desirable in beehives, they just need to be in sufficient numbers for fertilization. Therefore, drone larvae are not needed much. In some studies, it was determined that apilarnil showed biological activity.

DPPH· (1,1-Diphenyl 2-picryl hydrazil) radical scavenging method is one of the important antioxidant activity methods. This method is based on measuring the free radical scavenging activity of DPPH, a stable organic nitrogen radical of antioxidant compounds. In this study, antioxidant activity of drone larvae was determined by DPPH radical scavenging method. The results were found by comparing the standard antioxidant BHA (butylated hydroxy anisole), BHT (butylated hydroxy toluene), trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid),  $\alpha$ -tocopherol, ascorbic acid. As a result of DPPH radical scavenging of apilarnil, the IC<sub>50</sub> concentration was found to be 40,764. It was determined that apilarnil showed lower activity than ascorbic acid and higher activity than the other four standards. Thus, it is thought that drone larvae can be used as a natural source of antioxidants. Thus, it is thought that drone larvae can be used as a natural source of.

**Keywords:** Drone larvae, Apilarnil, DPPH, Antioxidants



## Thermal stability of phenolic compounds in breads enriched with bee pollen and bee bread

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### Abstract:

Over the last years, the rising concern on individual health lead people to search for changes in their way of live focusing attention in their diet, with a significant effect on body health, and increasing the interest in healthy food products. Bee pollen and bee bread are beehive products rich in carbohydrates, crude fibers, proteins and lipids, and, in minor concentrations, phenolic compounds, which are associated with several nutritional and biological benefits. This study investigated the addition of bee pollen and bee bread to conventional breads and the thermal stability of the bioactive compounds incorporated in this daily product. For that, functionalized breads were supplemented with raw bee pollen and bee bread in three different concentrations (1%, 3%, 5%) and the phenolic compounds were evaluated globally through spectrophotometry (total phenolics and total flavonoids) and its profile quantified by LC/DAD/ESI-MS<sup>n</sup>. Additionally, the antioxidant activity was determined through DPPH and reducing power antioxidant assays. Generally, enriched breads were found to contain higher bioactive compounds. For the total phenolics and total flavonoids there is a noticeable increase compared to conventional bread used as control. For antioxidant activity, the control group demonstrated  $0.81 \pm 0.0$  mg/mL which was the lowest antioxidant activity, while enriched bread samples increased the activity (lower values) to  $0.31 \pm 0.0$  mg/mL (5% enriched with bee bread) and  $0.33 \pm 0.0$  mg/mL (5% enriched with bee pollen). For the phenolic compounds, raw bee pollen contained quercetin-*O*-diglucoside, quercetin-3-*O*-glucoside and quercetin-3-*O*-rhamnoside, while enriched bread samples with bee pollen, only quercetin-3-*O*-rhamnoside was detected, which can be due to the high temperatures applied during cooking process.

**Keywords:** enriched bread, functional food, bee pollen, bee bread, phytochemicals

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Oral presentation

## Determination of Chemical Content of Bingöl Royal Jelly by LC-MS/MS<sup>#</sup>

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### Abstract:

Royal jelly is a very valuable bee product that is of vital importance for the queen bee and young larvae. Royal jelly is a special product secreted from the upper jaw and throat glands of five- to fifteen-day-old worker bees in order to feed the queen bee and young larvae. Royal jelly is produced in the hypopharyngeal and mandibular salivary glands of young worker bees as a result of partial digestion of pollen and nectar. Bioactive components in royal jelly; components such as vitamins, proteins, lipids, carbohydrates, free amino acids and 10-hydroxy-trans-2-decanoic acid (10-HDA). Royal jelly, which has a rich chemical content, is highly nutritious. Thus, it is important to determine the chemical content. LC-MS/MS technique is one of the advanced technologies that determine the chemical content in organic samples qualitatively and quantitatively. It gives safe results at very low concentrations. Hence, the chemical content of royal jelly was determined by this technique. Quantitative analysis of 53 different chemicals was made with the method validated method. As a consequence, in royal jelly (mg analyte/g sample) Quinic acid (6.573), Fumaric acid (3,927), Aconitic acid (0.043), Gallic acid (0.017), Protocatechuic acid (0.043), Chlorogenic acid (0.019), 4-OH Benzoic acid (2.91), Caffeic acid (0.023), p-Coumaric acid (0.332), Genistein (0.004), Quercetin (0.037), Luteolin (0.005), Apigenin (0.003), Acacetin (0.011), Chrysin (0.012) ) was found. It was determined that Bingöl royal jelly was moderately rich in phenolic and flavonoid compounds.

**Keywords:** Royal jelly, LC-MS/MS, Bingöl

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**Oral Presentation**

## Biosynthesis of Honey-mediated ZnO Nanoparticles and Investigation of Their *In Vitro* Cytotoxicity for Dermocosmetic Applications

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### Abstract:

Honey has been used since ancient times because of its antibacterial, antioxidant, anti-inflammatory and wound-healing properties as well as being a source of nutrients. Zinc oxide nanoparticles (ZnO-NPs), one of the most important metal oxide nanoparticles, are widely used in personal care products such as cosmetics and sunscreen due to their superior antibacterial, antimicrobial and excellent UV blocking properties. The aim of this study is to synthesize biocompatible and therapeutic ZnO nanoparticles coated with bioactive molecules in honey. The biosynthesized Honey/ZnO-NPs are characterized by UV-Vis spectroscopy, field emission scanning electron microscopy (FESEM), zeta potential and zeta size measurements. The obtained Honey/ZnO-NPs give a single peak in the 300–400 nm range due to surface plasmon resonance (SPR). The average zeta potential and hydrodynamic size of Honey/ZnO-NPs is found to be  $-39.7 \pm -5.2$  mV and  $26.7 \pm 2.3$  nm, respectively. Therefore, zeta measurements revealed that the synthesized particles were stable in aqueous solution. The functional groups analysis of the nanoparticles was investigated by a fourier transform infrared spectroscopy (FTIR) and results showed that the ZnO-NPs were coated and stabilized by bioactive compounds from the Honey. The thermal properties of Honey/ZnO nanoparticles have been analyzed using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). Also, the cytotoxic properties of Honey/ZnO nanoparticles were investigated by MTT assay according to ISO 10993-5 standard. MTT results revealed that nanoparticles at certain concentrations exhibited good biocompatibility for L929 fibroblast cells. Results reveal that Honey/ZnO-NPs synthesized in this study have a promising potential for biomedical and dermocosmetic applications.

**Keywords:** dermocosmetic, honey, *in vitro* cytotoxicity, zinc nanoparticles, wound healing.



## Identification of the entomological origin of European honey by high resolution melting analysis of a COI mini-barcode

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### Abstract:

Honey is widely consumed worldwide and highly appreciated for its organoleptic, nutritional and health properties. Honey is also considered one of the foods most prone to be adulterated, either by admixing of honey with lower quality, by sugars' addition, or by origin mislabelling, among other possible frauds. Recently, great attention has been paid to the development of techniques for authenticating honey through its entomological origin, which is also related to its geographical origin, since bees carrying mitochondrial DNA (mtDNA) from distinct ancestries can be found throughout Europe. Moreover, consumers are increasingly concerned with ethical and environmental issues, paying attention to issues such as the protection of biodiversity and the mode of production. For these reasons, the development of methodologies to authenticate the entomological origin of honey contributes not only to assure consumers rights and avoid unfair competition by the identification of frauds, but also to promote and valorise autochthonous honeybee subspecies.

In this work, a one-step approach based on HRM analysis of a 150 bp fragment of the COI gene was developed to establish the entomological origin of honey by discriminating A, M and C mtDNA lineages and differentiating a SNP associated with a high frequency of C1 or C2 mitotypes in the Italian honey bee *A. m. ligustica* and the Carniolan honey bee *A. m. carnica*. The method showed high analytical performance and was able to successfully identify the entomological origin of honeys of known origin obtained from research apiaries/beekeepers. Therefore, it was applied to 44 commercial honeys from different countries. It confirmed the entomological authenticity of French PDO honeys that should be produced by the Corse ecotype *A. m. mellifera*. For the remaining honeys, the results were also in good agreement with the declared geographical origin. This method is also capable of indicating the mixture of honeys produced by honey bees of different lineages, although not allowing to identify the lineages or mitotypes in the mixture. This was the case of three honeys from Slovenia that did not cluster with C2 mitotype *A. m. carnica* as expected, suggesting the mixture of honeys produced by honeybees of different mitotypes.

**Keywords:** Honey, authenticity, HRM analysis, *Apis mellifera* subspecies.

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Oral Presentation

**SHOW ME THE HONEY: MELITOURISM POTENTIAL OF BALI, INDONESIA AND BICOL, PHILIPPINES**

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**Abstract**

Melitourism, a special entomotourism featuring stingless bees as an agritourism attraction, remains less popular and underrated in Southeast Asian countries despite its economic benefits. This study assessed the melitourism potential of Bali, Indonesia, and Bicol, Philippines, through a qualitative approach using various indicators such as financial capital, market, social capital, environment, technology, policy, human capital, culture, and farm tourism as foundations for a melitourism social enterprise. Identification of these indicators was based on a literature review, and these became the basis of an interview guide designed for stingless bee operators. Findings showed that meliponiculture in Bali and Bicol offers a lot of promise as a social enterprise and tourism development. The stingless beekeeping industry dominated the aspects of environmental, technological, market, and social capital. Farmer groups provide a support system resulting in strong social capital. Compared to Bali, Bicol is quite advanced in agritourism integration in stingless bee farms. Melitourism is a good means of engaging the public to contribute towards a conducive entrepreneurial ecosystem for stingless beekeeping. Incorporating the tourism aspect in meliponiculture is a good economic diversification strategy that would enhance farm productivity and encourage farmers to engage in ethical practices that help maintain a healthy stingless bee population both on farms and in the wild. However, to hasten farmer engagement and melitourism development, local and national governments must strengthen their policy infrastructure and support this industry.

**Keywords:** melitourism, entomotourism, stingless beekeeping, apitourism, meliponiculture, stingless bees

## The New Animal Health Law Of the EU And Its Implications Of Honey Bees And Bumblebees

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### **Abstract:**

In order to ensure high standards of animal and public health in the European Union and the rational development of agriculture and animal farming, and to increase productivity, animal health rules should be laid down at Union level. The current European Union legislation on animal health consists of a series of linked and interrelated basic acts that lay down rules on animal health applying to intra-Union trade, entry into the Union, health certificate, disease notification, eradication and surveillance, veterinary controls, and financial support in relation to different animal species, but an overarching legal framework, laying down harmonized principles across the sector, is missing. The regulation 2016/429 (Animal Health Law) proposes the adoption of a single and simplified regulatory framework for animal health seeking convergence with international standards while ensuring a firm commitment to high standards of animal health. The aim of this regulation is to implement the commitments and visions provided for in the Animal Health Strategy, including the 'One Health' principle, and to consolidate the legal framework for a common Union animal health policy through a single, simplified and flexible regulatory framework for animal health. The new Animal Health Law of the EU and the correlated regulations available so far with implications for honey bees and bumblebees health and trade (diseases, surveillance, eradication, disease-free status, registration, establishment, certification, intra-Union trade, import) have been extrapolated, commented on and condensed for an easy and practical consultation by competent authorities, veterinarians, beekeepers and operators of the sector aiming at facilitating their application and compliance.

**Keywords:** bumblebee, certificate, disease, honey bee, trade.

## Physico-chemical characterization of *Quercus pyrenaica* honeydew honey

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### Abstract:

Honey is one of the oldest products used by humans. It is a natural mixture prepared by honeybees either from plant nectar, classified as nectar honey, or from exudates of living parts of plants or excretions of sucking insects living parts of plants, classified, in this case as honeydew honey. Both type of honeys has distinct physico-chemical and biochemical properties, greatly depending on their botanical source. Honeydew honeys are generally darker than nectar honeys and usually found with higher values in parameters such as enzymatic content, conductivity, phenolic compounds or bioactivity. The aim of this study was to characterize the production of honeydew honey with origin in *Quercus pyrenaica*, a specific oak forest highly abundant in the northeast region of Portugal. For that, 42 honey samples were collected in different apiaries of *Apis mellifera iberiensis*, located in Montesinho Natural Park, Bragança, Portugal, during September of 2021. The quality of honey was evaluated according to standard parameters such as: color, humidity, acidity, electrical conductivity, ash content, diastase index, HMF and proline. The results of the physio-chemical parameters analysis show that the color ranged from 130 to 150 mm pfund which corresponds to a dark amber color classification, while the moisture ranged from 14.4 to 18.5 %. The electrical conductivity varied from 0.93 to 1.4 mS.cm<sup>-1</sup> with ash levels between 0,45 to 0,74%. The amount of HMF range from 0 to 24 mg.kg<sup>-1</sup> while the diastase index varied from 9 to 33 DN. Finally, the proline content ranged from 0,90 to 8,1 mg.g<sup>-1</sup>.

**Keywords:** Honeydew honey, black oak, physico-chemical, *Apis mellifera*

<sup>#</sup>The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support by national funds FCT/MCTES to CIMO (UIDB/00690/2020), for the support through the project ACORNDEW (MTS/SAS/0099/2020) and through the institutional scientific employment program contract with Soraia I. Falcão. Thanks also to the European Regional Development Fund for project Norte-01-0145-FEDER-000042: “GreenHealth”



## Bee Microbiome: Insight into Host- Microbe Evolution and Bee Health

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### **Abstract:**

Bees play an important role as cornerstones for natural and agricultural ecosystems all over the world, Bees range from solitary wild species to highly social and managed species like honey bees. Honey has been in short supply recently due to the overuse of pesticides. Pesticide exposure has been linked to honeybees and wild bees increasing parasite burdens, habitat destruction, and low nutrition that has led to decrease in biodiversity worldwide. All animals including bees are associated with diverse community of microbes. Microorganisms associated with bees include a wide range of viruses, bacteria, and parasites some of which are significant bee pathogens whereas many bacteria contribute to the gut microbiota. The gut microbiota has a lot of influence on its hosts, but researching these interactions in humans is difficult. Honey bee gut communities are similar to mammalian gut communities. Both the microbiotas are predominantly made up of host-adapted facultative anaerobes and microaerophiles. The bee gut community is dominated by nine members of bacterial species clusters that are spread through social interaction between individuals. Recent developments, including the discovery of extensive strain-level variation, evidence of protective and nutritional functions, and reports of eco-physiological or disease-associated community perturbations, have drawn attention to the microbiota's role in bee health, as well as its potential as a model for studying gut symbiont ecology and evolution.

**Keywords:** Bee microbiome, Host-Microbe interaction, gut microflora

Oral Presentation

## Comparison of *Tetragona clavipes* (Apidae, Meliponini) Honey From Different Regions Of Brazil

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### Abstract:

The stingless bees of the species *Tetragona clavipes* (Hymenoptera: Apoidea), are widely distributed in Brazil, and are popularly known by its indigenous name “Borá”. It is a defensive bee that produces honey that is well appreciated by local communities; however, the production and commercialization this honey is compromised by the lack of adequate legislation that fits its properties without changing the original characteristics. Thus, the objective of this work was to analyze the honey of the *Tetragona clavipes* bee regarding its physicochemical properties in two regions of Brazil and to verify the characteristics of the honey of the same bee species in different regions, generating data for the development an adequate legislation that promote of the rational rearing of this bee. For this, a sample of honey was collected in the city of Seara (27°08’56’’S 52°18’39’’O), in the state of Santa Catarina (SC) and another sample was collected in the city of Atibaia (23°07’01’’S 46°33’’O), in the state of São Paulo (SP), both in the same period in the year 2021. These samples were analyzed regarding their physicochemical properties, which resulted in a honey with pH 4.29, acidity of 35.2 mEq/Kg, humidity 24.2, 5-hydroxymethylfurfural content < 0.4 and reducing sugars 46.65 g/100g for honey from Santa Catarina and pH 3.53, acidity of 181.3 mEq/Kg, humidity >25, 5-hydroxymethylfurfural content < 0.4 and reducing sugars 72.81 g/100g for honey from São Paulo. In addition, microbiological analyzes were performed to detect *Salmonella spp.* and Thermotolerant Coliforms, with negative results for contamination in both samples. We conclude that honey of the same species, produced in different regions, has very different properties, a more pronounced flavor and differentiated by high acidity, high humidity and low amount of reducing sugars, being possibly influenced by the climate and type of vegetation of the place, reflecting the identity the location, not just the species. In addition, a more comprehensive legislation that encompasses the variations of regions for the same species is necessary for the commercialization of this honey to become viable. Ideally, a new legislation should be based on data like the ones collected in this work.

**Keywords:** physicochemical properties; legislation; honey; stingless bees.

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## Bees and bee products-bio-indicators of environmental pollution

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### Abstract

Environmental pollution is one of the world's concerns, showing adverse biological effects on natural resources (plants, living organisms, ecosystems) and population through direct or indirect toxic actions through contamination of the trophic chain. The environment is constantly transforming due to natural (e.g., volcanic activities, forest fires) and anthropogenic (e.g., burning and combustion of fossil fuels, refining operations, waste incineration, intensive agricultural practices and degradation of uncontrolled waste) processes. Environmental pollution refers to water, atmosphere and soil pollution with mixture of toxicological compounds such as residues of pesticides and other toxic industrial compounds, petrochemicals, microplastics, heavy metals, pathogenic bacteria and yeasts. European Union recommends monitoring environmental pollution to assess regional variability of pollution on ecosystems and propose two methods, via active samplers or passive samplers (bio-monitors) represented by living organisms (plants, insects, etc.). Honey bees and their products (pollen, wax, honey, propolis) are regarded as a good bio-markers, reflecting environmental pollution because they are capable of capturing and accumulating contaminants, reside in diverse habitats and, within an area of several square kilometers centered on the hive to be monitored. Honey bees reveal the chemical impairment of the environment they live through: (i) high mortality (in the case of pesticides) or (ii) presence of different residues (heavy metals, PAH, pesticides) within their bodies or in bee products which can be detected by means of suitable laboratory analyses, such as chromatographic and mass spectrometric methods for organic contaminants and ICP-MS, ICP-AES and AAS for heavy metals detection. The determination of organic and inorganic contaminants in bees and bee products represent an efficient way to conduct ecological research, mostly in agricultural landscape, but also in the urban reference as well as in the highly polluted industrial regions.

**Keywords:** bio-indicators, bee-products, environmental pollution.

### Acknowledgements

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## Stingless bee honey: Potential tool in the floral induction of Mango

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### Abstract:

The alternatives to advance or induce flowering in fruit crops are varied, having to a greater extent the use of chemical compounds such as salts, hormones, phenolic compounds, among others. But the use of beehive products as an alternative in flower induction is little studied. The objective of this work is to evaluate the honeys of *Scaptotrigona mexicana*, *Melipona beecheii* and *Melipona solani* in the floral induction of mango crops (*Mangifera indica* L.). The study was carried out at the San Juan ranch, located in the municipality of Tapachula, Chiapas. 11 treatments consisting of 5 trees each 15 years old, separated by a row of trees each, were established. The total number of rows was 11. The application of the honey was in solutions of 0.25, 0.5 and 0.75% v/v, before flowering appeared with a manual backpack pump with a capacity of 20 L. They were used as a positive control, Nitrate of Potassium and negative, water. Shoots were evaluated in the 4 cardinal points of each evaluated tree and their growth, until the generation of well-formed flowers. The honey solutions that induced more flower buds were 0.5 and 0.75% v/v, corresponding to the honeys of *M. beecheii*. The first flower buds were observed a week after application in the treatments that received *S. mexicana* honey. In all the honey treatments, a well-formed flower about to open was found in the evaluated panicles, before the control treatment. It is concluded that honey solutions are viable at the field level to be used in floral induction in mango cultivation and that if more than one flowering is required, it is essential to use *M. Beecheii* honey.

**Keywords:** Meliponinos, honey, alternative, flowering, mango.

Oral Presentation

**Effects of Probiotics on Honeybee Diseases and Bee Products**

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Corresponding author:**Abstract:**

Probiotics are beneficial friendly bacteria found naturally and in a certain ratio in the digestive system. Living microorganisms, which provide microfloral balance and coordinate the regular functioning of the digestive system, provide improvements in the modulation and regulation of metabolic activities by acting on the immune system with their enzymatic and metabolic effects. As a result of these properties of probiotics, they are effective in preventing the colonization of sugar-rich digestive tracts of honey bees and the spread and development of acid-sensitive pathogenic bacteria. These specific microorganisms, have strong mechanisms of action in the immune system of the bee and become effective in the fight against various pathogenic microbial threats by entering into a fast and competitive struggle. Thus, bee colonies that are healthy and have a strong immune system will devote most of their energy to the reproduction of the colony population and the production of bee products. Especially adult honey bees have very rich microflora. Important point. While there are studies showing that the use of probiotics is beneficial, there are also studies reporting that incorrect probiotic application has harmful effects on bee health. For this reason, the types of bacteria to be administered probiotics to bees are very important. Microbiota controls of honey bees should be done in a planned manner, and a traceable honey bee microbiota and immune system map should be created. These data banks should be stored and used for health problems and scans with digital access when necessary.

**Keywords:** probiotics, effect, honeybee, diseases, product**Oral Presentation**



## Establishing Standards to Strengthen the International Trade of Bee Products

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### **Abstract:**

The international trade on bee products is mostly know for honey, nevertheless, the global demand for natural products is boosting the production and international transaction of other bee products, such as bee pollen, propolis and royal jelly. While for honey the market stands on the quality requirements defined on CODEX, the absent of international recognized quality specifications for the generality of the other bee products is a critical point for the traders and consumers, favoring fraud. Having that in mind, the international community set up, under the International Standard organization, ISO, a specific technical subcommittee ISO/TC 34/SC 19, solely engaged in the establishment of international standards for bee products. Following the approval of a norm for royal jelly specifications back in 2016, ISO 12824:2016, the international experts are developing specific standards for bee pollen, propolis, royal jelly management, but also for honey. This communication is an overview of the work under way in the different working groups, and will focus on the goals there are behind each standard, their scope and program, definitions, quality and production requirements, and other specifications related with packaging, labeling transport and storage. Although it is not possible to cover all the diversity of bee products, these standards represent a significant step forward to enhance the trade confidence and raise the quality on bee products within the global market. Furthermore, future work within the settled working groups, will enable the submission of new projects and additional standards on bee products.

**Keywords:** International standards; honey; bee pollen; propolis; royal jelly.

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**Oral Presentation**



## First Record of Chemical Content, Antimicrobial and Antioxidant Activity of Queen Bee and Drone Larvae of Yıǧılca Honey Bee Ecotype

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### Abstract:

Queen bee and drone larvae are a little-known bee product used in folk medicine to treat various health problems. They are very nutritious milky substance with high content of nutrients: proteins, lipids, fatty acids, carbohydrates, vitamins and minerals. Although their rich protein content, there are limited studies on these bee products. In this study, queen bee and drone larvae of Yıǧılca honey bee ecotype samples were analyzed for their antimicrobial properties, antioxidant activity, total phenolic content (TPC), and total protein amount. First, the activity of the samples against 19 different microorganisms was determined by the agar well diffusion method, then their zones were measured. The microdilution method was used to determine the minimum inhibitory concentration (MIC) for the antimicrobial activity tests. HPLC analysis was used to determine total phenolic content in the queen bee and drone larvae samples. Antioxidant activity was measured by the FRAP and DPPH methods. Phenolic compounds analysis showed that the amounts of queen bee larvae (14.869 mg GAE/g) higher than drone bee larvae (13.179 mg GAE/g). Stronger antioxidant activity was obtained from drone bee larvae. Total protein amount of the queen bee larvae (83.328 mgBSA Equivalent/g) was significantly higher in drone larvae (56.399 mgBSA Equivalent/g). No antibacterial and antifungal activities were noticed in the present study. As a result, it was concluded that queen bee and drone larvae of Yıǧılca honey bee ecotype can be used as a functional food because of their high amount of protein and strong antioxidant properties.

