



ICbees

International Congress on Bee Sciences

Second

International

Congress on Bee

Sciences

Abstract Book

www.beeandlifecongress.com

ONLINE

FREE
OF
CHARGE

14-15-16
June
2023

ISBN: 978-605-71368-7-9



SECOND INTERNATIONAL CONGRESS ON BEE SCIENCES ABSTRACT BOOK

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Editor: Ulaş ACARÖZ

Published: 17.07.2023

ISBN: 978-605-71368-7-9

Editor's Note

The second '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 33 invited speakers from 19 different countries. The scientific committee of the congress consisted of 274 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

Antioxidant Properties of Ultrasound Assisted Extracts of Propolis Produced in Ankara Region, Obtained in Different Solvents

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Abstract:

Propolis is an important natural product that has many biological activities, including antioxidants, and also contains polyphenolic compounds such as flavonoids and phenols. In the evaluation of the antioxidant status of propolis, total flavonoid substance (TF) (mg QE/kg), total phenolic content (TP) ((mg GAE/kg)) and 2,2-Diphenyl-1-picrylhydrazil (DPPH) (IC₅₀) levels are determined. In this study, TF, TP and DPPH (%) levels were evaluated using water extract (WE), methanolic extract (EE) and ethanolic extract (ME) in 20 propolis samples collected from Ankara region. As a result of the study, WE's TF level was 18.68 ± 0.32 , TP level was 13.05 ± 4.58 , and DPPH level was 21.34 ± 3.17 . The TF level of EE was 53.03 ± 1.3 , the TP level was 33.58 ± 1.34 , and the DPPH level was 18.57 ± 2.77 . The TF level of ME was 56.06 ± 0.99 , the TP level was 39.2 ± 1.09 , and the DPPH level was 15.28 ± 2.03 . It was also determined that the antioxidant property of the water extract of propolis was significantly lower than the other extracts. In this study, antioxidant levels of extracts of propolis samples in various solvents were successfully evaluated comparatively. As a result, it was predicted that the extraction of propolis sample by ultrasonic extraction method may provide significant advantages in the use of bioactive compounds of propolis in the medical field. In addition, it was thought that the active ingredients in propolis samples showed different biological properties in different solvents, and that it would provide various advantages to the pharmaceutical agents to be produced in terms of diversity and effectiveness.

Keywords: Ankara region, Antioxidants, Propolis, Total phenolic substance, Total flavonoid content, DPPH.

Molecular detection of honey bee pathogens in bee bread and royal jelly

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Abstract:

Due to their ecological and economic importance, honey bees have attracted much scientific attention, which has intensified due to the recent population decline of these insects in the several parts of the world. Among the factors related to these patterns, infection by various pathogens are the most relevant. Because of the possibility of dispersion of pathogens via bee products, this work aimed to identify the presence of six honey bee pathogens and parasites (*Paenibacillus larvae*, *Melissococcus plutonius*, *Nosema apis*, *Nosema ceranae*, *Ascospaera apis* and *Varroa destructor*) using conventional PCR assays in samples of bee bread and royal jelly. In this study, we extracted DNA from 12 bee bread and 4 royal jelly samples from different regions of the country and tested the presence of DNA. Of all examined samples, we were able to identify only *Nosema ceranae* and *Ascospaera apis* in two samples of bee bread. This study reported for the first time unconventional approach for molecular detection of pathogens and parasites in honey bee products - bee bread and royal jelly in Bulgaria. The use of these products (particularly, when it does not derive from a single colony, as in our case) might provide information based on the health situation in many colonies or at the apiary level or even at the regional level.

Keywords: honey bee, honey bee pathogens; alternative approach; honey bee health; monitoring of honey bee diseases.

This research was funded by the Bulgarian National Science Fund of the Ministry of Education and Science, grant number KII-06-H56/11 17.11.2021.