**Keywords:** Queen bee larvae, drone larvae, antioxidant, antimicrobial, Yıǧılca ecotype

Oral Presentation

**Characterization of 5 pollen samples from Castelo Branco**

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**Abstract**

Pollen is a natural food produced by honeybees (*Apis mellifera*), with valuable nutritional and medicinal characteristics. This work aimed to characterize the nutritional value of 5 pollen samples from different regions of Castelo Branco. Moisture content, aw, pH, ash, fibers, protein and total lipids, and carbohydrates (fructose and glucose) content were determined according to official methods. The fructose and glucose were determined by Performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD – ICS3000™ da Dionex®). The fatty acid profile was also analyzed by gas chromatography with flame ionization detector (GC SSL-FID). The other analytical parameters were evaluated by the official methods. The different pollen samples were classified as multifloral, obtaining one with 28 different floral species identified. The most abundant taxa found in the pollen samples was *Cistus ladanifer*, *Rubus* spp., *Sesamoides* spp., *Erica* spp. and *Salix* spp.. Thus, the different pollen characteristics associated with the different pollen collection regions influenced the nutritional parameters obtained. The variation observed was: humidity between 5.77 and 15.62%; aw between 0.36 and 0.69; pH between 5.15 and 5.80; protein between 14.06 and 20.44%; lipids 2.70 and 4.44%; fibers between 5.88 and 7.30%; carbohydrates between 66.26 and 71.16%; ash between 1.98 and 2.36%; glucose between 17.95 and 21.12 g/100 g; fructose between 13.50 and 19.14 g/100 g; energy between 339.47 and 388.88 kcal/100 g. The chemical composition of bee pollen depends on the flower nectar collected by bees in different regions. Our results suggest that the fructose content could be a good indicator of honeys' origin as well as the lipid profile.

**Keywords:** Pollen; Nutritional characteristics; fructose and glucose; fatty acid profile.

**Oral Presentation**





## Review on Honey Incorporated Polymer Hydrogels for Wound Healing Applications

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### **Abstract:**

Wound repair is an important biological process for regeneration of harmed tissue. Wound dressing materials have a significant role in the healing process. There is an increasing interest on the improvement and use of polymer based bioactive hydrogels for wound healing applications.

Natural bioactive agents such as curcumin, carrageenan, essential oils, honey, tannin, quercetin, vitamins, etc. can show significant wound healing process in wounds, diabetic ulcers and burns. In addition, these compounds can promote wound repair due to their good biocompatibility and several bioactivities. Honey, is widely used in traditional medicine, plays an important role in the treatment of wounds and regeneration of tissues. Among the studied natural compounds honey is widely used in wound healing applications because of being rich in phytochemicals. Honey, along with good biocompatibility and antimicrobial effects, can help to heal wounds. It has broad-spectrum activity against a wide variety of microorganisms. Its high osmolarity, low pH, hydrogen peroxide production potential and high viscosity provide antibacterial activity, while the phytochemical components such as flavonoids and phenolic acids provide antioxidant activity. By this way, honey prevents cell damage and thus reduces the inflammatory response.

The application of honey in wound treatment has been firstly developed with the use of honey and a gelling agent; however, its high osmolarity that would lead to absorb the wound exudate causes the honey to leak out of the wound bed. Hydrogels are the well-characterized tissue-engineering platforms formed at ambient temperature through the cross-linking of a polymer solution. In the previous studies honey incorporated different polymer matrices including alginate, chitosan, gelatin and poly(vinyl alcohol) as well as the combination of these polymers have been examined for wound healing applications. This review primarily provides an overview of researches realized on the development of honey-incorporated polymer based wound dressings for the potential acute and chronic wound healing treatments.

**Keywords:** Bioactive dressings, honey, hydrogels, wound-care.

**Oral Presentation**



## Comprehensive Phytochemical Profile Of Ordu Province Propolis By LC-MS/MS

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### Abstract:

Propolis is the sticky, gummy product in brown tones that honey bees create by mixing pollen and plant secretions they collect with the enzymes in their bodies and the wax they produce. Bees use propolis for hive disinfection, repair and bonding of honeycombs, removal of cracks, adjustment of hive temperature, and removal of different living things entering the hive. Propolis has different chemical content and aromatic odors according to the plant flora. Since the plant flora is very important in determining this content, the phytochemical content has been emphasized in this study. For this reason, its phytochemical content is important and it is known that these components show biological activity in the treatment of many diseases. The determination of the phytochemical profile was made by LC-MS/MS, which is one of the most advanced techniques. The qualitative and quantitative results of 53 different phytochemicals by LC-MS/MS of propolis obtained from Ordu province were found as follows: At the highest concentration (mg analyte/g sample); caffeic acid (47,779), p-coumaric acid (66,454), acacetin (10,865), quercetin (8,673), naringenin (8.468), ferulic acid (7,052), chrysin (6,029), kaempferol (5,727), vanilic acid (5,675), quinic acid (5,517) compounds and a total of 33 components were found. The concentration of the other components found was found to be less than 5. It was determined that the phytochemical content was both rich and quantitatively high.

**Keywords:** Propolis, LC-MS/MS, Phytochemical

Oral Presentation



## *Varroa Destructor* Infestation in Honey Bees in Ankara Region, and Antioxidant Properties of Produced Honey

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### **Abstract:**

Turkey is at the forefront of the world in terms of the number of hives. One of the most obvious reasons for the low amount of honey production per hive in our country is that bees are heavily infested with a pathogen, *Varroa Destructor*. In this study, 100 honey samples collected from honey producers in the Ankara region were used. Honey hives were divided into 3 groups considering the rate of infestation of bees with *Varroa* destructive mite. Hives with more than 12% contamination (n=33), heavily infested hives (group one); Hives with contamination between 3-12% (n=34), moderately infested hives (second group); Hives with 1-3% contamination (n=33) were considered as mildly infested hives (third group). Total phenolic substance, total flavonoid content, DPPH, ABTS and FRAP levels of honey samples used in the study were measured using the relevant assay methods. Statistically significant differences were determined between the total phenolic content and DPPH values measured in honey samples collected from three groups of hives (p <0.001). It was determined that the total flavonoid levels in the honey sample obtained from the third group of hives were different from the total flavonoid levels determined in the honey samples collected from the other two groups of hive (p <0.001). ABTS and FRAP levels in the honey sample obtained from the first group of hive were found to be different from the ABTS and FRAP levels determined in the honey samples collected from the other two groups (p <0.001). As a result of the study, current evaluations were made by comparing the severity of *Varroa Destructor* infestation in honey hives in the Ankara region and the antioxidant levels of honey samples collected from these hives. It was thought that the measurement of total phenolic substance, total flavonoid content, DPPH, ABTS and FRAP levels in honey samples could be useful in the diagnosis of *Varroa Destructor* infestation and in the evaluation of its pathogenesis.

**Keywords:** Ankara region, Antioxidants, Honey, Total phenolic substance, Total flavonoid content, *Varroa Destructor*.

This study supported by Tubitak 2209 Scientific Activities Support Program. (Project no: 1919B012002568)

**Oral Presentation**



## Investigation of the presence of virus infections in honey bee colonies in Afyonkarahisar Province

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### Abstract:

Bee colonies around the world incur significant losses associated with environmental and management factors. Among the possible reasons for these losses are parasite mites and several bee viruses are shown. To research the causes underlying the gradually decrease in honey bee colonies in world, it is important to focus on the key diseases that negatively affect bee health.

Afyonkarahisar is located in the inner Aegean region, that have important vegetation for beekeeping. Considering the geographical distribution of beekeeping enterprises in this province, research was conducted in 12 apiaries. In this study, a total of 252 bees were collected from 36 colonies (approximately 7 bees from each of colonies). The present study investigates the existence and prevalence of the infections of acute bee paralysis virus (ABPV), black queen cell virus (BQCV), Chronic bee paralysis virus (CBPV) and deformed wing virus (DWV) infections were analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR) method. Specific primers were used for the genome of each virus in order to use for the molecular detection of these four viruses. BQCV was detected in 30.55% (11/36) of colonies, and DWV in 63.88% (23/36), whereas ABPV and CBPV were not detected. The identification tests showed that many colonies were infected with one or two viruses: 77.22% of colonies were positive for a single infection and 11.11% a double infection. The study showed that viral infections infect honey bees in Afyonkarahisar province.

**Keywords:** Acute bee paralysis virus, black queen cell virus, chronic bee paralysis virus, deformed wing virus, honey bee, RT-PCR.

Oral Presentation



## **The Use of Biosensors in the Determination of Fenolic Compounds in Honey**

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### **Abstract:**

Oxidative damage caused by free radicals can lead to different levels of cell and tissue damage. Antioxidants, especially abundant in plant foods, contribute to the continuity of vital activities against oxidative damage in the body. This shows the importance of nutrition with natural products more clearly. The main sources that enable plants to have all these vital features for humans are phenolic compounds. We aimed to study a tyrosine kinase enzyme-based biosensor that will determine the amount of phenolic compounds in honey, since bees take propolis and flower essence containing abundant phenolic compounds while making honey. A biosensor based on tyrosine kinase enzyme was designed to determine the amount of phenolic compounds in honey. Tyrosine kinase enzyme is a group of enzymes that catalyzes the transfer of the gamma phosphate group of ATP to the phenolic hydroxyl group of tyrosine amino acids in target proteins. In this method, tyrosine kinase enzyme was immobilized to the graphite pencil tip electrode by bovine serum albumin (BSA) and gelatin. The principle of this method is based on the principle of electrobiochemical measurement of the potential change resulting from the ATP group transferring between the molecules with the help of a biosensor. During the nectar collection of bees, the amount of phenolic substances included in honey can range from 5 to 1300 mg/kg. The working range of the electrochemical biosensor used in the determination of phenolic substance was found to be 0.99 to 2710 mg/kg. When people buy honey, they want to make sure that the honey is real. Therefore, it is very important to find methods that are not laboratory dependent to show the quality of honey. Portable fenolic compounds measurement devices can be produced by developing biosensors. Biosensor method can be evaluated as an alternative method for diastase activity determination method.

**Keywords:** fenolic compounds, honey quality, biosensor

**Oral Presentation**



## Artificial Intelligence and Digital Technologies Produced and Developed for the Health and Future of Honey Bees and Bee Colonies

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### Abstract:

The number of honey bees in the world's ecosystems is decreasing. Those active in the beekeeping sector may fight the threat more effectively with the assistance of modern technology. Beekeepers are a vital component of a flourishing industry. They provide the hives for rent to farmers who recognize the importance of bees in the pollination process for their crops. Intelligent gadgets that provide beekeepers access to precise information on the condition of their hives are being developed by digital entrepreneurs. These technologies are being developed to decrease beekeeping losses and enhance the health of honeybees. There are now commercially available sensors powered by artificial intelligence and may provide beekeepers with early warnings if there are issues with their hives. This little, internet-connected sensor is tucked away within the beehive ceiling, where it monitors a variety of factors like temperature, humidity, sound, and movement. The information gleaned from the sensor is sent to the cloud, where it undergoes processing and analysis before being relayed to the beekeeper. Beekeepers will be able to control a much larger number of hives with the same number of workers and the same amount of money spent on supplies like feed and treat if they use the gadget. They will also significantly boost the quantity of honey production and pollination inside their businesses. The expansion of the pollination sector has led to an increase in demand for bees, which in turn has encouraged entrepreneurial businesses to focus more of their efforts on bee technologies. The field of computational intelligence known as swarm intelligence (SI) examines the collective behavior that arises inside self-organizing communities of individuals. This study highlights the significance of intelligent technologies for honeybees throughout the world, and technologies developed specifically for bees, particularly artificial intelligence, are investigated. After all, bees also play a significant role in the business world and marketing.

**Keywords:** Artificial intelligence, honeybees, swarm intelligence, bee technologies, marketing.

Oral Presentation



## Antimicrobial and Anti-quorum sensing Activities of Lavender (Karabaş) Honey

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### Abstract:

One of the 4 factors determining the antimicrobial activity of honey is the secondary metabolites in its structure. Secondary metabolites of honey vary depending on the honey flora. In this study, the antimicrobial activity of the fresh lavender (or Karabaş) honey collected from Ayvalık, Cunda Adası region in May, 2022 was investigated. As a result of palynological analysis, lavender pollen rate was 15%. For this reason, within the scope of the study, the antimicrobial activity of different dilutions of lavender honey against Gram negative *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 13883, *Chromobacterium violaceum* ATCC 12472, Gram positive *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Streptococcus mutans* ATCC 25175 and *Candida albicans* ATCC 10231 yeast was investigated by agar well method. Antiquorum sensing activity was performed using *Chromobacterium violacum* ATCC 12472. The results of the study determined that different dilutions of lavender honey have antimicrobial activity, especially an encapsulated bacterium and *K. pneumoniae* ATCC 13883, which is one of the pneumonia agents. In future studies, it is thought to increase the use of lavender honey in terms of biological activities and to increase the use of honey in the pharmaceutical industry as well as alternative treatment.

**Keywords:** Antimicrobial, Quorum sensing, Honey, Karabaş herbs



## Biochemical composition of *Eucalyptus camaldulensis* oil and solvent extracts from flora of Hatay, Turkey

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### Abstract:

*Eucalyptus* genus entered Turkey for the first time in 1885 with *Eucalyptus camaldulensis* species. *E. camaldulensis* is a tree belonging to the *Myrtaceae* family. It was brought by the French company that built the Adana-Mersin railway to be planted as an ornamental plant around the railway. Due to the fact that the species is an exotic species and grows rapidly, it has become widespread in parks and gardens. *Eucalyptus* leaves contain essential oil, resin, bitter substance and tannin. *Eucalyptus* wood, which is used in a wide variety of fields from perfumery to pharmacy, from swamp drying to the construction sector, is also used in herbal dyeing. *Varroa destructor*, a parasitic bee mite, causes serious damage to honey bees and causes significant economic losses to the beekeeping industry worldwide. These mites, which feed on the hemolymph of adult bees and pupae, cause serious physiological disorders. Misuse of pesticides such as fluvalinate, coumaphos and amitraz has led to the emergence of mite resistance. However, the accumulation of chemical residues in bee products can cause health problems for consumers and trade restrictions. These issues have prompted researchers to explore alternative measures, such as essential oils with acaricidal properties, that are safe and have less negative impact on the environment. Many studies have been conducted recently on the use of essential oils in the management of varroa. In a study, 1,8-cineole-rich *E. camaldulensis* oils were shown to be effective against varroa. The aim of the study was to determine the biochemical compositions of essential oil and solvent extractions of fresh and dried samples collected from Hatay flora of *E. camaldulensis*, which is reported to be effective against pathogens threatening bee health. Thus, it sheds light on the determination of both the sample period (fresh or dry) and the method of obtaining (essential oil or solvent extraction) with a higher effect potential in terms of biochemical composition in their use against pathogens in bee health.

**Keywords:** *Eucalyptus camaldulensis*, *Varroa destructor*, Biochemical composition, Eastern Mediterranean, Hatay



## Simulation and Decision-Making In a Bio-Hybrid Beehive #

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### Abstract:

To counter the adverse effects on the bees, the European Project HIVEOPOLIS aims at developing a bio-hybrid beehive that will be equipped with a wide range of sensors and processing power to monitor and support the health of the colony, as well as actuators allowing to influence the foraging behavior of the bees. An internal robotic actuator will interact with the bees in the colony through the imitation of a bee's waggle dance and vibrational actuators for suppressing certain waggle dances performed by the bees. With these actuators the bio-hybrid beehive is able to encourage the bees to forage at certain locations and to prevent them from flying to the undesired locations. This can be used, for instance, to send bees to a known foraging ground with high yield of nectar and pollen, leading to higher honey harvest. Sending bees to specific locations which require pollination could increase the quality of the pollination service provided by the bees. Avoiding potentially dangerous areas, such as those known to be contaminated with pesticides, could contribute to the health of the colony. The habitats and natural foraging grounds of wild bee species could be protected due to avoiding reserved areas. A decision mechanism steering the behavior of a whole bee swarm must be developed. Such a mechanism requires a model describing how waggle dancing generated by robots, which mimic honeybees' motions, would influence the behavior of the bees and their interaction with the environment. We present an agent-based simulation developed to study the control mechanisms for a bio-hybrid beehive in different scenarios. With the help of this simulation we analyze and discuss various possible scenarios of interactions of an augmented bio-hybrid beehive with the environment and other actors like wild beehives, as well as factors for competition and collaboration that arise from those situations.

**Keywords:** hiveopolis, beekeeping, simulation

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Oral Presentation



### Molecular Detection of Bee Pathogens in Honey Bee Pollen Samples in Bulgaria

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#### Abstract:

This study aimed to evaluate the honey bee pollen as a natural source of environmental DNA (eDNA), which allows for the detection of different honey bee pathogens and parasites. In the present study, we extracted DNA from 10 honey bee pollen samples from different regions in Bulgaria and tested for the presence of DNA of the ectoparasitic mite *Varroa destructor*, *Nosema ceranae*, *Ascospaera apis*, *Melissococcus plutonius*, *Paenibacillus* larvae, *Tropilaelaps* spp., *Lotmaria passim* by conventional PCR amplification of the part of the mtDNA *cox 1* gene. RT-PCR was used for molecular detection of some honey bee-associated viruses like ABPV, CBPV, BQCV, KBV, DWV and SBV. The obtained results shown that all collected pollen samples were negative for honey bee pathogens. In contrast, we identified DWV and SBV in collected honey bee pollen. Based on the results, it is clear how problematic the influx of non-sterilized honey bee-collected pollen in beehives. The obtained results from this study demonstrated that honey bee pollen eDNA can be useful for monitoring and evaluation of one of the most common diseases of honey bee populations.

**Keywords:** *Apis mellifera*, bee pollen, eDNA, bee pathogens

**Acknowledgements:** This study was supported by Bulgarian NSF KP-06-PN 51/7 17.11.2021.



## Conservation of European M-lineage Honey Bees Using Abdominal Colour as an Indicator of Subspecies Purity has Pitfalls

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### Abstract:

There are 31 honey bee (*Apis mellifera*) recognized subspecies, which have been grouped into four main lineages. Two of these lineages occur naturally in Europe: M in western and northern Europe and C in south-eastern Europe. In Europe, M-lineage groups only two subspecies, *Apis mellifera mellifera* and *Apis mellifera iberiensis*, both being black in colour. C-Lineage groups have instead eight subspecies, including one of the beekeepers-favored and phenotypically distinct, the yellow *Apis mellifera ligustica* from Italy. M-lineage honey bees' distribution has been changing and in some countries, the native bee is being replaced or hybridised with C-lineage subspecies. Honey bee abdominal pigmentation is one of the most recognisable traits and it has been used by beekeepers as an indicator of subspecies identity. However, this approach may negatively impact population diversity and is futile if there is no association between tergite colour patterns and genetic background. To test this approach, we calculated the introgression level of *A. m. mellifera* (N=162) and *A. m. iberiensis* individuals (N=559) with different colour phenotypes and from a wide geographical range using informative SNPs. In this study, many *A. m. mellifera* samples showed high levels of C-lineage introgression. The individuals collected in Iberia were revealed to be pure. Introgressed *A. m. iberiensis* individuals were all from the Azores, where a high frequency of C-lineage mitotypes exists in several islands. Our results showed that for both subspecies, it is not possible to directly identify introgressed individuals from observed colour patterns, as we found black honey bees with a considerable amount of introgression and honey bees with yellow banding that were pure or marginally introgressed. With this study, we hope to increase awareness among stakeholders of the need to use other tools to select honey bees for conservation and breeding purposes.

**Keywords:** *Apis mellifera mellifera*, *Apis mellifera iberiensis*, SNPs, yellow banding, conservation

This work was financed by the Native Irish Honeybee Society (NIHBS), Federation of Irish Beekeeping Associations (FIBKA) and FEDER (Fundo Europeu de Desenvolvimento Regional) through the program COMPETE 2020–POCI (Programa Operacional para a Competividade e Internacionalização) and by the Portuguese funds through FCT (Fundação para a Ciência e a Tecnologia) in the framework of the project BeeHappy (POCI-01-0145-FEDER-029871). FCT provided financial support by national funds (FCT/MCTES) to CIMO (UIDB/00690/2020). Dora Henriques is funded by BeeHappy and MEDIBEES which is part of the PRIMA programme supported by the European Union.

## Major Royal Jelly Protein 2 (*mrjp2*) gene as the potential honey authentication marker of *Apis mellifera* and *A. cerana javana*

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### Abstract:

*Apis mellifera* and *A. cerana* are common honey bee species for honey production in Indonesian Beekeeping. Based on a previous study, the entomological origin of honey using two sets of *Major Royal Jelly Protein 2 (mrjp2)* gene primers successfully differentiate honey from subtropical *A. cerana cerana* in China and *A. mellifera*. The molecular *mrjp2* markers are needed to applicate in *A. cerana javana* and *A. mellifera* honey from Indonesia, due to the high genetic divergence in *A. cerana*. Thus, this study aimed to explore the *mrjp2* gene for entomological origin detection in tropical honey of *A. cerana javana* and *A. mellifera* in Indonesia. The eDNA from honey and DNA from the thorax of honey bee were extracted using DNA extraction KIT. The targeted *mrjp2* gene was amplified using two pairs of M-F, M-R, and C-F, C-R primers and evaluated the presence-absence of the amplicon. To find the optimal temperature, amplifications were attempted at various annealing temperatures. This study found that M-F and M-R primers successfully differentiated honey of *A. mellifera* and *A. cerana javana* at 50°C. Furthermore, at 53°C, 55°C, 57°C, and 59°C annealing temperatures, these primers can differentiate both bees. However, at 47°C annealing temperature M-F and M-R primer could not differentiate the DNA of honey and bees from both species. This study revealed that the C-F and C-R primers are specific to amplify only the DNA of *A. cerana javana* for honey at an annealing temperature of 47°C and 50°C, whereas up to 55°C for the bees. Our results proved that those paired *mrjp2* gene primers are potential for *A. mellifera* and *A. c. javana* honey entomological detection markers. The C-F, C-R primers are also applicable for the high genetic diversity of *A. cerana* from wide geographical distribution.

**Keywords:** *Apis cerana javana*, entomological origin detection, Eastern honey bee, honey eDNA, molecular application



## Colonization patterns of *Nosema ceranae* in the Azores archipelago<sup>#</sup>

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### Abstract:

*Nosema ceranae* and *Nosema apis* are pathogens of honey bees that cause nosemosis, a disease implicated in colony losses worldwide. They are obligate intracellular pathogens infecting the midgut epithelial cells of adult honey bees. Although *N. ceranae* was originally a pathogen specific of *Apis cerana*, currently, it is found in *Apis mellifera* throughout most of the world. Due to their confined environments, which limited pathogen transmission and dissemination, islands are unique places for epidemiological studies. There are only a few *Varroa destructor*-free and possibly even fewer *N. ceranae*-free honey bee sanctuaries in the world, with the Azores being one. Even though with the exception of Santa Maria and Flores, nosemosis has been present in the Azores since 2008, the causal species has yet to be identified. Hence, this study aimed to determine the prevalence and infection levels of *Nosema* spp. in the Azorean honey bees. In 2014/2015, 474 colonies were sampled on Faial, Flores, Pico, Graciosa, São Jorge, São Miguel, Santa Maria, and Terceira. Additionally, São Jorge, Santa Maria, Faial, and Terceira were re-sampled in 2020 with a total of 91 colonies. DNA was extracted, and the diagnosis and *Nosema* spp. loads were obtained by multiplex PCR and RT-qPCR. The findings indicate that *N. ceranae* appears to be the dominant species in the Azores. *N. apis* was only detected in 2014/2015 with a very low prevalence (5.1%). *N. ceranae* prevalence varied between 2.7%, on São Jorge, and 50.7%, on Pico. In 2020, *N. ceranae* positive colonies increased significantly on Terceira (57.1%) and São Jorge (50.0%). *N. ceranae* was not detected on Santa Maria in both periods. Average infection levels in positive *N. ceranae* colonies were usually medium to high ( $>10^{-7}$  ng/ $\mu$ l), with São Jorge colonies displaying the greatest infection intensity ( $>10^{-5}$  ng/ $\mu$ l). This study highlights the Azores archipelago as a unique place for beekeeping, with islands free of *N. ceranae* and *V. destructor*, which are two important stressors that afflict honey bees in the world.

**Keywords:** *Nosema apis*, *Nosema ceranae*, *Apis mellifera*, real-time qPCR, prevalence, infection levels

<sup>#</sup> Financial support was provided by Portuguese funds through FCT (Fundação para a Ciência e a Tecnologia) in the framework of the project BeeHappy (POCI-01-0145-FEDER-029871). ARL was supported by a PhD scholarship (SFRH/BD/143627/2019) from the FCT. FCT provided financial support by national funds (FCT/MCTES) to CIMO (UIDB/00690/2020).

Oral Presentation

## Natural Bee Products and Apitherapy for Cancer Prevention and Treatment

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### Abstract

Cancer is a global health problem with a high incidence and mortality rate around the world, and therefore the global cancer burden is increasing significantly every year. About 12 million people are diagnosed with cancer each year, 7 million patients die from cancer, and 25 million people worldwide currently live with a cancer diagnosis. As a result of preventive medicine practices, early diagnosis of cancer, and treatment of cancer with appropriate treatment strategies, cancer survival has increased and cancer-related death rates have decreased globally. In the field of oncology, there are many treatment options from conventional medicine as well as complementary and alternative medicine (CAM) and more recently integrative medicine. One of the many complementary and alternative methods in oncology is apitherapy. Apitherapy is a field of CAM that focuses on treatment options using multifarious products obtained from the beehive, particularly honey, propolis, pollen, apilarnil, royal jelly and bee venom. With the support of scientific research on apitherapy, the interest in bees and bee products, known since ancient times and used as a source of healing by the public, is increasing day by day. Evidence concerning of cancer prevention potentials of bee products is mainly based on in-vitro studies and animal trials. A recent research on the anticancer properties of honey reveals that it consists of antioxidant components as well as phenolic acids and flavonoids that can be used to prevent cancer. Although there are some in vitro evidence that propolis may be effective in preventing cancer, especially with regard to colon cancer, studies are still ongoing to determine whether propolis is also clinically effective. The flavonoids found in the pollen, especially kaempferol, have been shown to have anticarcinogenic properties. In addition, clinical studies on propolis have revealed that propolis-containing drugs can reverse cervical dysplasia when administered intravaginally. As demonstrated by various studies, bee products may have different mechanisms of action that could be useful for cancer treatment. However, clinical trials are necessary before bee products can be recommended as supportive and complementary applications in the treatment of cancer. In this respect, the purpose of this review is to provide an overview of holistic apitherapy modalities based on cancer clinical findings, studies, trials and treatment concepts.

**Keywords:** Apitherapy, bee products, cancer, complementary therapy, oncology

## Determination Of The Effect Of Honey Bee (*Apis mellifera* L.) Venom In The Treatment Of Epilepsy

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### Abstract:

Insect poisons are a method of treating various diseases for many years. Honey bee (*Apis mellifera* L.) venom is also used in Traditional and Complementary Medicine in terms of its components in the treatment of autoimmune and inflammatory diseases such as chronic pain and diseases such as rheumatoid arthritis and osteoarthritis, in addition to neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), Parkinson's, Alzheimer's, multiple sclerosis (MS). In recent studies, the effects of honey bee venom on epilepsy, which is also a neuronal disorder, have been shown. In this study, Sprague dawley male rats (n=56) aged 2-3 months and weighing 230±30 g will be used to investigate the effect of honey bee venom on Acute Epilepsy. Animals including the control group will be divided into 7 groups and the effect of honey bee venom and Apami, the component of honey bee venom, on epilepsy will be examined. According to the results of the literature reviews, bee venom is thought to have potential antiepileptic properties because it inhibits epilepsy pathways and is involved in the regulation of voltage-gated ion channels such as sodium, potassium and calcium channels. Our study will also investigate the effect of honey bee venom and apamine on sugar levels in cells.

**Keywords:** Honeybee venom, Epilepsy, Antiepileptic, Epileptogenesis, *Apis mellifera* L.

## **Nutritional Composition and Macro Nutrient Digestibility of Bee Pollen and Bee Bread: A Simulated *In Vitro* Gastrointestinal Digestion Approach**

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### **Abstract:**

Beehive products such as bee pollen (BP) and bee bread (BB) have emerged as excellent functional foods rich in macro and micronutrients and exhibit bioactive properties. The magnificent properties of both bee products increasingly motivate their use as a natural resource for food applications. Nonetheless, researching and understanding their detailed physicochemical properties and fate in the digestive system could lead to new application facilities. This work aimed to comparatively evaluate the chemical and nutritional composition of BP and BB and their macronutrient digestibility, namely protein and soluble sugars, by applying *in vitro* gastrointestinal digestion. Although the nutritional composition of both bee products varied somewhat depending on the botanical origin of the pollen, they proved to be good sources of proteins (15.1–29.1 g.100 g<sup>-1</sup>), fibre (3.8–5.8 g.100 g<sup>-1</sup>), carbohydrates (64.5–68.4 g.100 g<sup>-1</sup>), different type of fatty acids, amino acids, including 8 essential and 10 non-essential, soluble sugars like sucrose, maltulose and maltose with high concentration of fructose and glucose, as well as minerals such as potassium, calcium, magnesium and iron. As to macronutrient digestibility, the digestibility scores for protein content were calculated on average as 69% and 76% for BP and BB, respectively, whereas digestibility scores for soluble sugars varied depending on bee product and sugar type. Overall, the findings from this study could expand the potential applications of both products in the food industry and provide baseline information for future research.

**Keywords:** bee pollen; bee bread; nutritional composition; micro-and macronutrients; digestibility degree

### **Funding:**

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## The Antimicrobial Properties of Poplar and Aspen–Poplar Propolis and Their Active Components against Selected Microorganisms, Including *Helicobacter pylori*

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### Abstract:

There is a noticeable interest in alternative therapies where the outcome is the eradication of the Gram-negative bacterium, *Helicobacter pylori* (*H. pylori*), for the purpose of treating many stomach diseases (chronic gastritis and peptic ulcers) and preventing stomach cancer. It is especially urgent because the mentioned pathogen infects over 50% of the world's population. Recent studies have shown the potential of natural products, such as medicinal plant and bee products, on the inhibition of *H. pylori* growth. Propolis is such a bee product, with known antimicrobial activities. The main scope of the study is the determination of the antimicrobial activity of ethanolic extracts from 11 propolis samples (mostly from Poland, Ukraine, Kazakhstan, and Greece) against *H. pylori*, as well as selected bacterial and yeast species. The most effective against *H. pylori* was the propolis from Ukraine, with an MIC = 0.02 mg/mL while the rest of samples (except one) had an MIC = 0.03 mg/mL. Moreover, significant antimicrobial activity against Gram+ bacteria (with an MIC of 0.02– 2.50 mg/mL) and three yeasts (with an MIC of 0.04–0.63 mg/mL) was also observed. A phytochemical analysis (polyphenolic profile) of the propolis samples, by ultra-high-performance liquid chromatography-diode array detector-mass spectrometry (UPLC-DAD-MS), was performed. An evaluation of the impact of the propolis components on antimicrobial activity, consisting of statistical analyses (principal component analysis (PCA) and hierarchical fuzzy clustering), was then performed. It was observed that the chemical composition characteristics of the poplar propolis correlated with higher antibacterial activity, while that of the poplar and aspen propolis correlated with weaker antibacterial activity. To summarize the activity in vitro, all tested propolis samples indicate that they can be regarded as useful and potent factors in antimicrobial therapies, especially against *H. pylori*.

**Keywords:** Galangin; *Helicobacter pylori*; Hierarchical fuzzy clustering analysis

## Seasonal changes in the circulation of viruses and mixed infection in the population of honey bees *Apis mellifera* L.

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### Abstract:

The article presents data on the circulation of seven species of viruses in the honey bee population in the summer (July) and autumn (September) periods in the apiary of the Central Federal District of Russia. It has been established that the SBV (sac brood virus) is the most common in the studied bee population - in the summer it can be detected in 100% of bee colonies. Two other viruses, wing deformity virus (DWV) and acute paralysis virus (ABPV), are also widespread, and circulate in approximately 50% of bee colonies in the summer. It is shown that in bee colonies in the autumn period, the percentage of families in which it is possible to detect at least one of the viruses decreases by an average of 2.5 times, the sac brood virus (SBV) is most susceptible to seasonal decline - 3.5 times. The wide distribution of mixed infections in bee colonies has been confirmed. The fact of clinical manifestations of sac brood disease was found in one of the 20 families studied, mixed infection was not observed in it, only the sac brood virus (SBV) was detected.

**Keywords:** seasonal circulation of viruses, honey bees, mixed infections

## Pesticides As A Leading Cause For The Increasing Honey Bee Colony Losses In Bulgaria<sup>#</sup>

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### Abstract:

The genetic richness among honey bee populations is threatened by: the uncontrolled introduction of foreign genes into the adapted local populations; the stress from the changing environment and its pollution; new pathogens and the global climate changes. In this aspect, during last years the Bulgarian beekeeping is under great danger because of: uncontrolled imports of bee queens with foreign origin and the usage by the agricultural producers of more new and diverse pesticides, which is related to the reported high mortality of bee colonies and whole apiaries. The aim of the current study was to look into the relationship between the increasing level of honey bee colony losses in Bulgaria and the presence of pesticides in honey bee colony food stocks. In order to study the rate and the reasons of colony losses, a standardized international COLOSS questionnaire was used. Data concerning: unsolvable queen problems; natural disaster and death or reducing in number to a few hundred bees were collected by beekeepers from different geographic regions in Bulgaria. The pesticides presence in honey bee samples and food stocks in the honey bee colony - honey, wax and pollen, collected from locations on signals of high mortality and severe loss of bee colonies was investigated by chemical chromatographic analysis (RESID 19/04 LC-MS / MS methods). Data from the present study showed that the honey bee colony losses in Bulgaria for the period 2017 - 2022 increased from 2.04% to over 30% and that the highest percentage of losses over the years was due to the indicator "death or reducing in number to a few hundred bees". At the same time, the investigation revealed the information concerning the presence of residues of a total of 27 pesticides in the tested samples – insecticides, fungicides, acaricides, herbicides and growth regulators. Based on the results obtained, it could be concluded that there is a clear relation between the various agrochemicals used for different purposes and the increasing losses of honey bee colonies in Bulgaria, as well as the need for future detailed studies of risk factors for the health and viability of honey bees.

**Keywords:** *Apis mellifera*, pesticides, honey bee colony losses

**#Acknowledgments:** This study was supported by the National Research Fund of Bulgaria through the contract KP-06-H5112/2021 "Complex assessment of genetic and environmental factors related to the losses of honey bees (*Apis mellifera* L.) in Bulgaria".

***Apis laboriosa* (Hymenoptera, Apidae) Confirmed by Morphometric and Genetic Analyses from Sites of Sympatry with *Apis dorsata* in Arunachal Pradesh, North East, India**

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**Abstract:** Two giant honey bee species are native to India: *Apis dorsata* (with bicolored metasomal abdomen) which is widespread over South-east Asia and *Apis laboriosa* (with fully black metasomal abdomen) which is restricted to the mountains of the Pan-Himalayan region. Although these two bees differ morphologically, behaviourally and genetically, the species status of *A. laboriosa* is still unresolved as there is no clear evidence of reproductive isolation. We sampled bees from three different regions in Arunachal Pradesh, North East, India, where they occurred in sympatry as they foraged in the same patches of flowers. Bees were preliminary identified at the time of collection based on thoracic hair and abdominal colour. We carried out the measurements of 20 standard morphological characters of 121 worker bees (73 from sites of sympatry and 48 from sites of allopatry) on which we performed cluster analysis, principal component analysis and MAVONA statistical analyses. In addition, we also sequenced approximately 500bp of mtDNA cytochrome oxidase subunit I (COI) and generated a phylogenetic tree. No bees with traits suggestive of hybridization could be found from sites of sympatry. The genetic sequences separated the bees into two distinct clades regardless of where they had been collected. Based on our morphometric and genetic analyses, we infer that *A. dorsata* and *A. laboriosa* are distinct, reproductively isolated species. However, still more studies are needed to clearly demonstrate reproductive isolations of the two forms. Most promising will be studies that document non-overlapping mating seasons or mating flight times.

**Keywords:** sympatry/ morphometry/ phylogenetic/ *Megapis*/ species

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## Dark European honey bee *Apis mellifera mellifera* benefits and its gut microbiome features

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### Abstract

The gut microbiome of the dark European honey bees *Apis mellifera mellifera* contains a limited number of evolutionarily formed representatives of gram-negative (genera *Apibacter*, *Bartonella*, *Commensalibacter*, *Gilliamella*, *Parasaccharibacter*, *Saccharibacter*, *Serratia*, *Snodgrassella*, *Frischella*) and gram-positive bacteria (genera *Lactococcus*, *Lactobacillus*, *Bifidobacterium*). Their functions and significance for the individuals and the local population has recently been the subject of research in laboratories around the world. Our interest in the study of the gut microbiome of the dark European honey bees *A. m. mellifera* is due to the expectation to find the microorganisms with increased antimicrobial, antioxidant, immunomodulatory, neuromodulatory potential of symbiotic microorganisms in bees highly adapted to long time (6 months) wintering in the extremely cold continental climate of northern Europe. Lactobacteria and bifidobacteria are classic probiotic bacteria. Their use as probiotics, paraprobiotics, postbiotics, pharmaceutical ingredients, and personalized food products is constantly expanding. Until now, the honeybee gut microbiome has not been used as a source of probiotics. A complete metagenomic analysis of the microbiome, using algorithms for searching for specified genes and their combinations, makes it possible to quickly and efficiently select promising strains of bacteria. Subsequent genomic, transcriptome, proteomic, and metabolomic analyzes make it possible to select the most practically acceptable strains. In the future, the proposed technological platform, tested at a convenient model of *A. m. mellifera*, allows it to be used on other animals.

**Keywords:** *Apis mellifera mellifera*, *Lactobacillus*, dark European honey bee, gut microbiome, metagenome, lactic acid bacteria, antioxidant potential, detoxification



## Invasion Patterns Of *Vespa velutina nigrithorax* In Southern Europe: A Genetic Perspective

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### Abstract:

*Vespa velutina* was seen for the first time in Europe in 2004, in pots imported from China into France. Since then, the population rapidly build up and expanded within France and, in 2010, started spreading across other countries, giving rise to one of the most phenomenal insect invasions ever seen in the European continent. Early studies suggested that the invasion originated from a single multi-mated queen introduced from China. However, little was known, until this study, about the genetics underlying the expansion towards south and east of the introduction point in France. Our aim was to further understand *V. velutina* invasion in Europe by surveying the Iberian and Italian peninsulas using microsatellites and mitochondrial DNA. Our results show that the French population was the source of the colonies expanding in Spain, Portugal and Italy, therefore leading to rejection of the hypothesis of multiple introductions from the native habitats. While Spain and Italy were invaded by leading-edge expansions from the French core population, in Portugal this invasion started from a long-distance jump. Both types of expansion were accompanied by a significant reduction in the population genetic diversity, with Portugal presenting the highest loss values ( $Ar = 17.4\%$ ;  $uHe = 42.3\%$ ) than Spain ( $Ar = 9.0\%$ ;  $uHe = 20.6\%$ ) or Italy ( $Ar = 16.3\%$ ;  $uHe = 26.8\%$ ). Population structure results and signatures of differentiation show that a secondary contact occurred between the front derived from the primary propagule introduced in France and the front derived from the secondary propagule introduced in Portugal. First-generation migrants were detected in Iberia and Italy, suggesting that a continuous gene flow is bringing in new alleles in the three invaded countries. This effect is more prominent in Portugal, as it is reflected by a 20.3% increase in allelic richness. Overall, this study provides new insights into the invasion genetics of the honey bee predator *V. velutina* in Europe, which can help developing strategies to manage this major threat to beekeeping.

**Keywords:** *Vespa velutina*, alien species, biological invasion, genetic diversity, molecular markers

This research was funded by the program POSEUR-03-2215-FC-000008, through the project “GesVespa: Estratégias de gestão sustentável da *Vespa velutina* no Norte de Portugal”. Fundação para a Ciência e a Tecnologia provided financial support by national funds (FCT/MCTES) to CIMO (UIDB/00690/2020).

## The beehive as biomonitor of pesticide residues in agroecosystems

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### Abstract:

When looking for food, bees flight long distances to reach and found nectar, pollen and resins and bring them to the hive. During the flight, they also come in touch with other components of the environment such as water and air. The whole agroecosystem environment as well as the food cropped is anthropogenically contaminated, and the main pollutant are the pesticides that were applied on it. Therefore, bees bring food and pesticide residues to the hive. Once in the hive, the pesticide residues distribute among the different beehive matrices according to their physicochemical properties. The analysis of the pesticide residues in each of these matrices could bring an idea about the agrochemicals applied in it. These facts are the basis for considering bees as pesticide biomonitors of the environment. Our group have been working in the last ten years developing this concept. During the presentation, the novel analytical methodology developed through the years for each of the hive matrices, wax, polen, honey and bees will be overlooked. The precise analytical methodology allowed trustable monitoring campaigns for pesticide residues in agroecosystems and selected results will be presented, linking the pesticide occurrence with the agricultural practices carried nearby the hive. According to the occurrence of residues in each matrix, pesticides applied in the field can be traced, and the results can be modelled combining chemical results and biological observations using support vector machines (SVM) to study the environmental status of the agroecosystem. Further, a rough indicator, Agroecology Environment Quality Quotient (AEQ), can be derived to get a predictable evolution of the environment and the hive living in it. Interestingly, a clear distinction between corbicular and stored pollen can be made. Pollen within the hive has been contaminated during beebread making but corbicular pollen is minimally contaminated with other hive matrices and the information it gives is much more precise. Combined with quantitative palynological observations, the incidence of crop pollen can be inferred, suggesting new refinements to the model.

**Keywords:** beehives as environmental monitors of pesticide residues, LC/GC- MS/MS analysis of beehives matrices, modelling the environmental quality of agroecosystems, residues in stored & corbicular pollen.

## Effect of Bee-Pollen Supplementation on Performance, Carcass Traits and Blood Parameters of Broiler

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### Abstract

One-day-old Hubbard broiler chicks were fed basal diet supplemented with bee-pollen at the rate of 0% (control), 0.2%, 0.4% or 0.6% for a period of 6 weeks with an aim to use them in broiler nutrition as a natural growth promoting substance. Significant ( $p < 0.01$ ) differences in live body weight and body weight gain were found between broiler chicks fed the basal diet and those having bee-pollen in their diets during the experiment period. Broiler receiving 0.6% bee-pollen had the highest significant ( $p < 0.01$ ) body weight and body weight gains. Bee-pollen supplementation resulted in less feed intake and improved feed conversion ratio compared to the control group. The relative weight of carcass were significantly ( $p < 0.01$ ) higher in chicks fed bee-pollen diets. Chicks fed 0.6% bee-pollen diet were found to have highest relative weights of thymus, bursa, and spleen, and the highest values of the packed cells volume, hemoglobin concentration, red blood cells, white blood cells, heterophils and lymphocytes. Serum total protein, albumen and globulin were significantly higher in birds fed on bee-pollen compared with the control group. The concentrations of serum uric acid, creatinine, triglycerides, cholesterol, GOT and GPT in chicks fed 0.2, 0.4 and 0.6% of bee-pollen were found to be lower than the control group. It was concluded, that supplementation of bee-pollen to the diets of broiler chicks improved the performance, carcass traits, and blood parameters.

**Keywords:** bee-pollen, blood parameters, broiler, carcass traits, performance.



## The Importance Of Citizen Science Protocols For The Formation Of Stingless Beekeepers

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### Abstract:

Citizen Science is a growing field of research defined as the active engagement of the general public in scientific activities to produce new scientific knowledge. Citizen Science is gaining popularity among Environmental Sciences. Evaluation is a very important step in Citizen Science projects. Accessing a volunteer's motivation is very valuable for planning the project's next steps. We accessed the motivations of 864 citizen scientists which participated in a meliponiculture and citizen science course. The citizen scientists answered two surveys, one before and another after the course. All the participants executed the Stingless Bees Flying Activity Monitoring Protocol, counting the number of bees leaving the hives, entering and carrying pollen using the platform BeeKeep. We found that among the volunteers, three motivation categories grew from the initial to the final survey. "Answer my own scientific questions regarding bees" (46,5%); "Do a leisure activity" (22,7%) and "Improving my bee's productivity" (12,5%). Volunteer's motivations are dynamic and changes are expected. The course shows up as a valuable tool for the formation of stingless beekeepers and citizen scientists and brings independence for them to ask their own questions about bees and their environment at the same time it may be a leisure activity. Unexpectedly, the participants found the Stingless Bees Flying Activity Monitoring Protocol as an asset to improve their stingless bees productivity because the flying activity is a good indicator of the colony strength and it makes it easier to compare one colony with another, or even the same colony along the time. This shows how valuable the protocol is not only to form citizen scientists but also to bring new skill assets to the beekeepers and make them more independent in their management. Our findings recommend the implementation of citizen science protocols as part of beekeeping formation courses and engage beekeepers in environment monitoring activities.

**Keywords:** stingless bees, citizen science, flying activity, volunteer assessment, motivation

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## Antifungal activity of “HO21-F”, a formulation based on *Olea europaea* plant extract, in honey bees infected with *Nosema ceranae*

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### Abstract:

*Nosema ceranae* is a microsporidium parasite that affects silently honey bees, causing a disease called nosemosis. This parasite produces resistant spores and germinates in the midgut of honey bees, extrudes a polar tubule that injects an infective sporoplasm in the host cell epithelium, proliferates, and produces intestinal disorders that shorten honey bee lifespan. The rapid extension of this disease has been reported to be widespread among adult bees, and treatments are less effective and counterproductive to weaken the colonies. This work aimed to evaluate the antifungal activity of “HO21-F”, a prototype formulation based on a non-toxic plant extract (*Olea europaea*) against *N. ceranae*. The effectiveness has been demonstrated by the reduction of infection levels observed in laboratory conditions at the concentrations of 0,5 and 1 g/L, and in-field conditions at the concentrations of 2,5 g/L. Honey bees were infected artificially and kept in cages for 17 days in the laboratory and in field experiments, we evaluated the therapeutic effect of HO21-F for 28 days in naturally infected colonies. In laboratory experiments, we observed a reduction in infection level of 83,6% without affecting the survival rate. A reduction of the infection level of 88% was observed in field experiments, higher than the other commercially available products, and increased the colony strength associated with population size and honey production. The antifungal activity of HO21-F presented in laboratory and field experiments will encourage new research to understand the mechanism of action, by the spore-inhibition effect and/or a stimulating effect in the natural response of colonies to counteract the disease.

**Keywords:** *Nosema ceranae*, honey bee, infection level, antifungal activity, *Olea europaea*.

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## Maturity evaluation in stingless bee honey; Soconusco, Chiapas

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### Abstract

Stingless bees are located in Neotropical areas around the world, where all their hive bee products are considered part of the ancestral pharmacopoeia. Recently, there are several studies focused in the elucidation of their bioactive compounds and their physico-chemical characteristics. In this study we performed a maturity evaluation in stingless bee honey along one year of study; *Melipona beecheii*, *M. solani* and *Scaptotrigona mexicana* honey from local producers in the Soconusco region. We determined the physico-chemical parameters during six months and after one year of collection on stingless bee honey, which were under different temperature treatments (-10°C, 25°C and 33°C). We also evaluated the antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* with fresh honey at 25°C, and after one year. Our results showed that pH of all the honeys was acidic, during the storage time, although it did not increase to higher levels than pH 4 in all the treatments. Free acidity increased in all treatments up to 180 days and after one year we observed pH values of 3.13-3.39°C and humidity of 25.97-29.13 %. This results showed us that there is an effect of time, temperature and bee species the maturity of honey. On the other hand antibacterial activity with fresh honey did not register antibacterial activity, however after one year of maturity we observed inhibition halos of 21.67-5.33 mm diameter against *E. coli*, and 3-22 mm diameter against *S. aureus*. After this, we can infer that maturity in stingless bee honey could be an option to explore bioactive properties.