Determination of hydroxymethylfurfural and proline amounts of Ardahan Raw Flower Honey

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Abstract:

The present study focused on the moisture content hydroxymethylfurfural (HMF) values and proline content of raw flower honey from Ardahan. According to the study, the raw floral honey from Ardahan demonstrated specific features that contribute to its quality and potential uses in the food and pharmaceutical industries. In 2020, 20 flower raw flower honeys collected and sold during the honey harvest season were purchased from local vendors. HMF and proline were analyzed in honey. Honey samples were analyzed for the detection of HMF and proline. In addition, it has been evaluated whether the results are in accordance with the Turkish Food Codex Honey Communiqué. The TSE 3036:2002 method was used to for the HMF analysis of the honey samples. The IHC technique was used to analyze the proline content of honey samples. The average HMF value of 7.1 ± 1.4 mg/kg demonstrated freshness and a minimal level of honey deterioration. The honey's legitimacy and purity were suggested by its proline concentration, which was 424.6 ± 36 mg/kg. The average HMF and proline values detected in honey samples were found to be in compliance with the Turkish Food Codex Honey Communiqué. These results provide valuable information about the raw flower honey from Ardahan, highlighting its quality, authenticity and potential applications. Further research can be done on these findings to explore the unique properties and potential benefits of honey from this region, which adds to the value of honey and its use in a variety of industries.

Keywords: Raw honey, HMF, proline, Ardahan

Chemical profiling and nutritional evaluation of bee pollen, bee bread, and royal jelly and their role in functional fermented dairy products

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Abstract:

Honeybee products, as multicomponent substances, have been a focus of great interest. The present work aimed to perform the nutritional and chemical profiling and biochemical characterization of bee pollen (BP), bee bread (BB), and royal jelly (RJ) and study their applications in the fortification of functional fermented dairy products. Their effects on starter cultures and the physicochemical and sensorial quality of products were monitored. A molecular networking analysis identified a total of 46 compounds in the three bee products that could be potential medicines, including flavonoids, fatty acids, and peptides. BB showed the highest protein and sugar contents (22.57 and 26.78 g/100 g), which cover 45.14 and 53.56% of their daily values (DVs), with considerable amounts of the essential amino acids threonine and lysine (59.50 and 42.03%). BP, BB, and RJ can be considered sources of iron, as 100 g can cover 141, 198.5, and 94.94% of DV%, respectively. BP was revealed to have the highest phenolic and flavonoid contents (105.68 and 43.91 µg/g) and showed a synergetic effect when mixed with RJ, resulting in increased antioxidant activity, while BB showed a synergetic effect when mixed with RJ in terms of both antioxidant and proteolytic powers (IC₅₀ 7.54, 11.55, 12.15, 12.50, and 12.65 cP compared to the control (10.55 cP)), reflecting their organoleptic properties and highlighting these health-oriented products as promising natural products for human health care.

Keywords: honeybee products; amino acid profile; chemical profiling; antioxidant potentials; proteolytic activity

Use of essential oil from *Calamintha nepeta*, a typical plant species of the Mediterranean basin, for varroosis control in *Apis mellifera*

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Abstract:

Varroa destructor is the main honeybees parasitosis. The mite is widespread worldwide and treatments are necessary for the survival of hives. Pest control strategies are mainly based on the use of synthetic acaricides. However, misuse has led to the emergence of resistance phenomena. The identification of control alternatives becomes mandatory. Possible solutions include the use of essential oils (EO). In Calabria (Southern Italy) there is a particular wealth of plants known for their pharmacological properties. In this study, it was decided to study the acaricidal effects of EO isolated from the native species *Calamintha nepeta* (whole plant). In Calabrian ethnobotany, this species was used as a vermifuge and insecticide against bugs, woodworms, and flies. EO activity was assessed by means of a bioassay in a closed chamber and in an open chamber. For the closed chamber, 5 female mites were transferred into an Eppendorf tube and exposed to the vapors from cotton balls soaked in EO solutions, which were inserted into the cap to close the Eppendorf tube. The mites were exposed to the vapors for 15, 30, 45 and 90 minutes. Subsequently, their mortality was evaluated under a stereomicroscope. Two-level cylindrical cages were designed for the open chamber. Twenty adult honeybees and ten mites were placed in the upper part of the cage. A filter paper soaked in EO solutions at different concentrations was placed in the lower compartment. Concentrations of 10, 20, 30 and 40 mg/mL were tested. Mite detachment was evaluated over time. In the closed chamber, the best results were achieved at 90 minutes. The open chamber experiment revealed that mite mortality depends on the EO concentration. To obtain a 76% decrease in *V. destructor* (similar to that obtained in the closed chamber), EO concentrations almost 20 times higher than the maximum used in the closed chamber were required. Phytochemical characterization showed that terpenes and monoterpenes were the most representative compounds. However, it cannot be excluded that the effectiveness of the phytocomplex is due to the interaction of these molecules with less represented ones, which could increase the effectiveness.