**Keywords:** meliponines, hive bee products, storage time, physicochemical, antimicrobial

## Exposure to Nepalese Propolis Alters the Metabolic State of Mycobacterium tuberculosis

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### Abstract:

Propolis is a natural product proved to be efficient against Mycobacterium tuberculosis. Although it is produced by bees, its active alcoholic-aqueous fraction contains plant derived molecules. To gain some insight into its mechanism of antimycobacterial activity, we studied the metabolic changes in bacterial cells treated with extract of Trigona sp. propolis from Nepal. The detailed metabolomic and transcriptomic analysis performed in this study indicated target points in bacterial cells under propolis extract influence. The profile of lipids forming outer and middle layer of the mycobacterial cell envelope was not changed by propolis treatment, however fluctuations in the profiles of amphipathic glycerophospholipids were observed. The enrichment analysis revealed bacterial metabolic pathways affected by Trigona sp. propolis treatment. The early metabolic response involved much more pathways than observed after 48 h of incubation, however the highest enrichment ratio was observed after 48 h, indicating long-lasting influence of propolis. The early bacterial response was related to the increased demand for energy and upregulation of molecules involved in the formation of the cell membrane. Transcriptomic analysis confirmed that bacteria also suffered also from oxidative stress, which was more pronounced on the second day of exposure. This was a first attempt to explain the action of Nepalese propolis extract against mycobacteria.

**Keywords:** flavonoids, metabolomics, LC-MS, sigma factors, oxidative stress, natural products

## **Surviving rate and cluster analysis of hives after winter in 2019 according to the statement of Turkish beekeepers**

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### **Abstract:**

A mail-based survey was conducted in Turkey, on a simple random sample of all registered beekeepers, to investigate survival rates of beehives and related factors at the exit from winter of 2019. Based on 650 fully completed questionnaires, the location of hives (at village level) over the winter were recorded. Further inquiries on sociodemographic characteristics of the beekeepers and their beekeeping practices were also documented. Wintering points of the hives were studied using geographic information systems (GIS) and survival rates were analyzed based on the number of beehives that came out alive through spring. Following interpolation of the data across Turkey, the ArcGis GIS performed for clustering analysis. Statistical significance was preset at  $p < 0.05$  for all analyses. Survival rates of the beehives were statistically significantly higher in Muğla, Uşak, Bartın and Eskişehir, whilst significantly lower in Trabzon, compared to those in surrounding provinces. Beehive survival rates were lower in Ankara, Burdur and Isparta provinces compared to neighboring provinces, yet differences did not reach statistical significance. The potential associations between beehive survival and bee feeding methods will be further investigated in the study.

**Keywords:** Beekeeping, cluster analysis, GIS, nutrition

## Diversity and Functions of Bee Microbiota

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### Abstract:

In general usage, both 'microbiome' and 'microbiota' are used. But the term 'microbiome' is generally accepted as the genome or general genetic material. Therefore, it is more correct to use the term "microbiota" to denote the microbial community. Microbiota is the internal ecosystem of the body. Microbiota is found in different parts of the body. The intestines are naturally the most populated environment for bacteria. Gut microbiota has important functions in metabolism, development, pathogen resistance and immune system development. *Apis mellifera* (*A. mellifera*) is the most important insect pollinator for ensuring world food security. In addition, various pathogens and environmental stressors threaten bee health. Gut microbiota has important effects on bee health, immune system development and metabolism. The gut microbiota of the honey bee contains less bacteria in numbers and types than in humans and other animals. The gut microbiota consists of eight major bacterial groups concentrated mostly in the hindgut. Two *Alpha*, one *Beta* -, two *Gammaproteobacteria*, two closely related *Lactobacillus* groups (Firm 4, 5) and a *Bifidobacterium* (*Bifidobacterium asteroidetes*). Some are found in all honey bees (*Lactobacilli*, *Beta*- and *Gammaproteobacteria*), but other bacterial groups may be absent completely in some individuals. The honey bee microbiota is very important for growth, development, metabolism and immune system. It is especially effective in inhibiting pathogens in bee health. Improper feeding, mistakes made in colony management and beekeeping practices, unnecessary and continuous antibiotic applications adversely affect the microbiota. Feeding and practices that support and strengthen the microbiota are extremely important for healthy, strong and highly productive beekeeping.

**Keywords:** Honeybee, Microbiota, Gut microbiota



**POSTER**

**PRESENTATIONS**

## **A Book on Resins and Propolis in Stingless Bee Life**

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### **Abstract:**

Pot-Propolis in Stingless Bee Ecology is a book project to be published by Springer Nature, It is about the plant resins and chemicals mixed with stingless bee wax, which fortify the colony in many ways that only now are beginning to be understood. Resins, gums are used make cerumen pots and several other elements of bee nest architecture, including the cells and matrices in which bees are reared, passages and portals that repel ants or other invaders, and cavity linings, where microbes continually may colonize and propagate. Forty chapters are distributed in the main sections planned for this book. The book addresses: 1. Stingless bees and their resin plant diversity, 2. Resin and propolis functional ecology, 3. Cerumen pots for food storage and ripening, 4. Chemical composition of cerumen, plant resins and pot-propolis, 5. Bioactivity of stingless bee cerumen, propolis and geopropolis, 6. Cultural uses and commercial products, 7. Sustainable stingless bee keeping and conservation, and 8. Marketing and standards of cerumen and propolis. Affiliations of authors are from Africa (Kenya, Nigeria), America (Argentina, Brazil, Colombia, Costa Rica, Mexico, Panama, United States, Venezuela), Asia (China, India, Indonesia, Malaysia, Philippines, Thailand), Europe (Bulgaria, Denmark, Italy, Germany, United Kingdom), Oceania (Australia). Scholars and the general public are mostly familiar with honey and pollen of *Apis mellifera*. However, Meliponini produce honey and pollen, have twice the evolutionary history, much greater biogeographical provenance, and 50 times the species of *Apis*, and are uniformly relished by indigenous peoples. Updated information, guidelines and references offer scientific background to pursue further investigations and surveys on the most ancient honey and pollen containers, the cerumen pots of stingless bees. The characteristics of the elastic, waterproof and resistant material of stingless bee nests has many uses and is a fascinating topic in their natural history and evolution.

**Keywords:** cerumen, Meliponini, propolis, resins, stingless bees.



## **Spirulina as for a natural source of protein for bee colonies *Apis mellifera caucasia***

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Spirulina is a genus of single-celled blue-green algae (cyanobacteria). Due to the high protein content (up to 70%) in its composition, it is of interest in beekeeping. The amino acid composition of spirulina is similar to pollen. Spirulina cell protein contains all 10 essential amino acids necessary for the development of bee. The aim of the work was to study the effectiveness of the use of spirulina as a protein supplement in the spring period of development of bee colonies *Apis mellifera caucasia*. The studies were carried out in the apiary on the Russia Krasnodar Territory of Sochi city. For testing, 3 groups of bee colonies were organized, formed by the method of pairs - analogues. The first group received sugar syrup in the amount of 500 ml per colonies with the addition of dry Spirulina (1.5g/l), the second - syrup with pollen (1.5g/l), the 3rd group became the control group and received syrup without protein supplements. The effectiveness of feeding was determined by the strength of colonies in the streets and the egg production of queen. The method for determining signs is presented in the collection of the International Scientific and Practical Conference “Beekeeping and Apitherapy: Modern Approaches and Development”, Rybnoe.2021;-st.55. Tests have shown an 8.3% increase in colony strength with pollen and an average of 5.7% with spirulina compared to controls. The indicators of egg production were similar in experimental groups, an increase in the on average 1.5 times was recorded compared to controls. Studies have shown that the most positive effect in the development of bee colonies is observed when using carbohydrate top dressing with pollen. However, top dressing with the addition of Spirulina is not inferior to pollen in terms of queen egg production. Blue-green algae spirulina with an acute shortage of a natural source of protein in the spring, can be used as an alternative to pollen.

**Keywords:** *Apis mellifera caucasia*, spirulina, protein.

## Use of Propolis Powder as a Functional Food Ingredient

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### Abstract:

Propolis contains numerous compounds with antioxidant and pharmacological activities that make it the ideal candidate to be used as a functional ingredient in foods. However, its solubility in alcohol and its strong and unpleasant taste limit its use as a food ingredient. The objective of the work was to encapsulate a propolis ethanolic extract by co-crystallization in a sucrose matrix to obtain an easily-handled alcohol-free powder to be used as a food ingredient. The effect of the propolis extract on colour, moisture content, solubility, particle size distribution, flow properties and spectroscopic characteristics (FTIR) of the co-crystallized powders (10-40% w/v extract) was determined. Polyphenols and flavonoids content and antioxidant capacity (ABTS and DPPH) during storage of the powders were also analysed. The powder was incorporated in a chocolate flavoured pre-mixed powdered dessert and the colour properties were assessed. The propolis co-crystallized powders showed moisture contents below 2% and solubility values higher than 95%. The dynamic angle of repose and Hausner ratio were between 41°-48° and 1.05-1.19, respectively, in samples with up to 30% w/v extract indicating a good flowability of the powders. The increase in the propolis content decreased the luminosity and made the powder more red-yellowish. The content of propolis influenced the particle size of the co-crystallized powders. As propolis content increased, the particle size distribution shifted to higher sizes. Thus, at low concentrations, the matrix structure was determined by the sucrose agglomerate, but as the volume of propolis extract increased, the encapsulated compound also had an impact. The FTIR results showed that the presence of propolis did not alter the crystalline sucrose structure. Entrapment yields higher than 84% (flavonoids) and 78% (polyphenols) were obtained. During storage of the powders, high stability of these compounds was observed under light, darkness and refrigeration conditions. The addition of the powder to the chocolate dessert did not change the luminosity but slightly decreased  $a^*$  and  $b^*$  colour coordinates. Thus, the co-crystallization technique becomes a low-cost alternative for the protection of bioactive compounds of propolis and to obtain a powder with suitable technological properties and high antioxidant activity to be used as a food ingredient.

**Keywords:** Propolis, Sucrose, Co-crystallization, Food ingredient.

This work was supported by CONICET (PIP 2017-0760)

## A Precise And Fully Automatic MATLAB-based Tool For Honey Bee Subspecies Diagnosis Based on Wing Morphometric Analysis #

Ernesto Angel-Beamonte<sup>1</sup>, Pilar Santolaria<sup>1</sup>, Susana Cortés-Calvo<sup>1</sup>, Irene Muñoz<sup>2</sup>, Pilar De la Rúa<sup>2</sup>, Jesús Yániz<sup>1</sup>

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### Abstract:

Wing morphometry has been found to be an invaluable tool for managing honey bee populations and promoting their conservation. In order to facilitate studies of honey bees, we present here a new software for the fully automatic and complete geometric morphometric analysis of the forewing venation structure used for identification of honey bee subspecies. This new software, called WingMarks2, requires a prior definition of a wing template, but all the images subsequently analyzed are automatically processed through formatting, orientation, and stylization, finally identifying the coordinates of each of the 19 landmarks that represent the wing vein union nodes using a spatial minutiae convolution algorithm. After landmark determination, WingMarks2 automatically performs a full Procrustes fit superimposition of the landmark coordinates and a Fisher's linear discrimination analysis to provide the results of subspecies classification. Two experiments were performed to evaluate the ability of WingMarks2 software to analyze wing images obtained with different optical equipment (experiment 1) to check its ability to discriminate between six subspecies of *Apis mellifera* (*A. m. iberiensis*, *A. m. mellifera*, *A. m. ligustica*, *A. m. carnica*, *A. m. intermissa*, and *A. m. sahariensis*) and between two evolutionary lineages within *A. m. iberiensis* (experiment 2). In experiment 1, the new software allowed the automatic and accurate precise analysis of 93.0%, 95.7% and 95.8% of the 645 wings analyzed when using Leica-microscope, Celestron USB-microscope and Xiaomi Smartphone images, respectively. Results of experiment 2 showed that, overall, the cross-validated correct identification rate of was 97.4 % for the subspecies as a whole and ranged between 94% and 100% for the individual subspecies. However, the evolutionary lineage identification within *A. m. iberiensis* was not sufficiently accurate, with an overall correct identification rate of 65%. It was concluded that WingMarks2 is a powerful and efficient tool for the complete analysis of wing morphometry in *A. mellifera* even using a smartphone camera for image acquisition.

**Keywords:** *Apis mellifera*, geometric morphometry, software, conservation.

# This work was supported by the Spanish AEI-MICINN (grant PID2020-112673RB-100), and the DGA-FSE (grants PRO-SOBRARBE and A07\_17R). I. Muñoz is supported by a MINECO Spanish postdoctoral grant "Juan de la Cierva-Incorporación" (JCI2018-036614-I).

## Morphometric Differentiation Of *Apis Mellifera Iberiensis* In The Balearic Islands #

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### Abstract:

The preservation of subspecies and ecotypes of *Apis mellifera* is important since they are better attuned to local flowering patterns, climatic variation and locally prevalent beekeeping methods. Honey bees of local origin also represent highly valuable biological capital for future selection in response to new ecological and production challenges. This study was designed to study the morphometric differentiation of *Apis mellifera iberiensis* in the Balearic Islands when compared to those in the Iberian Peninsula. Samples of 2970 workers were collected from 2000 to 2021 in 312 colonies distributed in the Iberian Peninsula (33 apiaries, 171 colonies, 1375 workers) and in the four Balearic Islands (31 apiaries, 141 colonies, 1595 workers). Wing images, obtained using a Leica optical microscope or a Smartphone, were processed with the WingMarks2 automatic software to obtain the subspecies classification and landmarks coordinates. These coordinates were subsequently processed in MorphoJ package. Results showed a morphometric differentiation of the *A. m. iberiensis* in the Balearic Islands when compared to those of the Iberian Peninsula. Cross-validation tests based on discriminant function correctly classified 100% of the colonies and 97.0% of honey bees. It was concluded that the honey bee in the Balearic Islands show a morphometric differentiation when compared to those in the Iberian Peninsula, and this may indicate the existence of a new ecotype of *A. m. iberiensis*.

**Keywords:** *Apis mellifera*, geometric morphometry, conservation.

# This work was supported by the Spanish AEI-MICINN (grant PID2020-112673RB-100), the DGA-FSE (grants PRO-SOBRARBE and A07\_17R), and the Serveis de Millora Agraria i Pesquera (SEMILLA, Govern de les Illes Balears). I. Muñoz is supported by a MINECO Spanish postdoctoral grant “Juan de la Cierva-Incorporación” (JCI2018-036614-I).

## Seasonal variation of quality parameters of the Brazilian red propolis from Alagoas: chemical and microbiological assays

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### Abstract:

Propolis is a natural product made by bees, which produce it by mixing their salivary secretion with the exudate of various plants. It can be present in nature in different chemical compositions, biological activities, colors and organoleptic properties, as the species of origin varies according to the region, and environmental factors influence the phytochemical composition. From this it was possible to identify 12 Brazilian varieties of propolis, later discovering the red propolis from Alagoas state, belonging to group 13, produced through the red exudate of the plant *Dalbergia ecastophyllum*. The red propolis has antibacterial, antifungal, anticancer and antioxidant activities, as well as a relatively high content of phenolic compounds. The aim of this study was to investigate the influence of seasonal variability on chemical and biological parameters of PVA collected over 12 months. The DPPH● radical scavenging capacity assay showed good results in all analyzed months (April 2017 to March 2018), surpassing the same effect in the red varieties of Maceió (Alagoas), Sergipe, Bahia and Rio Grande do Norte. It also showed high total levels of flavonoids, between 7.48% (March 2018) and 12.79% (January 2018), with a variable performance, in this test, in relation to the content in the other propolis. The broth microdilution test resulted in MICs of 128-1024 µg/mL against *S. aureus* (ATCC 25923) and 625-1250 µg/mL against *E. coli* (ATCC 25922). *S. aureus* was more sensitive to PVA than *E. coli*. The results against both strains, although high, were lower than those observed in other red propolis varieties from the Brazilian Northeast. The HPLC-DAD fingerprint showed a higher concentration of the markers formononetin, daidzein, liquiritigenin and isoliquiritigenin, compared to biochanin A, pinocembrin and pinobanksin. The analysis of the results with rainfall in the region allowed the distinction of two cycles of better results (April—June 2017 and October 2017—February 2018) from a longer cycle of lower results (July—September 2017 and March 2018 only). In general, biological activities and flavonoid content reduced in the rainy season, increasing significantly with low rainfall.

**Keywords:** Red Propolis; Seasonal variability; Biological activities.

#Acknowledgments to UFAL, FINEP, CAPES, and CNPq, the financing agencies of Brazilian scientific researches.

## Relevance of pH in Microbiological Quality Parameters of Honey from Native Stingless Bees

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### Abstract:

Honey from native stingless bees is used in Argentina for eating and medicinal purposes. *Tetragonisca fiebrigi*, commonly known as yateí, is the most exploited species, and its honey has been included in the Argentine Food Code (CAA, 2019). However, in our experience, the harvest season affects the honey properties, and that's why sometimes it does not achieve the established quality standards. This study aims to analyze physical-chemical and microbiological quality parameters in honey samples from native stingless bees extracted in different periods. Two species of bees, *T. fiebrigi* y *Melipona quadrifasciata*, were studied. Honey was taken from three hives per species from the same meliponary (max. 350 m around). The samples were extracted with sterile syringes from pots in aseptic conditions in dec-2019, mar-2020, jan-2021, aug-2021, and nov-2021. The parameter analyzed were: pH, titratable acidity, mesophilic aerobic bacteria (MAB), coliforms, thermotolerant coliforms, and *Escherichia coli*. *Tetragonisca fiebrigi* honey showed a wide pH range (between 3.40 – 5.98) associated with changes in titratable acidity (between 30 – 60 mEq/Kg) and in MAB count (1.9 – 5.8 log CFU/gram). The increase in pH showed a positive correlation ( $R^2=0.97$ ) with the MAB counts. Thermotolerant coliform bacteria and *Escherichia coli* were positive in the aug-2021 and nov-2021 periods, just when pH exceeded 5 points. In contrast, *M. quadrifasciata* honey presented lower pH values (between 2.6 – 3.8) than *T. fiebrigi*, while acidity was similar (20 – 55 mEq/Kg). MAB counts were also lower (1.6 – 2.8 log CFU/g) and coliform bacteria were not detected. We concluded the season and the harvest year affect the quality of honey, especially in *T. fiebrigi*. The pH has an important influence on the development of the total bacteria, coliforms, and *E. coli*. Nowadays, the CAA does not demand MAB count or pH determination. Given our results, we propose their incorporation as quality parameters in the honey from native stingless bees.

**Keywords:** honey, *Tetragonisca fiebrigi*, quality standards, *Escherichia coli*.

## The Use of Biosensors in the Determination of Diastase Activity in Honey

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### Abstract:

Diastase activity is an important parameter in determining the quality of honey. Diastase is an enzyme naturally found in honey that hydrolyzes starch. The aim of this study is to conduct a glucose oxidase-based biosensor study to determine the diastase activity. In order to determine the diastase activity, a biosensor was designed that measures the amount of glucose formed in the medium after the diastase enzyme hydrolyzes the starch. In this method, glucose oxidase enzyme and hydrogen peroxidase enzyme were immobilized to the graphite pencil tip electrode by bovine serum albumin (BSA) and gelatin. The principle of this method is bienzymatic and it is based on the principle of electrobiochemical measurement of the potential change resulting from the oxidation of hydrogen peroxide, which is formed as a result of the reaction of the glucose oxidase enzyme immobilized on the graphite pencil tip electrode surface, with glucose around +0,70 V with the help of a biosensor. Since glucose is naturally present in honey, glucose in the honey sample was first measured. Afterwards, starch was added to the medium and glucose measurement was made. The amount of glucose between both measurements was subtracted from each other. The glucose concentration in the working medium was found to be proportional to the diastase activity. All solutions were treated with iodine solution for control. When people buy honey, they want to make sure that the honey is real. Therefore, it is very important to find methods that are not laboratory dependent to show the quality of honey. Portable diastase activity measurement devices can be produced by developing biochemical sensors, which is one of the new generation methods that are not laboratory dependent. Electro biochemical biosensor method can be evaluated as an alternative method for diastase activity determination method.

**Keywords:** diastase, honey quality, biosensor



## Genomic DNA Isolation Methods From Honey Bee (*Apis mellifera* L.) Spermatheca

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### Abstract:

The honey bee queen (*Apis mellifera* L.) has a polyandrous mating system, meaning that the queen mates on average with 17 drones from the surroundings in a congregation area. After the mating event, the spermatozoa of the drones are stored in an organ called spermatheca. Genetic analysis of the spermathecal content can provide an estimate of the genetic diversity and purity of the surrounding honey bee populations. This can be particularly useful for conservation and mating centers that need to monitor their populations' genetic backgrounds. However, isolating enough DNA for genomic applications from such a small and complex matrix can be a challenge. Here, we compared the quantity and quality of DNA isolated using five methods: (i) phenol-chloroform-isopropanol, (ii) QIAamp DNA Minikit, (iii) QIAamp DNA Microkit, (iv) Macherey-Nagel Nucleospin Tissue, and (v) NEB Monarch Genomic DNA Purification Tissue. For each kit, when appropriate, variations including different isolation protocols, lysis incubation times, and the addition of RNA carrier were assayed. The quantity and quality of DNA extracted was assessed by spectrophotometric (SpectroStar®Nano LVis Plate) and fluorometric methods (Quantus™ Fluorometer). Spectrophotometric quantification indicated nucleic acid concentrations ranging from 2.00 to 55.58 ng/μL, and in 91.43% of the cases, the A260/280 ratios were over 2.00, indicating an elevated presence of RNA. The fluorometric quantification, specific for double-stranded DNA, provided values ranging from 0.02 to 2.30 ng/μL. From the five methods, two alternative protocols of the commercial kit QIAamp DNA Microkit produced a sufficient DNA quantity ( $\geq 1.7$  ng/μL measured by Quantus) for applications involving SNP genotyping, namely: the Tissue protocol with 6 hours of lysis incubation and the Tissue protocol with 3 hours of incubation, both with addition of RNA carrier. In contrast, overnight lysis decreased the DNA yield. The other methods generally produced low and/or inconsistent DNA recovery. According to our results, QIAamp DNA Microkit with the use of RNA carrier and lysis incubation times between 3 to 6 hours produce the required DNA quantities for SNP genotyping.

**Keywords:** DNA isolation, *Apis mellifera*, spermatheca, conservation

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## An overview of the chemical contaminants in honey

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### Abstract:

Honey is defined as the natural sweet substance produced by honey bees (*Apis mellifera*) from the nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honeycomb to ripen and mature. Honey contains more than 170 substances, and the main compounds are sugars, water, and macro and micro elements. Honey also contains small amounts of phenolic compounds, vitamins, organic acids, enzymes, amino acids and this substance have a high benefit in the human health. Also, many studies demonstrated different therapeutic effects that honey can have (anti-inflammatory effects, antibacterial properties, anti-proliferative effects as antioxidants etc.). On the other hand, heavy metals/metalloids, radionuclides, antibiotics, PAH (polycyclic aromatic hydrocarbons), polycyclic aromatic hydrocarbons, herbicides, and neonicotinoids in honey might pose toxic effects, which would be a risk to human health. According to EU legislation that is in force, the use of antibiotics in beekeeping is forbidden. Residues of pesticides such as organochlorine (OCs) and organophosphorus (OPs), carbamates, and pyrethroids have been detected in honey in different studies. In the present work, overview of recent literature data on chemical contaminants in honey was performed.

**Keywords:** honey bee products; honey; pesticides; toxic elements; antibiotics; radionuclides.

<sup>#</sup>This paper was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia (451-03-68/2022-14/200050 from 04.02.2022)

## Antimicrobial Activity of Bee Bread

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### Abstract:

Bee bread is natural honey bee product obtained from the fermentation of bee pollen mixed with honey bee saliva and flower nectar inside the honeycomb cells of a bee hive. Also, bee bread is defined as a functional food. Primarily, honey bees use bee bread for brood growing (nutrition), but humans use bee bread in apitherapy according to the bread's powerful healing properties and content of different molecules. Bee bread contains about 300 different substances, such as proteins, macro- and microelements, lipids, free amino acids, fatty acids (linoleic, linolenic and arachidonic), flavonoids, phenolic compounds, vitamins, and enzymes. Research on bee bread has been rather limited until now. In recent years, there is an increasing interest regarding the antimicrobial properties of bee bread, due to emerging antimicrobial resistance by different pathogenic microorganism. Different studies demonstrated that bee bread inhibited Gram positive and Gram negative bacteria, virus, yeast and parasite. The high antimicrobial activity of bee bread was correlated to the phenolic content. Phenolic compounds are defined as secondary plant metabolites with protective mechanism. They include a large number of phenolic acids, flavonoids, proanthocyanidins, etc. The main phenolic compounds determined in bee bread were quercetin followed by kaempferol, myricetin and luteolin. This review aims to present up to date research findings regarding antimicrobial activity of bee bread.

**Keywords:** honey bee products; bee bread; functional food; nutritional value; antimicrobial compounds.

<sup>#</sup>This paper was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia (451-03-68/2022-14/200050 from 04.02.2022)

## Synthesis of Green Copper Nanoparticles: Antimicrobial Properties

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### Abstract:

In this study, Chilean honey has been applied to develop green honey-copper nanoparticles (honey-Cu NPs) and evaluate their antimicrobial properties. The synthesized honey-Cu NPs using ascorbic acid, honey, and the assistance of the sonochemical method were characterized by UV-visible, FT-IR, and TSEM techniques. The maximum absorbance was found to be near 600 nm for honey-Cu NP due to surface plasmon resonance. FT-IR spectra confirmed the presence of sugars and proteins in the honey-Cu NPs. The spectral band around 668 and 2900  $\text{cm}^{-1}$  are characteristics of the interaction between Cu and the biomolecules of the honey. TSEM micrographs showed spherical-shaped honey-Cu NPs. ImageJ analysis found the average particle size of the honey-Cu NPs to be  $2.8 \pm 0,5$  nm. It demonstrates that honey has the ability to stabilize and control the size and shape of nanoparticles. The antimicrobial test performed on *Escherichia coli* (gram-negative) and *Staphylococcus aureus* bacteria (gram-positive) showed a  $\text{CIM}_{50}:250$  and  $\text{CIM}_{50}:125$   $\mu\text{g/ml}$  respectively, demonstrating the potential of honey-Cu NPs for infectious diseases caused by tested pathogens.

**Keywords:** Honey mediated, copper nanoparticles, antibacterial.

## Critical levels of Pb and Cd accumulation in the bees' bodies stimulating gatherings of bee colonies

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### Abstract:

The purpose of the study is to establish the cause of autumn-winter gatherings of bee colonies and, in particular, the effect on the accumulation of Pb and Cd in the body of bees. The studies were carried out in laboratory and field conditions. The accumulation of Pb and Cd in meliferous plants, the body of bees and beekeeping products was studied by the atomic absorption method. The specificity of the observed autumn-winter gatherings of bee colonies is expressed in the fact that the bees fly out of the hives one by one at intervals from several seconds to minutes. The gathered bees do not delay at the notch and do not make tentative overflights, which is typical for bees flying out of their hives in the spring-summer period. Flying colonies leave food supplies in the nests, and even brood. Bees react to carbohydrate feed contamination by refusing to consume it. The sensitivity threshold of bees to Pb in a 50–60% sucrose solution is at the level of  $5 \pm 1$  mg/L, and to Cd,  $0.2 \pm 0.05$  mg/L. Gatherings of bee colonies in the autumn-winter period are caused by the accumulation of pollutants in the body of bees. In particular, bees begin to leave the hives when the accumulation of Pb or Cd in their head sections reaches critical levels. Their value for Pb is 1.6–1.8 mg/kg, for Cd, 0.5–0.8 mg/kg. The gathered bee colonies have a high content of Pb and Cd in the winter food reserves left in the nests. Relatively low contamination is typical for honey (~2.5 mg/kg Pb and ~0.1 mg/kg Cd) and high contamination – is for bee pollen (11 mg/kg and 0.3 mg/kg, respectively).

**Keywords:** gatherings of bees, Cd, Pb, critical levels

## Comparative Study of the Prevalence of Nosemosis in Honey Bees in Bulgaria and Estonia

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### Abstract:

Nosemosis is a disease of honey bees, affecting bee colonies worldwide, including in Bulgaria and Estonia. The present study aimed to determine the prevalence of *Nosema* spp. (nosemosis) in Bulgaria and Estonia in 2017, the degree of invasion, as well as to compare the prevalence of nosemosis in countries with colder and temperate climates. For each sample, 60 forage bees were collected from the flying boards of hives. The samples were placed in plastic tubes, cooled immediately for transportation, and frozen at  $-20^{\circ}\text{C}$  until laboratory analyses. Diagnostic methods used to proof spores and identification of *Nosema* spp. - *N. apis* and *N. ceranae*, including light microscopic examination (native and stained smears and counting spores in haemocytometer / flow cytometer) and multiplex PCR. During this period in Bulgaria 114 samples of bees from 82 apiaries located in different regions of the country were studied. The results showed 85 (74,6%) positive for nosemosis samples and 29 (25,4%) negative. Of the positive samples, 47.4% had an invasion rate of 2 to 10 million spores / bee, followed by those with up to 1 million spores / bee ( 17.5%) and the smallest number of samples showed an invasion rate of over 10 million / bee ( 9.6%). Among the 30 apiaries surveyed in Estonia the median number of spores per worker bee ranged from 1.6 to 14 million. *N. ceranae* has replaced *N. apis* in many countries. Estonia seems to be one of the few countries in the world where *N. apis* (43%) is still individually prevalent, while in Bulgaria in 98% of cases nosemosis caused by *N. ceranae* predominates.

**Keywords:** *Apis mellifera* L.; *Nosema* spp., Bulgaria, Estonia, pathogens

**Acknowledgements:** This study was supported by Estonian-Bulgarian joint research project: “Field and experimental studies of actual diseases of honey bees (*Apis mellifera* L.) from Bulgaria and Estonia.”

## Biomonitoring of industrialized areas (tanning industry) in northeastern Italy using bee-collected pollen <sup>#</sup>

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### Abstract:

Honey bees are excellent biomarkers of environmental pollution. Sentinel apiaries were positioned in areas close to tanning industries and in “white” hilly areas located in north-eastern Italy. Between April and September 2021, nine pollen samplings have been carried out and analyzed for heavy metals, volatile organic compounds (VOCs) and pesticides. Heavy metals were searched with inductively coupled plasma mass spectrometry (ICP-MS), VOCs with solid phase microextraction followed by gas chromatography coupled with tandem mass spectrometry (SPME-GC-MS/MS) and pesticides with liquid and gas chromatography coupled with tandem mass spectrometry (LC- and GC-MS/MS). The presence of heavy metals does not appear to be significantly different between control and industrial areas and no elements have been detected in relevant concentrations. VOCs have also been detected in both study areas, being volatile compounds, the meteorological conditions, the atmospheric pressure, the precipitations, and the air currents contribute to carry these pollutants even towards less exposed areas. The industrialized areas show greater contamination both in terms of the amount of volatile compounds and concentrations detected. Regarding pesticides, the greatest contamination occurs in industrialized areas, which border on agricultural areas and coincides with the season of greatest crop treatment. This preliminary study has highlighted the ability of different pollutants to diffuse into the environment, the sampling scheduled for 2022 will be able to provide further data and information in order to improve our knowledge in this territory thanks to the role of bees in environmental monitoring.

**Keywords:** Bee-collected pollen, heavy metals, VOCs, pesticides.

<sup>#</sup>The project is financially supported by the Province of Vicenza (Italy) and carried out in collaboration with the Beekeepers Association of Vicenza province.

## Establishing of Taxonomic Affiliation and Hybridization of Honeybees (*Apis mellifera* L.) in Belarus Using Molecular Genetic Methods

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### Abstract:

Traditionally, morphometric techniques are used to differentiate and evaluate the purity of bee colonies. However, in the conditions of increasing crossbreeding of bees, traditional methods of identification are not enough, and it is necessary to use DNA analysis techniques. Belarus has launched systemic molecular-genetic studies of bees. The purpose of this study: accurate DNA identification of honey bees in Belarus, as well as purity and hybridity evaluation. An individual DNA analysis of apiary worker bees, as well as forest populations of the south and north of Belarus, was carried out. Analysis of the COI-COII locus of mitochondrial DNA (PCR, Dra-I test) was performed. Polymorphism of nuclear DNA microsatellites was analyzed. Statistical data were processed using the STRUCTURE v.2.3.4, GenAIEx v.6.5 software package. Analysis of polymorphism of the mtDNA COI-COII locus revealed two variants — PQ and Q. The PQ variant is characteristic of the subspecies *A. m. mellifera* (evolutionary branch M), while the variant Q is characteristic of the species of southern origin (evolutionary branch C). It was established that most of the studied apiary bee colonies originate from subspecies *A. m. caucasica*, *A. m. carnica*, and *A. m. ligustica*. DNA analysis data differed from classical morphometry data. Analysis of the variability of microsatellite loci of the nuclear genome made it possible to identify a complex of five highly effective molecular markers with the highest, according to the  $F_{ST}$  criterion, differentiating potential (A88, A24, HB-C16-05, A113, HB-THE-03). The clustering accuracy was 79.3-99.3%. Bee colonies with a high and medium degree of crossbreeding were identified. The calculated FIS value (0.107 on average) indicated the predominance of heterozygotes in the studied bee colonies, and the  $H_o < H_e$  value indicated an intensive interbreed hybridization process.

Bee colonies with the PQ locus variant (Western European origin, *A. m. mellifera* subspecies) were found. The Dra-I test showed that the haplotype of *A. m. mellifera* in the southern regions of Belarus differs from the haplotype of *A. m. mellifera* in the northern regions of Belarus. In Europe, *A. m. mellifera* is represented by a large number of ecotypes. Each ecotype has a number of genetically determined traits that allow adapting to certain living conditions. Further studies of forest populations of *A. m. mellifera* will be performed and genetic features associated with adaptation to local climatic and honey harvest conditions of Belarus will be determined. Under the conditions of increasing hybridization of bees, classical morphometry data are not enough to establish the exact subspecies affiliation. A set of five SSR markers with high differentiating potential was determined. The clustering accuracy was 79.3-99.3%. An intensive process of interbreed hybridization was established. Based on the DNA analysis, the existence of populations of the subspecies *A. m. mellifera* was reliably established for the first time in Belarus.

**Keywords:** *Apis mellifera* L., hybridization, identification methods, mitochondrial DNA analysis, nuclear DNA analysis.



## Bee food: are the supplements and substitutes quality granted?

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### Abstract:

Honey bees are strongly dependent, to obtain pollen and nectar for their development and survival, upon the availability of floral resources. Unfortunately, loads of foraging areas are nowadays altered by the landscape modification and the agriculture intensification. Furthermore, the beekeeping sector is facing multiple criticalities involving, among others, climate change, new pests, and parasites. The beekeepers frequently perceive supplemental feeding as a highly effective strategy to reduce multiple stressors risks for their colonies. On the other hand, it is extremely complex to understand the real nutrition needs of a honeybee colony and make the right choice among the countless proposal of bee feed supplements or substitutes offered by the market that present highly variable and sometimes even undefined composition. The COLOSS (Colony LOSSes, Honey Bee Research Association, [www.coloss.org](http://www.coloss.org), Switzerland) and NUTRITION Task Force (part of COLOSS activities, [www.coloss.org/projects/nutrition/](http://www.coloss.org/projects/nutrition/)) members, are actively working to propose an action on honey bee feed control and monitoring. This Task Force is elaborating a methodology to study bee aliments and is creating a network of laboratories using the proposed protocols and methodologies. More importantly, it is promoting a close interaction to the multiple stakeholders to understand the type of analyses requested, depending on their real needs. Finally, the COLOSS Nutrition Task Force will elaborate guidelines to support and assist food companies and regulators to proceed the effective quality and safety control of honey bees' supplements and substitutes.

**Keywords:** Honey bee, nutrition, supplements, quality, monitoring



## **Ethanolic extracts of propolis from *Apis mellifera* and *Scaptotrigona mexicana* in the inhibition of pathogenic microorganisms in the silage of tropical pastures**

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### **Abstract:**

Propolis are natural resins collected by bees in a physical way that forms a barrier that seals cavities and prevents the growth of bacteria and other pathogenic microorganisms, due to its phenolic compounds: Flavonoids, Flavones, Isoflavones and Flavonones up to 50%. That gives their inhibitory capacity against bacteria, fungi and yeast. Silage is vulnerable to pathogenic fungal and bacterial contamination from the growth of microorganisms such as bacteria, yeasts and filamentous fungi such as *Fusarium*, *Aspergillus*, *Penicillium* and others. Etiological cause of diseases in animals or their products for human consumption. Propolis extracts from two species of bees (*Scaptotrigona mexicana* and *Apis mellifera*) were evaluated as inhibitors of contaminating microorganisms in the silage process of *Panicum maximum* and *Digitaria Zwazilandensis*, which showed up to 90% inhibition ( $p < .05$ ), against bacteria and fungi at *in vitro* tests. It was found that type of grass silage is related to inhibition capacity of propolis. Physicochemical and organoleptic characteristics are related to the inhibition of microorganisms included and by the type of propolis studied ( $p < 0.05$ ).

**Keywords:** Native bees, forage, microbial contamination

## Analytical Method for Pesticide Residues Quantitation in Honey by LC-MS/MS

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### Abstract:

The concern with the management of pesticides is related to both food safety and damage to the environment. Bees are extremely sensitive to environmental disturbances and its products (such as honey) can be used as bioindicators to define impacts resulting from anthropogenic activities once bees can transport pesticide residues to the hive and contaminate it. The development of analytical methods for the detection and quantification of residues of these contaminants is essential. Honey is a complex matrix with more than 200 known substances. Therefore, to analyze pesticide residues in this matrix, the analytical method should be developed and validated to provide reliable results. This study proposes a method for the quantification of six insecticides in honey (cypermethrin, permethrin, dimethoate, imidacloprid, clothianidin and chlorpyrifos). The use of the Plackett Burman approach in the sample preparation step proved to be effective in reducing the matrix effect. Thus, a modified QuEChERS method followed by LC - MS / MS analysis was validated in accordance with the European Union SANTE 11813/2017. The method was applied for the determination in 51 samples of honey from different botanical origins and countries. The occurrence of residues was verified in 37% of the samples, with residues of imidacloprid, clothianidin and dimethoate.

**Keywords:** pesticide residues; honey; analytical methods; LC-MS/MS

## ISOLATION OF YEAST IN PROPOLIS AND GEOPROPOLIS FROM NATIVE BEES WITH FERMENTATIVE POTENTIAL

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### Abstract:

Bees are very important pollinators of cultivated plants all over the world, guaranteeing diverse ecosystem services and having great economic and ecological importance. Native stingless bees produce honey, wax, cerúmen, propolis and geopropolis, in addition, inside the hive there are many organisms that live in symbiosis with bees. Among these organisms are yeasts, which helps with pollen fermentation, increasing the nutritional value and the preservation of the hive. Yeasts are interesting for biotechnology because these organisms are explored in pharmaceutical, food and alcoholic beverage industries. Within the alcoholic beverage market, the brewery stands out and has been growing in Brazil and in the world with the development of new technologies and brewing production techniques. Despite the size of this market, Brazil has few national supplies for beer production, including the most used yeast strains. In this context, the ongoing work aims to isolate and analyze yeasts present in the propolis and geopropolis of native stingless bees, and to explore their fermentative potential. Thereunto, the yeasts were isolated from 12 samples of 9 different bee species that were collected in two meliponaries. Yeasts were found in 8 samples, and submitted to fermentative tests. The results showed the presence of yeasts in propolis associated with native bees in 6 species and fermentative potential of approximately 46% of the isolated yeasts, being those of the species *Melipona rufiventris*, *Partamona críptica*, *Plebeia droryana*, *Scaptotrigona bipunctata*, *Melipona quadrifasciata* e *Plebeia remota*. New additional tests will still be developed, but the preliminary results so far are very promising regarding the biotechnological potential of these yeasts. Furthermore, the exploitation of this microbiota should still add value to by-products derived from bees, encouraging their conservation.

**Keywords:** Bees; Propolis; Yeasts; Fermentation.

\*This project is financially supported by the Office of Provost of Culture and Extension of University of São Paulo.

## Modeling pesticide exposure on honeybee population dynamics with seasonality

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### Abstract:

One third of the food that we consume relies on pollination by bees for its production. Pollination by honey bees is valued between \$15 and \$20 billion annually in the U.S. Substantial evidence indicates population of honey bees are declining in Europe and the Americas. Honey bees being exposed to pesticides has been considered as one key factor in the causes of declining. Motivated by experiment work in Harrison lab, we developed a five-dimension age structure compartmental model (delay differential equations) for honey bee and pollen/pesticides with seasonality equation in Queen egg-laying rate to explore (1) how may pesticide influences colony dynamics by regulating pollen consumption rates and mortality rates of brood level bees and adults; and (2) how seasonality of queen egg-laying may contribute to colony dynamics with pesticides exposures. Our results suggested that 1) pesticide can influence the colony population, for example, honey bee population decline more; 2) pesticide may influence the nutrient absorption; 3) pesticides may cause colony to respond less to seasonality. Our model is validated with experimental data to provide useful insights of proposed questions above and suggest that we should consider the possible negative impacts of pesticides exposure on pollinators in the light of seasonality.

**Keywords:** pesticides, seasonality equation, delay differential equations model, experimental data fitting

This research is partially supported by NSF-DMS (Award Number 1716802\&2052820); NSF- IOS/DMS (Award Number 1558127) and The James S. McDonnell Foundation 21st Century Science Initiative in Studying Complex Systems Scholar Award (UHC Scholar Award 220020472); USDA NIFA (Award Number 2022-67013-36285) and ASU Graduate Complete Fellowship.

## Impacts of Climate Change on the Honeybee-Parasite System

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### Abstract:

Both seasonality and parasites have huge impacts on honey bee population dynamics. To explore impacts of seasonality and parasite, we develop honeybee-only and honeybee-parasite models with or without seasonality to explore (1) how seasonality affects the colony dynamics and (2) how parasites affect the colony dynamics with/without seasonality. Our preliminary work suggests that the length of seasonality and strength of seasonality have huge impacts on colony dynamics. More specifically, (a) The length and strength of seasonality influence each other on colony dynamics and the effects are not monotonic. (b) In the honeybee-only model, there exists a critical value of seasonal strength. Under the critical value, increasing the length of seasonality can survive the colony; conversely, above the critical value, increasing the length can collapse the colony. (c) In the honeybee-parasite model, either increasing the length of seasonality under the critical value of the strength of seasonality or decreasing the strength of seasonality under the critical value of the length of seasonality can make the colony survive. (d) lower conversion rate of mites can have both mites and honey bee survive. Our results exposure the climate has the ability to support or suppress the colony survivor depending on varied conditions. It is important to control the positive impacts of the climate on the colony survivor.

**Keywords:** climate change, parasitism, seasonality equation, mathematical modeling, computational simulations

This research is partially supported by NSF-DMS (Award Number 1716802\&2052820); NSF- IOS/DMS (Award Number 1558127); The James S. McDonnell Foundation 21st Century Science Initiative in Studying Complex Systems Scholar Award (UHC Scholar Award 220020472); USDA NIFA (Award Number 2022-67013-36285) and ASU Graduate Complete Fellowship.

## Health properties of crushed honey

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### Abstract:

In recent years, an increase in bacterial resistance to the antibiotics has been observed. It is related to the problem of treating people suffering from various diseases. Scientists are looking for natural compounds that would fight bacterial and fungal diseases. One of such substances is honey. The aim of our experiments was to check the selected physicochemical and antimicrobial properties of three crushed honeys taken from the urban area, i.e. Urban Artistic Apiary (summer - sample A; spring - sample C) in Lublin and from the rural area, i.e. Czerniejów (sample B). The pH and electrical conductivity were checked according to the methodology provided by the International Honey Commission Methods (2009). The colour of honey was determined using the Pfund scale. The antibacterial activity of honeys against *Staphylococcus aureus*, *Escherichia coli*, *Serratia marcescens* and *Pseudomonas aeruginosa* as well as antifungal activity (*Aspergillus niger*) was checked by radial diffusion based on the zones of inhibition of growth of a given bacterium or fungus. On the basis of the obtained results, samples of honeys B and C were dark amber in colour, while the sample of honey A was amber. Honey from the Urban Artistic Apiary collected in spring was characterized by the highest electrical conductivity, 161.6  $\mu\text{S}/\text{cm}$ , and the highest pH, ie 4.83. Moreover, this honey showed the highest activity against *E. coli* (6.5 mm) and *S. marcescens* (4.1 mm). Honey from rural areas inhibited *S. aureus* (4.7 mm) and *E. coli* (6 mm). In contrast, honey sample A was only active against *E. coli*. No activity was observed against *P. aeruginosa*. It was also found that the tested honeys show a similar activity against *A. niger*. Due to the wide spectrum of activity, the above-mentioned honeys may be an alternative to antibiotics used in bacterial or fungal diseases in the future.

**Keywords:** honey, antimicrobial activity, antifungal activity, crushed honey

## Antifungal activity of poplar, green and red propolis: a screening study in preservation of table grapes

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### Abstract:

Propolis is a resinous mixture, with a complex composition, collected by honey bees from leaf buds, shoots and petioles of leaves from different plants, being constituted by exudates, components of bee metabolism, pollen and waxes. Propolis has been described as having a broad spectrum of biological properties, including antimicrobial, antioxidant, anti-inflammatory, anti-tumor and anti-neurodegenerative, among others, and these are specific to their botanical origin. This multifunctionality of propolis can be explored for the development of different applications in fields such as food preservation, offering a promising alternative to the global and growing use of synthetic preservatives that are known to considerably harm human health. The aim of this work was to evaluate the antifungal activity of the phenolic propolis extracts obtained from the most common types: European propolis from *Populus* (PP), Brazilian green (BGP) from *Baccharis* and red propolis (BRP) from *Dalbergia/Clusia*, having in mind its potential application as a preservative in food, replacing synthetic additives. A preliminary screening of antifungal activity was done in vitro, using the agar diffusion method. Propolis extract was tested at concentrations ranging from 0.5 to 15 g/L. Several fungi isolated from grapes were tested: *Alternaria* sp., *Botrytis cinerea*, *Cladosporium* sp., *Penicillium* sp., *Aspergillus carbonarius* MUM04.46, and *Aspergillus carbonarius* MUM04.52. Commercial fungicides were used as positive controls. In this screening test, PP and BRP, at 15 g/L, showed antifungal activity and were selected to be tested on red and white table grapes for the ability to reduce fungal growth and fruit rot. Grapes were immersed in the extracts, inoculated with fungal spore suspensions and stored for 15 days under refrigeration. The fruit rot diameter was measured after 15 days of storage. The propolis extracts has variable effects on the different fungi. PP and BRP generally showed antifungal activity when compared with the negative control, but the difference was only significant for *Botrytis cinerea*.

**Keywords:** propolis, antifungal activity, food preservative, grapes

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## Mead production utilizing wild yeast from stingless and solitary bees

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### Abstract:

The interactions among bees and wild yeast are poorly understood, especially when considering stingless bees and solitary bees, with an enormous variety of not yet identified yeast living together with different species of bees. This work aims in exploring the potential of wild yeast for isolation and characterization of their fermentation capacity and the potential of producing alcoholic beverages. For that, we made an initial sample collection of yeasts from native Brazilian stingless bees and also some solitary bees. After the growth period, we performed a yeast selection, since they required resistance to osmotic stress and ethanol stress, as both were present in the wort utilized in this work. By means of growth in high dextrose conditions, and in alcoholic conditions similar to that of the final product we were able to obtain a variety of yeast, and we chose 2 among them for the next step. One of them originated from the mature honey of *Melipona quadrifasciata* bees, the other one originated from the larval food of the *Euglossa* sp., and in the end, we were able to obtain two distinct meads, one of which possessed 7,5% of alcohol percentual, and had a sweet profile, while the second one had 16% of alcohol percentual and had a floral profile and tasted like peach. This initial step indicates the great potential for exploitation of these organisms, in several different fields, like biotechnology and space exploration.