Keywords: *Varroa destructor*, *Apis mellifera*, *Calamintha nepeta*, essential oil, fumigation toxicity

Honey Loaded Electrospun Nanofibers for Antibacterial Applications: A Review

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Abstract:

Bee products, especially honey and propolis, are considered as complementary medicines to treat the skin wounds and burns, related to their biological activities. Honey has antibacterial, anti-inflammatory, antioxidant, antiviral and biocompatibility features. Therefore, it is utilized therapeutically in both conventional and modern medicine. Modern wound dressings are special materials in promoting wound healing, preventing infections and minimizing the healing time and finally improving skin restoration. In particular, it is important to fabricate suitable wound dresses for chronic skin wounds which cannot heal generally on their own. Among the wound dressings, electrospun nanofibers are gaining importance in wound care applications as promising solutions for better treatment. Nanofibrous scaffolds can bring the advantage of the ability to mimic the skin extracellular matrix (ECM). In addition, the high surface area to volume ratio and greater porosity of these fibers made them precious. These structural properties provide transportation of wound fluid and breathability for cellular growth and cell proliferation. Moreover, many bioactive compounds can be loaded to these systems. The recent researches focused on the evaluation of different types of honey to create electrospun nanofibers as potential wound dressings. In the previous studies, honey loaded several polymer matrices including chitosan, gelatin, polycaprolactone, polyethylene oxide, polyvinyl alcohol, and silk fibroin as well as the combination of these polymers have been investigated for the development of electrospun nanofiber based materials. In this review, recent advances in honey loaded bioactive electrospun nanofibers for wound healing applications are summarized in detail.

Keywords: Biomaterials, electrospun nanofibers, honey, tissue engineering, wound dressing.

Bees under interactive stressors: the novel insecticides flupyradifurone and sulfoxaflor along with the fungicide azoxystrobin disrupt the gut microbiota of honey bees and increase opportunistic bacterial pathogens

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Abstract:

The gut microbiome plays an important role in bee health and disease. But it can be disrupted by pesticides and in-hive chemicals, putting honey bee health in danger. We used a controlled and fully crossed laboratory experimental design to test the effects of a 10-day period of chronic exposure to field-realistic sublethal concentrations of two nicotinic acetylcholine receptor agonist insecticides (nAChRs), namely flupyradifurone (FPF) and sulfoxaflor (Sulf), and a fungicide, azoxystrobin (Azoxy), individually and in combination, on the survival of individual honey bee workers and the composition of their gut microbiota (fungal and bacterial diversity). Metabarcoding was used to examine the gut microbiota on days 0, 5, and 10 of pesticide exposure to determine how the microbial response varies over time; to do so, the fungal ITS2 fragment and the V4 region of the bacterial 16S rRNA were targeted. We found that FPF has a negative impact on honey bee survival, but interactive (additive or synergistic) effects between either insecticide and the fungicide on honey bee survival were not statistically significant. Pesticide treatments significantly impacted the microbial community composition. The fungicide Azoxy substantially reduced the Shannon diversity of fungi after chronic exposure for 10 days. The relative abundance of the top 10 genera of the bee gut microbiota was also differentially affected by the fungicide, insecticides, and fungicide-insecticide combinations. Gut microbiota dysbiosis was associated with an increase in the relative abundance of opportunistic pathogens such as *Serratia* spp. (e.g. *S. marcescens*), which can have devastating consequences for host health such as increased susceptibility to infection and reduced lifespan. Our findings raise concerns about the long-term impact of novel nAChR insecticides, particularly FPF, on pollinator health and recommend a novel methodology for a refined risk assessment that includes the potential effects of agrochemicals on the gut microbiome of bees.

Keywords: Gut microbiota, pollinator decline, pesticide, dysbiosis, synergistic effect, pathogen, yeast

Evidence for Bee Products Efficacy on Liver Disorders in Animal Studies

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Abstract:

Apitherapy is the therapeutic or preventative use of bee products to prevent or delay the progression of various disease. Recent studies have shown that bee products potential benefits for liver disorders. It has been demonstrated that bee venom inhibits adjuvant-induced arthritis and methotrexate hepatotoxicity in rats due to its inhibitory effect on TNF- α and NF- κ B. It has also been discovered that honey protects against acute metanil yellow induced liver injuries by antioxidant and anti-inflammatory properties. In another study, chestnut bee pollen has been shown to protect the rats against carbon tetrachloride induced liver injury. It is thought that honey, propolis and royal jelly have a similar effect against liver disorders. In addition, recent research suggests that bee bread may have anti-oxidant, anti-inflammatory, anti-steatotic, and anti-fibrotic properties that protect against the progression of non-alcoholic steatohepatitis (NASH) and fibrosis in the liver by attenuating oxidative stress and inflammation. Bee products are recognized as a potential source of natural antioxidants such as flavonoids, phenolic acids or terpenoids. The strong effects of bee products on liver diseases may be due to these components. However, additional detailed pre-clinical and clinical research is required to thoroughly comprehend the medicinal value of bee products. This review examines the potential effects of various bee products on liver disorders in animal studies.