**Keywords:** yeasts, native bees, mead.

# This project is financially supported by the Office of Provost of Culture and Extension of the University of São Paulo.



## Floral visitors of *Thevetia peruviana* (Pers.) K. Schum in the Soconusco of Chiapas, Mexico

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### Abstract:

*Thevetia peruviana* is a native species of Central and South America that can function as a reservoir plant of many food resources for the attraction of beneficial insects for the pollination of this and other species. Knowledge about the entomophilous fauna that surrounds *T. peruviana* can provide information to help its maintain and establishment, as well as the conservation of the insects native visiting. The objective of this research was studying the floral visitors and type of visitors of *T. peruviana* in the South of Mexico. The study site is a traditional agricultural landscape in the city of Tapachula, in the Soconusco region, Chiapas, Mexico. The visiting insects were collected from 10 plants for 2 min from 6:00 a.m. to 6:00 p.m. for 20 min intervals with entomological nets for identification. The visiting insects were classified as pollinator, nectar collector, resin collector and catcher by observations considering the following criteria: behavior in the flower, percentage of individuals collected by species, time of visit, frequency of visit, movement of a flower to another and identification of the resource collected. The diversity of visiting insects of *T. peruviana* flowers consisted of 16 species, which were grouped into three orders, three families and 11 genera. The most diverse and dominant family was Apidae with 87.5 % and 89.19 %, respectively. The most diverse genera were *Trigona* and *Xilocopa* (17.64 %), followed by *Euglossa* and *Eufriesea* (11.76 %). The most dominant genera were *Trigona* and *Euglossa* (IAR = 35.86 % and 30.93 %, respectively). Among the insects visiting the flowers were bees, wasps, and a beetle, of which pollinators, efficient nectar collectors, resin collectors and nectar robbers were recognized. The highest activity of visits was 7:00 a.m. to 10:00 a.m. Similarly, the greatest species richness was recorded from 7:00 a.m. to 11:00 a.m. The results of this research could guarantee the conservation of native insects and pollinating potentials through *T. peruviana* cultivation areas, since the plant guarantees a source of resources necessary for the development and conservation of these insects, so it is necessary to establish strategies that allow it to maintain and establishment.

**Keywords:** insects, floral visitors, pollinators, robbers, corolla.



## Preparation and characterization of green propolis granulates from Alagoas-Brazil

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### Abstract:

Propolis is a resinous substance, chemically complex, made by bees, whose variability and physicochemical characteristics are dependent on the environment where they are produced. In view of, the natural products market has attracted the attention of alcohol-free solid dosage forms, the aim of this work was to obtain and characterize granules of Commercial Green Propolis of Beekeeping of Alagoas. It was developed pharmaceutical granules by cold granulation and subjected to characterization processes. In this way, a degree of encapsulation ranged between 71.92 and 83.27%. The antioxidant activity ranged between 92.94% and 97.55% at a concentration of 100 µg/mL with IC50 values of 12.78 µg/mL for the extract and between 18.47 µg/mL and 26.13 µg/mL for granules. The total phenol content ranged between 9.06% and 9.49% and the total flavonoids showed values between 3.17% and 3.5%. Therefore, the samples evaluated were within the standards established by Brazilian legislation. The thermal analysis data showed mass loss processes in the TG curves between 50°C and 150°C and in the DSC curves endothermic volatilization enthalpies that are suggestive of loss of volatile components such as cinnamic acid esters present in the green propolis extract. Furthermore, by SEM it was possible to identify the formation of spheres with a rough surface and particle size below 100 µm. In view of the results obtained, it was possible to observe the importance of monitoring the quality of green propolis encapsulates to avoid losses due to volatilization or decomposition during processing steps and the need for special conditions of packaging and storage of the extract in solid form.

**Keywords:** Green Propolis, pharmaceutical granules, physicochemical characterization and quality control.

**Acknowledgements:** UFAL, FAPEAL, CAPES, CNPq, FINEP.

## Investigating the Correlation Between the Antioxidant and Colour Properties of Honeys of Different Botanical Origin

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### Abstract:

Honey is a natural sweetener that has been consumed over thousands of years for its high nutritional value and therapeutic potential. Mainly due to its phenolic compounds, honey serves as a source of antioxidants in human nutrition. In our study, the total phenolic content (TPC) and total flavonoid content (TFC) of sixteen honeys of different plant sources were determined spectrophotometrically, the colour of which varied between dark amber and light yellow. The CIELAB colour parameters ( $L^*$ ,  $a^*$ ,  $b^*$ ) of the products were measured by a Minolta CR-100 device. The linear relationships between the tested parameters were investigated by Pearson correlation. A great variance can be observed between the honeys in terms of TPC and TFC. Based on our results, buckwheat honey is by far the richest in phenolic components (143.67 mg GAE (gallic acid equivalent)/100 g), while acacia, silk grass, phacelia and chestnut honeys appear to contain relatively low amounts of polyphenols (<10 mg GAE/100 g). The flavonoid content of honeys range between 3.19 and 46.57 mg QE (quercetin equivalent)/100 g. A strong positive correlation can be observed between the total phenolic and flavonoid content of honeys. Our results confirm that the colour of honeys is affected by the amount of polyphenols, because negative correlations were observed between the lightness values ( $L^*$ ) and total phenolic contents as well as between the lightness values ( $L^*$ ) and total flavonoid contents. As consumers prefer light honeys to dark honeys in many countries, there is a need for informing consumers about the potential health-beneficial properties of darker honeys including buckwheat, honeydew, goldenrod, larch and coriander honeys.

**Keywords:** honey; colour; phenolic; flavonoid; Pearson correlation

This research was supported by the National Research, Development and Innovation Office of Hungary (OTKA, contracts No. 135700).

## The Red Propolis of Alagoas State/Brazil:

### A study of the production and value chain

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#### Abstract:

The present work is a preliminary study of the production and value chain of a beekeeping product in the State of Alagoas-Brazil, with emphasis on the Red Propolis of Alagoas, the first in Brazil to receive the Geographical Identification (Appellation of Origin). The goals were to map and identify the characteristics of the red propolis production and value chain: its potential, difficulties faced, infrastructure, governance, appropriate technologies, talents, territorial area of production and other aspects. The research allowed to evaluate the level of integration between the red propolis value chain with the government, research and teaching institutes, universities and S System units (SEBRAE and others). For the development of the study, an extensive data collection was carried out, involving several references from master's dissertations, doctoral theses and scientific articles on R&D, information that contributed to the creation of a questionnaire. Still, in an exploratory way, *in loco*, visits were made to companies and beekeepers, and government agencies, interviewing managers, entrepreneurs, technicians, researchers and specialists. From this initial diagnosis of the interviews, action proposals will be developed to enhance the production and value chain, with short, medium, long-term actions and respective indicators, which will be transformed into a governance plan for this productive sector, with incentives for the productive chain of Honey and value chain of Red Propolis in the State of Alagoas-Brazil.

**Keywords:** Red Propolis from Alagoas/Brazil. Geographical Indication in Beekeeping. Value Chain in Beekeeping.

**Acknowledgements:** PROFNIT, CAPES, CNPq, FAPEAL.

## Effectiveness of Honey From Bees *Melipona Beecheii* And *Melipona Solani* As Alternative Treatment For The Healing Of Neuropathic Diabetic Foot Ulcers In Wagner Stages I And II

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### Abstract:

Diabetes mellitus represents high costs for health systems worldwide due to complications derived from poor control of the disease and inaccessibility to medical services. The appearance of chronic ulcers due to neuropathy is one of the main complications and the treatment of which can be expensive. The aim of this study was to evaluate the effectiveness of *Melipona beecheii* and *Melipona solani* stingless bee honeys in healing ulcers in diabetic patients. Twenty diabetic patients with ulcers in Wagner stages I and II were selected, who agreed to participate through informed consent. Treatments Mb, n=5 patients, Ms, n=5, Mb+U (n=5) and Ms+U (n=5) were randomly assigned. Through a registration table, the characteristics of the wound and the effectiveness of the treatments were followed up. The average number of weeks for patients to be discharged was  $7.6 \pm 5.1$  (mean  $\pm$  standard deviation). A positive correlation was found between the rate of epithelialization and the presence of bacteria. The permutation test showed no significant differences in epithelialization rates between treatments (F3, 20=0.03, p=0.98). This study demonstrates that honey from stingless bees contributes to the healing of lesions in diabetic patients. When contrasting our results with previous studies in *Apis mellifera* honeys, a higher epithelialization rate is observed: 3.3 cm<sup>2</sup> per day compared to 0.019 cm<sup>2</sup> per day, respectively. The cost of treatment with honey per patient was on average of \$150 Mexican pesos while the conventional treatment It costs approximately \$2,000. Thus, we argue that honey from native bees should be seriously considered as a low-cost alternative for the treatment of diabetic foot ulcers in Mexico.

**Keywords:** Stingless bees, Native bees, Diabetic patients, Epithelialization

## Amitraz Residues in Honey: A case study of Natural Park of Montesinho

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### Abstract:

Bees and other pollinators are decreasing in abundance in many parts of the world mainly due to intensive farming practices, mono-cropping, excessive use of agricultural chemicals, the presence of other environmental contaminants, and higher temperatures associated with climate change, affecting not only crop yields but also other variables such as nutrition and bee diseases. The Varroa destructor mites are a major threat to bees around the world. Amitraz is a formamide exhibiting both acaricidal and insecticidal activity and is frequently used by beekeepers to protect honeybee colonies against Varroa destructor mites. The application of acaricides in honeybee colonies leaves residues in honeybee products. Most acaricides are fat-soluble which means they can accumulate in wax and could be transferred into honey. The issue of residues can raise concerns in beekeepers and consumers who normally see honey and beeswax as pure products. This study aims to monitor the presence of amitraz residues in honey samples from the Natural Park of Montesinho in Portugal. For the first time, a modified QuEChERS approach for the amitraz extraction was validated and applied to 20 honey samples. The method was evaluated regarding the linearity, precision, recovery, and matrix effects. The results proved that ultrasound extraction coupled with one-step QuEChERS was the most efficient method. In this study, we used GC-MS instrument for the identification/quantification of amitraz and its metabolite (2,4-dimethylaniline). In a quantitative validation, acceptable performances were achieved with good linearity (>0.99) acceptable recovery and <20% RSD. After analytical validation, the methodology was applied to 20 honey samples. No amitraz residues were detected in the honey samples, however, its metabolite was identified by GC-MS in several honey samples.

**Keywords:** Amitraz, Honey, Monitoring, QuEChERS, GC-MS

### Acknowledgment

This work was funded by the project MTS/SAS/0077/2020 - Honey+ - New reasons to care honey from the Natural Park of Montesinho: A bioindicator of environmental quality & its therapeutic potential.

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## DOES ROBBER BEES HAVE A HIGHER POTENTIAL FOR ANTIMICROBIAL ACTIVITY IN THEIR PROPOLIS? A CASE STUDY WITH *LESTRIMELITTA LIMAO*

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### Abstract:

In stingless bees, wax is normally mixed with propolis and resins forming cerumen, which presents better rigidity and is used in constructive functions in hives, like food pots, brood combs, nest entries, and involucre. Propolis is the most important chemical defense against pathogens within the nests, used to close walls, cracks, and to embalm the bodies of dead invaders. More than three hundred chemical constituents were identified in propolis, although propolis from stingless bees is poorly studied. Evolutionarily, several species have lost the characteristic of collecting resources in flowers and plants, and parasitize or pillage nests of other bees, a behavior known as kleptoparasitic behavior. Among these species, *Lestrimelitta limao*, a stingless bee, is mostly unwanted and often exterminated by beekeepers. Among the main targets of *L. limao* are the species *Melipona quadrifasciata* and *Tetragonisca angustula*, the two most bred species in the Brazilian meliponiculture, whose propolis and wax, which are plundered by *L. limao* bees, have interesting pharmacological properties. The materials of the plundered nests are mixed in the *L. limao* nests and used to build nest structures. Based on this information, the objective of this work is to investigate the antibacterial activity of propolis and cerumen. For this, we used the agar diffusion disk methodology and minimum inhibitory concentration (CIM), performing the tests in comparison with propolis from *M. quadrifasciata* and *T. angustula*. Data on agar diffusion disk indicates no differences among the three compared species, but the CIM tests indicate a greater efficiency of the *L. limao*, when compared to the two normally robbed species. The next steps are to evaluate the efficiency of cerumen in antimicrobial activity, since *L. limao* bees use large amounts of this material in the nest construction and can be used as a strong supplier of this material.

**Keywords:** Propolis, Antimicrobial Activity, *Lestrimelitta limao*

## Selection of Strategies for Voluntary Engagement in the Formulation of Citizen Science Projects

### A Suggestion for Bee Conservation Projects

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#### **Abstract:**

The main objective of the work is to propose the best strategies for engagement and voluntary permanence in a national scenario for the development of citizen science projects involving the study and conservation of native bees, through a bibliographic review on the theme "Citizen Science", a fairly young term in academic literature and consistently cited today as an effective method for disseminating scientific knowledge, promoting awareness of sensitive topics, and ensuring action and participation by large groups in social and scientific movements.

Considering the prominence of this method of scientific production, which fundamentally depends on the voluntary and effective participation of partners outside the usual means of academic production, it was necessary to understand which examples of methodological strategies bring greater engagement and commitment on the part of the participants. Thus, through a systematic literature review, exploring different works carried out over the last decades in different countries and fields of science, a selection of suggestions for materials and methods focused especially on the task of maximizing the engagement of voluntary participants who initially do not master data collection and analysis techniques.

A summary of the most successful strategies among those presented is also presented, as well as a suggestion of a methodological tool developed in the Brazilian scenario and its characteristics, according to the planning, design and application choices in future research.

**Keywords:** Voluntary Participation; Engagement; Scientific Communication.



## The functional study of honey bee (*Apis mellifera*) olfactory receptors that detect phenethyl acetate, which triggers hygienic behavior<sup>#</sup>

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### Abstract:

Chalkbrood is one of the rapidly spreading diseases in honeybees (*Apis mellifera*) caused by a fungal pathogen (*Ascosphaera apis*). The disease significantly represses brood growth and reproductivity of honey bee colonies. Phenethyl acetate has been known to be a key compound associated with chalkbrood-infected larvae that induce hygienic behavior. The mechanism of hygienic action is triggered by the detection of chemical stimuli in diseased larvae and proceeds with the removal of infected broods from the nest. Insect odorant receptors (ORs) are responsible for detecting of the relevant cues from diverse odorants including pheromones. Previous studies in *Drosophila melanogaster* odorant receptors (DmOrs) have revealed that DmOr67a (NP\_524005) and DmOr85d (NP\_524281) act as phenethyl acetate receptors. Five candidate odorant receptors in *Apis mellifera* (AmOrs) were selected via protein BLAST search (blastp) of those receptors. In addition, one more candidate olfactory receptor was selected through the phylogenetic tree of *Aedes aegypti* receptor AaOr15 (NP\_001345110.1), *Culex quinquefasciatus* receptor CqOr4 (XP\_001842214.2) with AmOrs and DmOrs. 6 candidate odorant receptors for phenethyl acetate were cloned and tested against 8 odorants stimuli by using *in vivo* cell calcium imaging after transfection into HEK-293 cells. Experiments were carried out using AmOr B, D, and F, three of the six candidate receptors, and they all showed high reactivity to phenethyl acetate.

**Keywords:** chalkbrood, disease, odorant, cell response, calcium imaging

<sup>#</sup>This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (PJ014762)" Rural Development Administration, Republic of Korea.

## Overview Of Manufactured Goods Derived From Stingless Bees Products In The Brazilian Market

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### Abstract:

Brazil is the country with the greatest biodiversity of stingless bees. They are essential for the maintenance of natural habitats and for the possibility of commercial exploitation of their products, which can be obtained directly from meliponiculture (stingless beekeeping), such as honey, pollen, propolis, or via indirect manufacture of goods. The present study aimed to find the widest variety of goods derived from Brazilian stingless bees for sale over the internet to the general public. The searches were carried out on several websites and marketplace platforms. Products from all over the country were found, mainly food (7), medicinal (6), and personal hygiene (6). Food products were the most diversified, ranging from honey bread and chocolates with stingless bee honey to drinks such as beers, meads, and kombuchás. Among the medicinal and personal hygiene products, moisturizing, healing, and burn ointments and a myriad of body soaps were found, products that could contain honey, propolis, and/or stingless beeswax. The species that provided the resources for the products are *Melipona mondury*, *Melipona quadrifasciata*, *Tetragonisca angustula*, *Melipona bicolor*, *Scaptotrigona* sp., and *Melipona compressipes*. Although the raw materials come from all over Brazil, the main states involved in the commercialization are Rio de Janeiro, São Paulo, Espírito Santo, and Pará. The state of São Paulo has the largest number of brands (8). Meliponiculture has become more popular and, as an activity of great socioeconomic and environmental potential, has been growing beyond the commercialization of direct products, presenting a diversification in derivative products. Few brands sell products derived from stingless bees, and there is still a great possibility of expansion into this market.

**Keywords:** meliponini, beekeeping economy, bees products, innovation, Brazilian market

#If the study financially supported, the information should be given here.

**Behavioral dynamics of pollen storage in *Melipona quadrifasciata***

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**Abstract:**

Stingless bees (Apidae, Meliponini) make up an important group of eusocial insects, occurring in tropical and subtropical areas of the planet. The fermentation process that occurs with pollen collected by stingless bees during storage is little known and described in the literature. Therefore, the objective of this work was to observe the behavior of pollen storage inside the pots in the colonies, in order to obtain information that helps in the understanding of its fermentation process. The observation was carried out for 30 days, with an observation colony of *Melipona quadrifasciata*, from the Meliponário Experimental da Universidade Federal de São Paulo, on Diadema campus (Brazil – SP). Every day, observations were carried out in the morning and evening. The pollen pots were marked daily and an energy supplement was offered on the first day of the experiment, with sucrose syrup. According to the observations, we visualized that the pollen pots analyzed during the experiment were not closed for long periods. The observations reveal that there is a lack of pattern regarding the opening and closing dynamics of pots. The work of bee workers moving constantly in and out of the open pollen pots. This may indicate a continuation of the fermentation process in relation to the exchange of gases and even maintenance of the flow of nutrients. However, it should be noted that there is a great diversity among stingless bees, which may indicate variables in behavior. We were able to conclude in a preliminary way that when it comes to the behavior of storage and work with pollen in species of *M. quadrifasciata* bees, it is quite remarkable the fact that there is no regularity in the treatment of this food resource. Data such as these are of extreme importance for the advancement of techniques that improve beekeeping throughout the country, such as the development of protein supplementation for stingless bees.

**Keywords:** pollen, stingless bee, behavioral, fermentation

**Financial support:** Capes

## Development Of Rapid Test Kit For Pure Honey Kelulut

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### Abstract:

This study has designed and developed a portable rapid test kit that is suitable for consumers usage to detect adulterated honey. It is user-friendly and very fast, the result can be obtained in 3 seconds. There have been many devices developed to identify pure honey, however, the detection methods used required expensive analytical instruments, and can only be performed in the laboratory by competent personnel. In this study, the device is intended for public usage to safeguard their right as a consumer to detect the honey product that they procured in the market is authentic. The device used an infrared detection circuit consisting of a transmitter and receiver to read the wavelength value of a pure honey sample. There are five samples of honey kelulut prepared from the species *Heterotrigona Itama*, the infrared detection circuit in the device will read the wavelength from the original sample and compare it with another four samples that were mixed with 50% of sugar syrup, 50% of vinegar, 25% of sugar syrup, and 25% of vinegar. The results showed the device was able to compare the wavelength of the first sample of pure honey kelulut *Heterotrigona Itama* with the adulterated samples. For the first sample, the result displayed was “not adulterated” and for all four samples that were mixed with adulterants, the result displayed ‘adulterated’ on the screen. The finding showed potential to protect the integrity of honey health benefits from being manipulated by adulterated product. It also contributed to protect the well-being and consumer rights of the public from being deceived to consume adulterated product that could harm their health.

**Keywords:** adulterated honey, pure honey kelulut, detection method, rapid test kit, heterotrigona itama

## Trap-Nests For Stingless Bees In An Atlantic Forest Region

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### Abstract:

Stingless bees are the most diversity among eusocial bees, and most species build their nests inside preexisting cavities such as tree trunks, other abandoned nests (termites nests), exposed on walls or even tree branches. The choice of an adequate location for nesting therefore represents a critical aspect of colony reproduction. For eusocial bees, the construction of new colonies happens through the swarming process. Regarding stingless bees, this process is slow, gradual and different from *Apis mellifera*. Seeking to understand the swarming process, trap-nests or bait-nests are shown to be effective, as in addition to being useful in meliponiculture to capture stingless bees, they also provide the possibility of measuring some variables, such as the period of best capture of these individuals, which species can be attracted and which resources will be needed for successful attractiveness. Therefore, this work has as main objective to evaluate different types of trap nests, testing different volumes of PET bottles (1L, 2L and 3L), the use of attractants in traps and understanding the best period of the year for the installation of nests-trap. Of the installed trap-nests (90 in total), we found that 23 were visited by bees and started colonization. Of these nests, only in two of them the foundation and installation process were permanent. We also observed that the volumes chosen for the permanent installation of the bees were 3L bottles. Thus, of the 23 nests visited, 21 were abandoned. Thus, the data collected during 11 months show bees visiting the trap nests with the principle of nest foundation. We did not get any relevant conclusions regarding the visitation preference of the social bees in the studied area. Further work needs to be done to verify if the low establishment is due to the high humidity of the area (because it is Atlantic Forest) or if, because it is an area (in some points still young), it does not support the presence of eusocial bee species in sufficient numbers.

**Keywords:** Meliponini, stingless bees, swarming, trap-nests.

## Acetolyzed and Natural Pollen Preparations for Melissopalynology

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### Abstract:

Preparations of the honey sediment for melissopalynology are based on acetolyzed and natural pollen. To assign a correct botanical origin of sporomorphs, it is necessary to identify the types of pollen, represented by taxa at the level of family, genus and even species for the best known pollen grains. This is achieved with pollen reference collections, visual comparisons with photomicrographs from scientific literature, and experience. It is also necessary to know the ecology, which plants are nectariferous and which are polliniferous, or if they offer both nectar and pollen rewards for pollinators. Some plants such as the *Clusia* genus offer floral resin. For example, if a high pollen count in honey is caused by contamination with pollen from nectarless flora, it should be removed as honey is made from nectar. In those cases the following taxon is used to propose that botanical origin. Examples of polliniferous plants are: *Mimosa caesalpinifolia*, Melastomataceae, *Myrcia*, *Solanum*, *Borreria verticillata*, *Piper*. Contamination with anemophilous pollen needs to be eliminated because it did not provide nectar for the analyzed honey. For example some Asteraceae genera, *Casuarina*, *Cecropia*, *Celtis*, Chenopodiaceae, Cyperaceae, *Pinus*, Poaceae, Podocarpus, *Trema*. The representation of pollen in the nectar of a certain taxon in a honey causes hypo- and hyper-represented pollen in honey. For example, high quantities of nectar in the *Bombax ceiba* flower with large pollen explains low pollen counts in unifloral honeys of Bombacaceae. The fine tuning of pollen analysis, using acetolyzed or natural material, of course, comes with experience. General observations worthy of documentation: 1. Dye is not needed with acetolysis, the oxidative darkening of all surface features is sufficient, 2. Delicate exines of Marantaceae, Zingiberaceae and Heliconiaceae are destroyed by acetolysis, 3. Tropical flora are composed of roughly 20% tricolporate, reticulate grains, of many families, 4. Pore and exine structures are of particular importance and require immersion oil magnification at 1000X, and 5. Good phenological data as to flowering schedule can greatly improve possible identification. It is possible to identify most of the bee plant families of pollen grains prepared by both methodologies.

**Keywords:** acetolyzed pollen, melissopalynology, natural pollen

## Determination of Total Phenol, Total Flavonoid Amounts and Antioxidant Activity of Commercial Turkish Propolis Extracts<sup>#</sup>

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### Abstract:

Propolis is a resinous substance that is produced by bees via bee enzymes, from the exudates of buds and plants. Propolis contains phenolic and flavonoid compounds, so it is a wellknown antioxidant source. This study was carried out to determine the total phenol, total flavonoid and antioxidant activities of commercial propolis, extracted in ethanol, sold in the markets. In this study, 4 different commercially available propolis extracted in alcohol (Turkish propolis) were used. To determine the amount of total phenol, Folin-Ciocalteu method was used while aluminium chloride colorimetric method was used to determine the total flavonoids. Antioxidant activity of the propolis extracts were examined by CUPRAC spectrophotometric method. The total phenol and total flavanoid amounts were determined between 5893.33-27138.19 mg/GAE/L and 14.58-118.39 mg/QE/L, respectively. The antioxidant activity was found between 4.82-66.91 µg/ml when the CUPRAC method was used to evaluate the propolis extracted with ethyl alcohol. In conclusion, solvent type, total phenol, total flavonoid contents and antioxidant activity should be taken into account when purchasing propolis from the market.

**Keywords:** Antioxidant activity, Commercial propolis extracts, Total Flavanoid, Total Phenol

<sup>#</sup> This study was supported by Kirikkale University Scientific Research Coordination Unit (Project No: 2021/79). This study was master thesis Project carried out in Kirikkale University Health Sciences Institute.

## Poster presentations

## STINGLESS BEE KEEPERS GROUP CHARACTERIZATION FROM THE WEST AND SOUTH AREA OF YUCATAN; FUTURE IMPLEMENTATION OF BIOTECHNOLOGICAL INNOVATION#

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**Abstract:**

In the Yucatan Peninsula, meliponiculture has been developed since pre-Colombian times, and the bee "Xunan kaab" (*Melipona beecheii*) has been the most widespread because it is one of the most productive and easy to manage. The objective of this study was to identify the areas of opportunity where biotechnological innovation is part of the production of honey and beehive products with participatory analysis tools with beekeepers from the west and south of the state of Yucatan, Mexico and thereby contribute to economic and social level, the study was carried out in different communities: in the municipality of Tekax in the community of Xaya with the group of meliponiculturists "Flor de Jazmin", in the municipality of Opichén with the group of meliponiculturists Jaanil Kaab and in the municipality de Kinchil with the group of Kaabil Chaak meliponiculturists, different social and participatory activities were used, such as a timeline, resource map, prioritization matrix and problem tree, as part of the results the beginnings of the groups of meliponiculturists were identified, As they have evolved over time, a high percentage of species of pollinating plants of native bees, problems such as loss of hives, contamination and pests were highlighted, in the area of by-products their main problem is the training of marketing, which does not have sales at the market level, the annual production of honey is from 5 to 15 liters of *M. beecheii* honey, which they sell and use to make by-products, such as; soaps, shampoo, candles, gums, honey sticks, among others. As part of biotechnological innovation, tests have been carried out on safety and quality standards for honey and by-products of the *Melipona beecheii* bee species through the NOM.

**Keywords:** Meliponiculture; innovation; biotechnology; honey.





## The phytochemical composition and antioxidant properties of bee-collected pollen originated from poppy (*Papaverum somniferum*) plant #

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### Abstract:

Poppy (*Papaverum somniferum*), a flowering plant belonging to the Papaveraceae family is well known in the Asia and the Europe as a source of opium and poppy seeds as well as for ornamental purposes. However, its pollen also can be collected by bees and used as source of valuable nutrients for them as well as for humans. In that sense, the aim of this research was to determine phytochemical composition of monofloral bee-collected poppy pollen gathered in Serbia (Belgrade region). For that purpose the homogenized and powdered sample was extracted with 80% methanol (1:10 sample to solvent ratio) to obtain free (extractable) fraction of different phenolic compounds as well as with 80% acetone to obtain the carotenoid pigments. Several parameters (total phenolic (TPC), flavonoid (TFC) and carotenoid content (TCC), the content of derivatives of hydrocinnamic acid (HCAs) and rutin) were determined by application of standard spectrophotometric assays. In addition, antioxidant properties of collected pollen was examined through application of the following standard assays: DPPH radical scavenging, total antioxidant phosphomolybdenum capacity (TAC), ferric reducing power (FRP) and cupric antioxidant reducing power (CUPRAC). It was determined that TPC, TFC and TCC had the following values: 11.59 mg/g gallic acid equivalents (GAE), 6.03 mg/g rutin equivalents (RE) and 65.05 µg/g of dry weight (d.w.). Besides, several phenolic acids presence were confirmed with HCAs assay with value of 5.96 mg/g chlorogenic acid equivalents (CGAE) of d.w. In addition, the rutin content, as one of the main phenolics presented in pollen was also determined with value of 15.90 mg/g RE of d.w. In addition the following values were obtained for applied antioxidant tests: 4.18 mg/g trolox equivalents (TE) (for DPPH<sup>•</sup>), 28.92 mg/g ascorbic acid equivalents (AAE) (for TAC), 5.58 mg/g AAE (for FRP) and 68.99 mg/g AAE (for CUPRAC) expressed on dry weight. Based on all it can be concluded that poppy bee-collected pollen can be used as good source of different phytochemicals with expressed antioxidant activity.

Keywords: bee-collected pollen, poppy, phytochemicals, antioxidant properties.

# This work was supported by the Ministry of Education, Science and Technological Development of Serbia, grant no. 451-03-68/2022-14/200116.

## Aliphatic Organic Acids in *Geotrigona*, *Melipona*, and *Scaptotrigona* honeys: Potential markers of microbial associations with stingless bees

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### Abstract:

Honey metabolites have diverse origin: 1. Visited Plants, 2. Bee secretions, 3. Associated microbes, 4. Honey processing in the nest, and 5. Storage after harvest. They are studied with diverse scopes such as standards for quality control, biological properties, chemical profiling, identification and quantification techniques, markers of botanical, entomological and geographical origin. Non-pathogenic bacteria and fungi found naturally in honey have an ecological role inside the stingless bee nests, and offer sources of bioactive metabolites. Acetic and lactic acid are produced during the fermentation of pot-honey, as a preservation method used by stingless bees. Aliphatic organic acids (AOA) of *Geotrigona*, *Melipona* and *Scaptotrigona* honey from Ecuador were studied with targeted <sup>1</sup>H-NMR. The advantage of NMR spectroscopy is the chemical profiling of metabolites with organic functional groups, with simultaneous quantification. The <sup>1</sup>H-NMR spectra of three representative pot-honey of *Geotrigona*, *Melipona* and *Scaptotrigona* were contrasted. Differences in the number and/or intensity of the peaks correlated with the stingless bee genus. *Geotrigona* had very high intensities in the aliphatic region, showing the high concentrations of acetic acid and lactic acid. Major acetic and lactic acids, besides other six AOA (citric, acid, formic acid, fumaric acid, malic acid, pyruvic acid, and succinic acid) were identified, quantified and plotted with Principal Component Analysis (PCA). Quinic acid was absent. The AOA botanical and microbial origin is discussed, as well as their functions in the stingless bee nest. Future scientific research will answer how pot-honey is produced and kept within the cerumen pots in the nest.

**Keywords:** aliphatic organic acids, *Geotrigona*, <sup>1</sup>H-NMR, *Melipona*, pot-honey, *Scaptotrigona*



**INVITED  
PRESENTATIONS**

## Therapeutic Potential of Pollen

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### Abstract:

The research already developed with Pollen samples that had the well-identified floral origin gives important data on its therapeutic Efficacy. This information allows that in a near future it could be possible to apply for Pollen Monographs (one for each taxon), for instance, in the Herbal International Pharmacopoeia (at World Health Organization) and on Herbal Medicinal Products Committee (HMPC) at European Medicines Agency (EMA). Until now, although eighteen flower herbal drugs have a monograph for Quality Control, for example, at European Pharmacopoeia only one, *Typha angustifolia* L. is correlated with pollen as an herbal drug. Its percentage content of total flavonoids is expressed in typhaneoside, which is an isorhamnetin-3-2''-rhamnosylrutinoside. This information could be used as a base template to develop other monographs and provide methodologies for flavonoids when they are the main biomarkers for pollens which could be validated as herbal drugs. This is clearly an opportunity once flavonoids are pollen constituents for the majority of the floral sources collected by bees. In fact, it is undeniable that therapeutic bioactivities such as anti-inflammatory, antimicrobial, and antineoplastic are associated with these compounds, going from free acids, flavonoids, and, more recently phenolamines (these hydroxycinnamic acid amines, include *p*-coumaric, ferulic, caffeic and sinapic acids). From the previous works to the most recent research, pollen from various botanical proveniences is described with potential bioactivities which allow new drugs discovered in a more tangible endpoint to be validated as an Herbal Drug itself or even being used as a source of new chemical structures for drug development. Antitumoral, antiosteoporosis, and as prebiotic are some of the possibilities. With the advance in the Quality Control of Pollen by ISO/TC34/SC19, or by the Pharmacopoeias Monographs, a window is open for its use as an herbal drug. Nevertheless, data on risk assessment still scarce, and more information in Safety still be needed. For the investigation of new molecules in Drug Research, pollen continues to be a huge source of new bioactive molecules that will increase the potential harvesting of various botanical sources. In conclusion, the development of pre-clinical and clinical data still needs to be improved and the Companies should to do the necessary investment to leverage pollen to the next pharmaceutical level.

**Keywords:** Pollen, therapeutic, antiinflammatory, cancer, flavonoid, phenolamine, cinnamic

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## Bee pollen as a healthy food and food ingredient

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### Abstract:

Bee pollen is a healthy food and also a food ingredient for humans, produced by honeybees (*Apis* genus). Its nutritional value is related to the balanced composition of proteins, lipids, free sugars, minerals, phenolic acids, flavonoids, and a variety of vitamins. Since pollen is also an important source of macro and micronutrients for bees, human harvesting should not harm the health of the hive, implying care to avoid larvae being nutritionally disadvantaged. In this work, the quality of pollen stored under different conditions and the use of this product as a food ingredient will be discussed. Nowadays, consumers are more informed and are interested in choosing sustainable and natural food. However, concerning some products, and given the lack of standards or specific rules, the knowledge related to their origin, quality and harvested methods must be improved. To fill with this gap, a technical subcommittee for Bee products was created, ISO/TC 34/SC 19, which ensure the quality of these products. There, the Working Group (WG3) is dedicated to Bee pollen and is working in a proposal to standardize the requirements of quality parameters, which can be followed in the international trade. This normalization also includes the methodologies of analysis, storage and transport. The working group aims to continue their work and standardize the production process of bee pollen and the bee bread quality. As aforementioned, bee pollen is gaining a lot of interest from the consumers, given their natural origin, variability (related to the different floral species where is harvested) and their bioactive substances associated to healthy benefits. Besides this, the industry looks for natural food ingredients with biological activities and functional properties, due to the consumers concerns about additives. These compounds appear as a long list in label, with unfamiliar names and people think of them as complex chemical compounds. In this way, pollen became a suitable alternative, despite some allergic issues (like with other food products) that must be very clear in label packages which will include pollen as additive.

In conclusion, bee pollen is a good and healthy food that has a high nutritional value and biological activities. Concerning their properties, it could also be a good food additive for the industry acting mainly as antioxidant. Despite this actual knowledge, further more possibilities could be explored in a near food to improve its impact in food industry.

**Keywords:** Pollen; food; food ingredient; Quality; Nutritional characteristics; standardization.

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## Functional effect of royal jelly and apilarnil on reproductive health

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### **Abstract:**

Royal jelly is a product secreted from the mandibular and hypopharyngeal glands of young worker bees (5-15 days old) to ensure the nutrition and development of the queen bee. In fact, during the first three days of their life, the young of all three castes (drones, worker bees and queen bees) are fed royal jelly. After the first 3 days, drone and worker bee larvae are fed with a mixture of honey and pollen. The queen bee continues to be fed with royal jelly. It is reported that royal jelly is a strong supporter of healthy aging and longevity. Because it is known to increase the general health and productivity of queen bees, which can live up to five years and lay 3000 eggs per day, compared to infertile worker bees, which live an average of 45 days. As an antioxidant, royal jelly has been shown to reduce oxidative stress damage in reproductive organs. Apilarnil is a bee product with biologically active properties. It is obtained by filtration and pulverization of drone larva homogenate harvested at the 7-day larval stage before the comb cells are closed. Since apilarnil contains male-specific hormones, it is reported to have male sex-specific enhancing effects. For example, it is recommended as a natural anabolism stimulator in men as it increases muscular body weight. In some cultures, honeybee larvae have been used to treat impotence. While its success in infertility treatment is not attributed to its high protein content, the presence of sex hormones has been demonstrated in later studies. It is reported that eighty million people worldwide are affected by the inability to have children. Approximately 15% of couples in the reproductive period have infertility problems. While it is estimated that 25-40% of infertility in couples is due to male factors such as oligospermia and/or asthenospermia, it is stated in more recent publications that the male factor ratio is around 50% and one out of every 20 men will be affected by subfertility. For this reason, it is thought that royal jelly and apilarnil are potential bee products that can support infertility treatment due to their beneficial biological activities.

**Keywords:** royal jelly, apilarnil, infertility, reproductive health

## Is the honeybee venom an effective agent against cancer?

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### Abstract:

Honey bee venom is a special mixture of protein, lipid and low molecules that honey bees secrete by their venom glands in order to defend their colonies against the enemies and inject through their sting. Bee venom has been using in traditional and complementary medicine for centuries to treat immune-related diseases. Honeybee venom contains melittin, apamin, adolapin, MCD peptide, Phospholipase A2, etc., biologically active amines (histamine and epinephrine) and nonpeptide components. The most active ingredients of honey bee venom determined as; melittin, apamine and phosphalipase A2. This specific mixture has been known by its positive effects on chronic pain, arthritis, antitumor, skin diseases and neurodegenerative diseases. Malignant gliomas are fast-moving tumors consisting of anaplastic oligodendroglioma, anaplastic astrocytoma, mixed anaplastic oligoastrocytoma, and glioblastoma. There is no clear treatment strategy for the progression of primary tumor cells. Current treatments can significantly reduce patient's survival and quality of life. Therefore, new treatments are being investigated in traditional complementary medicine. In this study, it was aimed to review the antitumoral effect of bee venom on tumor cells. Melittin is the major component of bee venom and known to have anti-inflammatory, cancer properties. Although the biological and physiological effects of melittin have been investigated in detail in previous in vivo studies, the desired effect has not been achieved in therapeutic preclinical studies. The reason for this is that melittin is unstable and degrades quickly in plasma. In addition, due to its cytolytic effect, melittin breaks down blood cells and targeted research cannot be carried out. With the obtained data, cytotoxicity, cellular death mechanisms, oxidative stress mechanisms have been revealed. The market for honey bee venom in Türkiye has not been established yet. In the world, it is generally used in the field of cosmetics. The results obtained from this review will lead to clinical studies on the use of bee venom in cancer treatment. As a result of the study, it was determined that honey bee venom has an antitumoral effect not only on maling cells, but also healthy cells. Based on this result, it is planned to reveal the mechanism of action of bee venom on cells through molecular pathways and to brought together nanoparticles with honey bee venom specific components to reveal their anti-cancer activities in biomedical use.

**Keywords:** Antitumoral effects, honeybee venom, tumor cell, cancer

## Use of honey bee (*Apis mellifera*) colonies as a bioindicator for human, animals and plant diseases

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### Abstract:

Honey bee (*Apis mellifera*, L. 1758) colonies are frequently used as bioindicators in environmental monitoring plans, thanks to their morphology and behaviour, such as the high mobility within the explored area, the frequent flights of forager bees and their body covered by hairs and bristles, capturing pollen and other particles during the flight. That makes possible the detection of environmental contaminants: radioactive fallouts, heavy metals, pesticides, and plant pathogens by the analysis of honey bee bodies and their products, in particular pollen and bee bread. Among plant pathogens, honey bee colonies can be useful to detect and prevent outbreaks of the fire blight disease caused by the Gram-negative bacterium *Erwinia amylovora* and mainly hitting pomaceous fruit trees in several countries.

Besides, among human pathogens, honey bee colonies were used for SARS-CoV-2 monitoring in Italy. The presence of airborne viral particles linked to the particulate matter (PM), enhanced the use of foragers that capture the PMs during their flying activity thanks to their bodies covered by hairs and bristles.

Considering human and animal diseases, antibiotic resistance represents an important threat becoming very relevant to public health. Within the Italian project “BeeNet – monitoring the environment through bees and biodiversity”, forager bees were used as bioindicators of environmentally-present antibiotic-resistant bacteria. From the isolated bacteria strains, the highest antibiotic resistance values were found for amoxicillin, vancomycin, penicillin and erythromycin.

A lot of studies supported the use of *Apis mellifera* colonies as bioindicators for human, plant and animal diseases. This use must be improved to better know all possible applications.

**Keywords:** bioindicator; environmental microorganism; SARS-CoV-2; *Erwinia amylovora*; One-Health



## **Pharmacological properties, chemical composition and potential applications of Algerian propolis**

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### **Abstract**

Propolis is one of the most well documented hive product extensively used since ancient time. Egyptian used propolis as embalming material and explore its anti-putrefactive properties. Roman and Greek used propolis as antiseptic. While, Incas used it as antipyretic. Nowadays, propolis is used as a complementary or alternative medicine. With its pharmacological properties such as anti-inflammatory, antioxidant, antimicrobial, hepatoprotective, immunomodulatory... etc propolis can be a good alternative to synthetic drugs. In addition, propolis is regarded as a safe substance with no side effects. Propolis chemical composition is extremely complex and depends mainly on geographical origin, climate conditions and bee species. Several studies have been performed on Algerian propolis in the last two decades. However, it is still unknown and its use is very limited. We reported in the present presentation our main results concerning our investigation of pharmacological properties, chemical composition, botanical origin and potential applications of Algerian propolis.

**Keywords:** Algerian propolis, pharmacological properties, chemical composition, potential applications

## **Newly discovered probiotic product: perga**

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### **Abstract:**

Bee bread is a bee product that is not easily harvested because it is not easy to harvest and the honeycomb is destroyed during harvest. “Bee bread (Perga) is obtained as a result of lactic acid fermentation, which is formed by covering with a thin layer of honey and bee wax after the pollen collected from plants and mixed with digestive enzymes are stored in the honeycomb cells. Honeybees use perga especially for feeding larvae and young bees producing royal jelly. The nutritional value of bee bread is higher than that of bee pollen, and it is more digestible due to its higher free amino acid content and the presence of easily assimilable sugars. This event is provided by the degradation of the pollen wall, which restricts the digestion and bioavailability of pollen, by fermentation in bee bread, and the part containing nutrients comes out. In addition, despite the biochemical transformation that occurs during the fermentation of bee bread, phenolic compounds are not affected and remain unchanged. Studies on the chemical content of bee bread generally show that it consists of water, proteins, free amino acids, carbohydrates, fatty acids and other bioactive molecules. It is also shown to have components such as phenolic compounds, alpha-tocopherol and coenzyme Q10. The composition of bee products may vary depending on the plant source from which the pollen is collected, climate and seasonal conditions. This feature makes its standardization difficult. The biological effect of bee bread is probably associated with the presence of antioxidants such as phenolic compounds, especially flavonoids. After a period full of processed and additives/preservatives in the food industry, people's return to natural foods is accepted all over the world. The expectations of consumers who do not want chemicals in the food they consume are increasing. Consumer demand is the introduction of functional foods, which are characterized by health-oriented properties, are effective in increasing the overall efficiency of an organism and even prevent the development of certain diseases.

**Keywords:** bee bread, perga, bee products, bioactivity

## Functional food properties-can bee pollen help us? #

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### Abstract:

Functional food can be defined as food item that can effect on human health besides nutritional and energetic increment as regular diet. Consequently, bioactive compounds presented in the food are the most influential on functional properties of food. In that sense, plant based components, including bee-collected pollen (BP), can be a great source of food bioactivities. The richness of pollen in the content of various bioactive compounds is mostly determined by its botanical origin. Although there are no any official recommendation daily intake of pollen should be in the range from 20 to 40 g/day for adult. However, for those who have pollen allergies a great precaution should be applied. On the other side there are much more reason to use BP not per se but as food enricher. In the last decade, there are significant numbers of examples of functional food improvements with BP addition or application as functional ingredients in different types of foods. In particular, there are proved BP application in the dairy products as well as meat based products. In both cases BP addition has improved antioxidant properties of final food products, bioaccessibility of bioactive compounds (in particular phenolics) while in the case of meat based products decreased lipid peroxidation is also observed. However, due to presence of strong membranes (intine and exine) and sporopollenin layer a significant part of different BP's nutrients and pharmaceuticals will show a limited bioaccessibility. In that case, some pre-treatment (in particular some microbial eco-friendly pre-processing) proves to be very effective. Additionally, due to its ambiguous nature (both lipophilic and hydrophilic properties depending on botanical origin and methods of processing and storage) BP can significantly contribute and modify techno-functional properties of final food product which is extremely important for food industry. For instance, it has been determined that BP possesses good anti-foaming properties important during production of several food stuff. Also, it possesses good oil absorption capacity. In that sense, this naturally easily available material can serve as excellent improver of different functional food products for wide range of consumers.

**Keywords:** bee pollen, functional food, bioactive compounds.

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Invited Oral Presentation

**Romanian propolis: past, present and future directions. How geographical and botanical origin influence the chemical composition and bioactive properties**Otilia Bobiș, Victorița Bonta, Adela Moise, Claudia Pașca, Daniel Severus DezmireanFaculty of Animal Science and Biotechnology, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca,  
RomaniaCorresponding author: Otilia Bobiș, [obobis@usamvcluj.ro](mailto:obobis@usamvcluj.ro)**Abstract:**

Propolis is a resinous product that bees create to protect hive, especially for wintering. It contains a mixture of substances from plants and trees (cherry, poplar, fir, spruce, willow, oak), bee saliva and beeswax. According to geographical and botanical origin, propolis is classified in several categories: brown propolis (the most widespread on earth, made from different resins of poplar species and coniferous or deciduous trees), red propolis (made from resins of *Dalbergia ecastophyllum*, *Clusia* sp.) and green propolis (from *Baccharis dracunculifolia* resins). As most of the propolis samples from Central and Eastern Europe, Romanian propolis plant sources are resins secreted by buds of *Populus nigra*, *Quercus*, *Aesculus hippocastanum*, *Ulmus*, *Picea*, and *Fraxinus*, being classified as “Poplar type propolis”. Studies on propolis around the world are made since long time, after the medical practice noticed the benefits of this special bee product. The scientific studies start because because more and more, the scientific community and not only, was interested in finding natural products that can be used in treatment of different diseases, to eliminate some of the antibiotics, which give resistance if they are used long time. Propolis is known mostly for its therapeutic properties. This is the reason that Romanian research on propolis several decades ago and published studies are available since early 80’s. Early Romanian studies on propolis were focused on the chemical composition and effects on certain blood constituents and lymphatic system, hepatotoxic and hepatoprotector effect, antibacterial, antiviral, and anti-inflammatory effect. Present studies are focused on the effect of Romanian propolis on human and bee pathogens as well as apoptotic effect on different cancer lines. Also the use of different nanoemulsions based on aqueous propolis in skin’s protective mechanism are investigated. These approaches include preparation of different nanoparticles: gold, silver, magnetic nanoparticles, liposomes, liquid crystalline formulations, solid lipid nanoparticles, etc. Nano-propolis may be used for the potential benefits in the development of futuristic useful products such as sunscreens, creams, mouthwashes, toothpastes, and nutritional supplements with improved solubility and bioavailability.

**Keywords:** propolis, chemical composition, properties, geographical origin

Invited Oral Presentation

**Metabolomics Applications in Bee Science: A Suspected *Starmerella* Yeast Association with the Stingless Bee *Scaptotrigona vitorum* Engel 2022**Patricia Vit

Apitherapy and Bioactivity, Food Science Department, Faculty of Pharmacy and Bioanalysis,  
Universidad de Los Andes, Merida, Venezuela

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**Abstract:**

Metabolic networks are investigated combining chemical information from analytical approaches with multivariate statistics. Two segments of this talk comprise a review and a discovery. First, metabolomic studies in bee science were reviewed: 1. Bee health: Gut microbiota, nutritional stress, virus. 2. Bee pollen: Chemical profiling, 3. Cerumen: Chemical profiling. 4. Honey: Authenticity, botanical and geographical origin, honey maturation. 5. Pesticides: Impact of phytochemicals in bees exposed to pesticides. 6. Pot-honey: Entomological origin, spatial memory activity. 7. Pot-Pollen: Chemical profiling. 8. Propolis: Antibacterial activity of bee collected resins, chemical profiling, review of honey bee and stingless bee propolis. 9. Royal jelly: Chemical profiling. Scientific research of this review on metabolomics was done with the following species of bees: *Apis mellifera*, *Austroplebeia australis*, *Bombus terrestris*, *Heterotrigona itama*, *Meliponula ferruginea*, *Tetragonula carbonaria* and *Tetragonula hockingsi*. Second, biosurfactant activity was discovered in *Scaptotrigona vitorum* honey from Ecuador. A routine honey authenticity test by interphase emulsion (HATIE) produced the unusual pattern not previously detected. One phase instead of the three phase signature for genuine honey. Only a microbiologist could comment that some yeasts synthesize surfactants as Professor Carlos Augusto Rosa did from Universidade Federal de Minas Gerais, an expert in yeasts associated with stingless bees, know to name the new genus *Starmerella* C.A. Rosa & Lachance, 1998. *Starmerella bombicola* and other yeasts of that clade biosynthesize sophorolipids. Therefore, it was suspected *Starmerella* yeast type association with the Ecuadorian stingless bee *Scaptotrigona vitorum* Engel 2022, called 'Catiana' in El Oro province. The HATIE was tested in 51 honeys of *Apis cerana*, *Apis dorsata*, *Apis mellifera*, and nine species of stingless bees from the collection at the Apitherapy and Bioactivity Group. It was observed one phase in *Scaptotrigona depilis* from Argentina, Bolivia and Brazil, *Tetragonula carbonaria* from Australia, and a "Kelulut" honey from Malaysia. Honey-microbiome are needed to identify the suspected microbial associations and metabolomics to identify and quantify the biosurfactants of microbial origin, causing one phase in the test.

**Keywords:** biosurfactant activity, honey authenticity test, metabolomics, pot-honey, *Scaptotrigona*

**Belgian action limits for pesticides in beeswax to preserve bee health****Advice 18-2018 Scientific committee FASFC**Reybroeck Wim<sup>1</sup><sup>1</sup> Research institute for Agriculture, Fisheries and Food (ILVO), Brusselsesteenweg 370, 9090 Melle, BelgiumCorresponding author: Wim.Reybroeck@ilvo.vlaanderen.be**Abstract:**

After problems of bee brood mortality due to wax adulteration with stearin and analysis results of beeswax showing high concentrations of pesticides, beekeepers are worried about the quality of beeswax and the impact on bee health. So the Scientific Committee of the Belgian Federal Agency for the Safety of the Food Chain (FASFC) has been asked to answer the following questions:

1. What are the known beeswax contaminations and adulterations?
2. Which substances are likely to pose a risk to bee/colony health following wax contamination or adulteration?
3. Regarding the possible presence of these substances in wax, can an action limit be proposed in order to preserve bee health?

A group of experts first identified several substances that may adulterate or contaminate beeswax. 18 Different compounds were selected based on toxicity by contact or oral, lipophilicity and occurrence in beeswax. The risk to bee health posed by these substances has been assessed based on three exposure scenarios. The first scenario corresponds to the exposure of larvae following their close contact with the wax constituting the cells in which they develop. The second scenario corresponds to the exposure of larvae following consumption of royal jelly and bee bread that have been contaminated via the wax during storage in the wax cells. The third scenario corresponds to the exposure of adult bees to beeswax following the building and fixing of cells and based on a worst-case scenario (consumption (= ingestion) of wax).

Finally for each selected compound an action limit for re-melted beeswax was calculated for each scenario and the most strict value was proposed as final action limit.

**Keywords:** beeswax, action limits, pesticides, adulteration.

## Integrated management of varroosis in European honeybee (*Apis mellifera*)

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### Abstract:

Implementation of integrated pest management requires good knowledge of the parasite-host relationship. Its aim is to provide an effective control of the parasite while reducing the amount and/or number of chemical treatments. This results in the lower pressure for the selection of resistant strains and reduces the accumulation of acaricide residues in the bee products. *Varroa destructor* mite is a widespread parasite of European honeybee. Recent data indicate that it primarily feeds on fat body tissue of adult bees which has a significant impact on the longevity of winter bees and can result in the colony collapse. *V. destructor* reproduces inside the capped bee brood, with a strong preference for drone brood. Before implementing an effective integrated management for varroosis, economic threshold should be set at which the mite levels need to be controlled. Mite infestation level can be assessed using reliable procedures, such as a sugar shake or ether roll method. Thereafter, a combination of preventive, biotechnological and chemical treatment methods is used to control varroosis. Traditional biotechnological methods such as removal of capped worker or drone brood, comb trapping method or queen caging (brood interruption) disrupt the *V. destructor* developmental cycle thereby reducing the number of mites. This might enable the beekeeper to postpone the need for the chemical control until the honey harvesting has finished. In addition, a combination of brood interruption method with a subsequent chemical treatment offers high efficiency due to the absence of brood. Some biotechnological methods require high beekeeping skills and are rather time consuming. Better education of beekeepers will allow for the easier implementation of integrated management programs for varroosis.

**Keywords:** varroosis, integrated management, biotechnological methods, economic threshold

## **Propolis as cariostatic agent and connection between oral cavity health and systemic diseases**

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### **Abstract:**

Dental biofilm especially subgingival dental plaque among patients with periodontitis is associated with many systemic diseases. Periodontal pathogens may be associated with cardiovascular diseases, diabetes mellitus, respiratory diseases, Alzheimer's disease, rheumatoid arthritis and recently with COVID-19. Inflammation processes involved in periodontal disease are the same as in systemic diseases. Altered microbiome in oral cavity leads to activation of immunocompetent cells in order to produce and release inflammatory mediators initiating inflammatory process. Therefore, improving the condition of the oral cavity plays a significant role in the overall body condition. Propolis as a natural substance, may be useful in dentistry and oral health management, bringing advantages in maintaining oral health and in prophylaxis of systemic diseases. Propolis possesses antibacterial, anti-adherent, and anti-inflammatory properties as well as inhibits glucosyltransferase activity. Extract of propolis reduces cell adhesion and biofilm formation. Mouthwash, toothpaste, and dentifrices containing propolis brings excellent effects in preventing gingivitis, tooth decay, periodontitis, and biofilm reduction.

**Keywords:** propolis, oral cavity health, dental biofilm, systemic diseases.



## Bee Pollination And Its Economic Value For Food Production

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### Abstract:

Bees as an insect play a crucial role in crop pollination alongside other animal pollinators. Bees contribute to the global food supply via pollinating a wide range of crops, including fruits, vegetables, oilseeds, legumes, etc. Bee pollination improves the quality and quantity of fruits, nuts, and oils. Currently, 5–8% of all global crop production would be lost without the pollination services provided by bees, necessitating changes in the human diet and the expansion of agricultural lands to resolve shortfalls in crop production. One-third of a person's diet comes from insect-pollinated plants, and honeybees are responsible for the pollination of over 80% of flowering plants. Without honeybee pollination, crop yields will decrease by >90%. Bee colonies are faced with many challenges that influence their growth, reproduction, and sustainability will be discussed.

**Keywords:** Bee pollination; crop production; bee visitation; challenges; economic value

## Pollination of Fruit Crops –Challenges and Opportunities

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### **Abstract**

Fruits play a significant role in economic development, nutritional security, employment generation and overall economic growth of a country. They provide a dynamic tool for enhancing economic returns, creating employment avenues and ensuring ecological sustainability. Our population is growing, but the per capita availability of land and water resources are going down. It is obvious that we will have to produce more and more from less and less land and water. This can be achieved only through the ever-green revolution pathway which can help us to increase productivity in perpetuity without associated ecological harm. There is widespread concern about malnutrition in our country and it is now clear that for every nutritional malady, there is a horticultural remedy. There is a need for enhanced growth and productivity and quality of temperate, tropical and sub-tropical fruits. Production constraints include two diverse but mutually interlinked approaches such as attack of pests on one hand and inadequate pollination on the other. The widespread use of pesticides in modern agriculture throughout the world have become necessary for the protection of the plants against insect pests and diseases to obtain higher yields to meet out the food requirement of increasing population but the injudicious use of pesticides has resulted in contamination of agroecosystem and agriculture produce including nectar and pollen and caused heavy losses to the pollinators. Such contaminated nectar and pollen when brought to hive may cause damage to brood besides the contamination of the stored honey. Pollinators provide an ecosystem service that enables plants to produce fruits and seeds. About 70% of the world's plants require a pollinator to produce fruits/seed of which 35% are crop species and this account for one in three mouthfuls of food and drink we consume.

**Keywords:** Fruit crops, honeybees, pollination, food security, pollinator decline

## **Antioxidant Potential in Apitherapy**

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### **Abstract:**

Chronic diseases are the leading causes of morbidity and mortality worldwide. Recently, increased demand on the healthcare systems due to the chronic diseases has become a major concern. Reducing risk factors can help prevent some of these conditions. One of the best ways to prevent and manage chronic disease is to maintain a healthy diet. There is good evidence that a diet with high in antioxidants may reduce the risk of many diseases including cardiovascular and neurodegenerative diseases. It is known that when oxygen molecules split into single atoms that have unpaired electrons, they become unstable free radicals. When produced in excess, free radicals generate oxidative stress that can seriously alter the cell membranes. Contrastingly, antioxidants scavenge free radicals and prevent or reduce the damage caused by oxidation. Apitherapy is a type of complementary therapy that uses products that come directly from honeybees. Honeybees produce several products that contain bioactive constituents like honey, propolis, royal jelly, and bee pollen which have been used for a long time for their nutritional value and a broad spectrum of disease prevention and treatment purposes. Scientific studies attribute to apitherapy products an extensive range of beneficial health effects, including antioxidant properties. Therefore, apitherapy has an important potential in the primary prevention of chronic diseases.

**Keywords:** antioxidant, apitherapy, chronic diseases, oxidative stress, primary prevention

**Physico- chemical and biological functionalities of bee products : An approach that warrant clinical investigations**

Pr Badiaa Lyoussi

Laboratory of Natural Substances, Pharmacology, Environment, Modeling, Health and Quality of Life (SNAMOPEQ), University Sidi Mohamed Ben Abdellah, Fez, Morocco

Corresponding author: lyoussi@gmail.com**Abstract:**

Bee products are inexhaustible sources of bioactive molecules. They are extensively used in folk medicine for the prevention and self-treatment of several diseases and has become actually the objective of many scientific investigations. Different biological and pharmacological effects of honey, pollen, propolis, royal jelly, bee bread and bee venom have been referred to their antioxidant, antibacterial, antitumoral, anti-inflammatory agents, antihyperglycemic effect and renal disease protection. Oxidative stress is believed to be responsible for the occurrence of several pathologies. Scientific reports from our laboratory have shown that bee products have a wide chemical composition and multi-functional properties. In this context, and in order to understand the relationship between biomolecules from beehive products and their functional potential, we will investigate the antioxidant properties of Moroccan bee products, their capacities for preventing lipid peroxidation and scavenging free radicals was generally correlated with their phytochemical screening. *In vivo*, propolis and honey preparations were able to attenuate diabetic hepato-renal damage, probably through antioxidant and detoxification properties. The protective role of some honeybee products against reactive oxygen species-induced damage and nephrotoxicity in diabetic rats, gives hope that some of these products will have similar protective action in humans. In the rat diabetic nephropathy model, honey, propolis and pollen also showed significant effect on glucose homeostasis and improving kidney function. The possible mechanism of action is discussed. A compilation on therapeutic properties of honeybee products in experimental animal models and human health will be presented. It might be concluded that bee products are a potential target, to be used in the management of chronic kidney diseases, proteinuria, diabetes, cancer and inflammation. Overall, chemical characteristics of bee products may allow the extracts to be used as bioactive ingredients in the food industry, but they also present potential for the pharmaceutical or nutraceutical sectors for the prevention and/or treatment of health disorders.

**Keywords:** Propolis, Bee pollen, Honey, Royal jelly, Bee venom, Quality control, Phenolic compounds, Antioxydant activity, Therapeutic efficacy

**Unique Kelulut Factor? A Review of Potential Nutritional Parameters for Grading Stingless Bee Honey**

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Corresponding author: [norhasnida@upm.edu.my](mailto:norhasnida@upm.edu.my)**Abstract:**

Stingless bee honey has a long history of traditional indigenous use with a range of purported therapeutic properties. More than 60 species of stingless bees have been discovered in the Indo-Malayan clade and 10 in the Australasian region of Australia, with the associated honey traditionally harvested from the forest by the indigenous people for their medicinal properties. Currently, two species of stingless bees are commonly reared in managed hives for their honey in Malaysia — *Heterotrigona itama* and *Geniotrigona thoracica*. The physicochemical properties of honey produced by these two species currently do not completely comply to the International CODEX Standards for honey albeit partially comply to the Malaysian Standards for Kelulut Honey (MS2683) as reported previously. In this review, physicochemical properties of stingless bee honey produced in other countries such as Ecuador, Brazil, Southeast Asia, Australia and the recent study of *H. itama* honey from Malaysia are compared and discussed. Although all honey samples were freshly collected, the physicochemical qualities cannot meet all of the requirement set by the International CODEX Standards. However, the same samples can comply with the MS2683 standards. The presence of trehalulose sugar in the 50 samples of *H. itama* honey was found as the main unique properties of this honey that can be used as a grading factor for marketing. More studies on the physicochemical properties and quantification of trehalulose in stingless bee honey across the globe is needed to be able to develop a new standard specifically for this honey and highlight trehalulose as a unique quality indicator.

**Keywords:** Unique Kelulut Factor, Stingless Bee Honey

## Geographical Indication of Brazilian Red Propolis and the value chain and importance on the Beekeeping Economy for the State of Alagoas-Brazil

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### Abstract:

The present work carries out a bibliographic survey and published information from the management institutions of the Geographical Indication (Appellation Origin) of red propolis in Alagoas. Historical and evolutionary aspects of the Local Productive Arrangement based on traditional beekeeping are raised during the period Before- Geographical Indication and Post-Geographical Indication between the period from 1980 to 2022. The organizational structure of the shared hybrid model of management of the Geographical Indication of red propolis are presented. The Geographical Indication as a driving force for the development of a new business model based on biotechnological product and of Brazilian biodiversity enhanced by scientific, technological and innovation research aimed at greater national and international competitiveness are discussed. The transformation of the Productivist Local Productive Arrangement model to the shared business model based on scientific and technological knowledge enhanced by socio-environmental values are also discussed. The post-Geographical Indication social and economic advances for the State of Alagoas/Brazil are presented and discussed. Post-Geographical Indication obstacles in the production chain, in beekeeping companies, in Research Institutes, in the institutions that manage the red propolis GI and in the regulatory bodies and quality indicators for the GI product of Brazilian biodiversity are discussed.

**Keywords:** Brazilian Red Propolis. Geographical Indication – Red Propolis. Shared Value Chain.

Acknowledgements: UFAL, FAPEAL, CAPES, CNPq, FINEP, SEBRAE, INPI.

Invited Oral Presentation

## **Quality Criteria in Queens Reared by Commercial Queens Enterprises and their Importance For Turkish Beekeeping**

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### **Abstract:**

Honey bees are social insects that live in communities called colonies. Under normal conditions, there are tens of thousands of worker bees and a few hundred drones, the number of which varies according to the seasons, with a queen in this social structure. The queen is the only individual of the colony capable of laying eggs, the mother of all colony individuals and the genetic source of all hereditary characteristics of the colony. Because it is the source of the hereditary structure, the strength of the colony, its hard work, being sensitive or resistant to diseases, being good-natured or combative, wintering ability, honey yield, tendency to swarm and propolis collection, etc., depend on the queen and the drones that mate with her. A good quality queen should have a high body weight, multiple ovaries and a large spermatheca. In addition, the queen must mate successfully and store sufficient spermatozoa in the spermathecae sac and be free from diseases and pests. On the other hand, it should not be forgotten that the performance of the colony varies depending on the genetic structure of the queens as well as the genetic structure of the drones mating with her. This shows us that to increase colony performance, it is not only sufficient to rearing quality queens, but also to rearing quality drones. Especially in commercial queen rearing, rearing drones in sufficient quantity and quality is the most important factor that closely affects the quality of the queen produced.

**Keywords:** Honeybee, Queen bee, Queen rearing, Quality criteria, Turkey

Invited Oral Presentation

## Conserving honey bee diversity on islands: the case of the Canary Islands

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### Abstract:

The honey bee has been of immense socio-economic value since prehistoric times and is now recognised as a major player in the pollination of flowering plants, wild or cultivated. Recent studies have shown that 35% of global food production depends on insect pollination of crops (Klein et al. 2007). This essential process has been quantified at 153 billion euros in 2005 (10% of the total economic value of crop production). Furthermore, pollinators are essential for maintaining natural and agricultural ecosystems as they facilitate gene flow between pollinated plants. In the mid-1990s, beekeepers in the Canary Islands, specifically on the island of La Palma, became interested in studying their honey bee populations when they began to see honey bees with a different morphology to the local black Canary Island honey bee. Since then, several research groups, especially that of the University of Murcia, have carried out studies that have made it possible to characterise a Canarian ecotype included in a Macaronesian sub-lineage of the African evolutionary lineage. The results of the molecular (mitochondrial DNA and microsatellites markers) and morphometric studies also showed an important genetic differentiation of the Canarian bees with respect to the Iberian populations, probably due to microevolutionary processes such as the founder effect, the bottleneck effect and local adaptation. Therefore, we propose the classification of these Canarian populations as a distinct ecotype within *A. m. iberiensis*. Taken together, the studies presented in this invited presentation highlight the idiosyncrasies of Canary Island honey bee populations and their need for protection, especially in the face of the current global crisis of biodiversity loss.

**Keywords:** biodiversity, conservation, local populations

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## Antimicrobial activity of bee products produced in Greece: the case of pine honey and bee bread (perga)

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### Abstract:

Pine honey is a honeydew honey produced in the East Mediterranean region (Greece and Turkey) from the secretions of the plant sucking insect *Marchalina hellenica* feeding on living parts of *Pinus* species. Nowadays, honeydew honey has attracted great attention due to its biological activities. One of our aims was to study unifloral pine honey samples produced in Greece regarding their antibacterial activity against nosocomial and foodborne pathogens as well as elucidating antimicrobial mechanisms using cutting-edge RNA-sequencing (transcriptomics) methodology. Higher levels of H<sub>2</sub>O<sub>2</sub> were estimated compared to other types of honeydew honey, whereas protein content was similar. All tested honeys exerted variable but significant antibacterial activity, expressed as MIC and MBC values, comparable or even superior to Manuka honey for some tested samples. Honey antibacterial activity is mainly attributed to hydrogen peroxide and proteins in some cases. Data regarding RNA-sequencing will be presented indicating multiple mechanisms of antibacterial activity exerted by pine honey. Beebread (perga) is derived from bee collected pollen, which is processed by the bees, fermented and stored in the hive. Few studies have shown the antimicrobial activity of beebread against major bacterial pathogens and fungi. Data regarding the bioactivity (antibacterial, antiviral and antioxidant) and their correlation with botanical origin will be presented for the first time for 18 samples of bee bread collected from apiaries across Greece, two of which were monofloral. The antimicrobial activity of each sample was tested against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Salmonella* Typhimurium. Our data demonstrate that all samples exert inhibitory and most of them bactericidal activity against at least two pathogens. Furthermore, all samples exert significant anti-oxidant activity, where the monofloral *Castanea Sativa* sample demonstrated superior antioxidant activity. Nevertheless, the antioxidant and antimicrobial activity were not strongly correlated. However, machine learning methods demonstrated that the palynological composition of the samples is a good predictor of their TPC and ABTS activity. Last but definitely not least, data regarding the antiviral activity of bee bread against Enterovirus D68 (provoking serious disease sometimes in children) will be presented for the first time.

**Keywords:** Greek bee products, pine honey, beebread, antibacterial, antiviral, antioxidant, RNA-sequencing, enterovirus

This work was partly funded by the Hellenic General Secretariat for Research and Innovation under the emblematic action “Honeybee Routes”

## **Biochemical and Physiochemical Properties of Turkish Forest Honeys**

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### **Abstract:**

Honey is one of the most natural and sweetest products produced from nectar and secretions collected by honeybees from nature. Due to its geographical location, Turkey is very rich in terms of honey plants and is a habitat for a wide variety of flower and secretion honeys. It has a large variety of honeys such as blossom honeys, dew honeys, forest honeys and industrial honeys etc. Forest honey is the honey produced from trees and is divided into two classes as flower and secretory honeys. Chestnut, acacia, linden, rhododendron, naringin, blackthorn and heather honeys are blossomed forest honeys. Pine, oak, cedar, spruce, and fir honeys are forest honeys with secretory honey properties.

**Keywords:** Forest honey, Turkiye, oak, pine, dew

## **Antiglycating and Antiaggregating properties of natural products from Honey**

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### **Abstract:**

Diabetes has become a major health concern for many countries. It either originates from or manifests the dystegulation of glucose metabolism. The consequences of diabetes are realised due to the biochemical process of glycation, a nonenzymatic process of interaction between carbonyl and amino groups of sugars and proteins respectively. Advanced glycation end products are implicated in many metabolic and neurodegenerative disorders because of their potential in altering the structure and function of biomolecules. Although these products have been identified several decades ago there is a single approved drug to control the formation of AGEs. In recent times the focus has been shifted to identify natural products for the prevention of Glycation and its downstream processes like protein aggregation and glycooxidation. Several studies have reported the antidiabetic potential of honey and its constituents. Some of the important bee products which have been shown to suppress the formation of glycation products are chrysin, honey bee venom and bee propolis. They have been found to be effective in reducing the glycated hemoglobin, HbA1c, level as well as oxidative stress. Further studies are needed to identify the active ingredients from the bee products and their mechanism of action in suppressing the prevention of glycation products and its downstream consequences like protein aggregation and glycooxidation.

**Keywords:** Aggregation, Diabetes, Glycation, Honey bee products, Propolis

## Interactions of honey bee viruses with other stressors

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### Abstract:

More than 70 viruses have already been described in honey bees, resulting in frequent co-infections, some infections remaining as covert infections. Interactions with other stressors could be key factors to induce the increase of viral loads, then causing clinical signs. Nutrition has been shown to impact viral loads via modulating the immune response of honeybees: when diversity and abundance of pollen increase, viral loads may decrease. Temperature also modulates viral loads, with elevated temperatures causing a decrease in Deformed wing virus (DWV) loads. Drastic impacts of co-exposure to virus and pesticides have been demonstrated on foraging behavior and lifespan. A high reduction in total foraging time has been observed for DWV infected bees when exposed to 1ng of Thiametoxam. A high proportion of co-exposed bees (> 50%) never return to the colony after their first exit. Co-exposure causes also premature foraging trips, then changes in labor division may impact the homeostasis of the colony. Beekeeping practices need to avoid the accumulation of stress, whatever biotic or abiotic stressors.

**Keywords:** interactions, stress, neonicotinoids, virus loads, epidemiology