Keywords: animal models, apitherapy, bee products, liver disorders

Honey Reduces Virulence Factors and Biofilm Formation of *Pseudomonas aeruginosa* Clinical Isolates

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Abstract:

Quorum sensing (QS) is crucial for *Pseudomonas aeruginosa* in the production of virulence factors and biofilm formation. Strategies that targeting virulence factors and biofilm formation are known to be more sustainable than antibiotics to attenuate the infectivity of bacteria and less likely to induce resistance. Although honey has been shown to impact the growth of *P. aeruginosa*, the information about the antipseudomonal activity of *Apis cerana* honey is still very limited. Hence, the objective of this study was to evaluate the inhibitory effects of honey on the QS virulence factors and biofilm formation of antibiotic resistant *P. aeruginosa* clinical isolates. Results showed honey has significantly reduced the growth, protease activity, pyocyanin production and exotoxin A concentration of ATCC 27853 and four clinical isolates. Other than biofilm formation, the swarming and swimming motility of all isolates were also significantly inhibited by the honey. Remarkable morphological changes of bacterial cells were also observed by using a scanning electron microscope. This study revealed the effectiveness of *A. cerana* honey to diminish QS virulence factors and biofilm formation of *P. aeruginosa*.

Keywords: honey; antibacterial; virulence factors; biofilm formation; *Pseudomonas aeruginosa*

Consequences of pesticide exposure on gut bacterial diversity in honeybee queens #

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Abstract:

Honeybees (*Apis mellifera*) face multiple environmental stressors that have consequences on their physiology, fitness and health. Among them, pesticides are a major threat to pollinators due to direct mortality or sublethal effects on a wide variety of traits. Honeybees are exposed to pesticides mainly by foraging and ingesting resources, such as pollen and nectar, often contaminated with numerous agrochemical compounds. Most of the studies on pesticides and gut microbiota (e.g., bacterial diversity, community structure) were conducted on worker bees. Gut microbiota is involved in digestion, immunity and pathogen protection for example, and therefore plays a major role in honeybee health. However, to our knowledge, the effects of pesticides on queens' gut microbiota are unknown. Honeybees are social insects in which a single individual, the queen, produces all the offspring. Moreover, the fate and performance of the colony is highly dependent on queen health. In this study, we investigated the consequences of pesticide exposure on queen gut microbiota. Pesticide concentrations were based on pesticide residues in pollens found in the literature. Queens were exposed to the insecticide cypermethrin, either alone or in combination with two fungicides, in the laboratory for two days after emergence. Queens were collected after two weeks to assess their gut bacterial community. Alpha- (observed richness, Shannon, inverse Simpson) and beta-diversity (Bray-Curtis and Weighted UniFrac) indexes were not significantly affected by queen exposure to pesticides. However, we identified bacteria belonging to important genera in the honeybee queen microbiota that were differentially abundant between pesticide treatments. Brief oral exposure at very low dose of pesticides can therefore induce changes in the honeybee queen microbiota. In addition, these pesticides could have long-term effects on queen health and reproductive performance that need to be assessed.

Keywords: microbiota, insecticide, fungicides, sublethal effects, *Apis mellifera*

#This work was financially supported by the French National Program 'Ecophyto II+'.

Differentiation Of Bee Pollen Samples According To Its Bioactive Compounds By Using A Canonical Discriminant Analysis

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Abstract:

Bee pollen has been used in the human diet for many centuries. Its ever-increasing consumption results from its nutritional value and its health-promoting effects, like those relating to its antioxidant, anti-inflammatory, anticarcinogenic, antibacterial or anti-fungal properties. The nutritional value/quality and health properties of bee pollen are linked to its constituents, which include proteins, amino acids, lipids, carbohydrates, phenolic compounds, vitamins, and minerals, among several other compounds. However, its composition varies greatly according to several factors, like botanical and geographical origins, climatic conditions, the type of soil, or harvesting and processing conditions. This is quite important to prevent one of the main problems currently affecting the commercialization of bee pollen and thus the beekeeping industry, which is the fraudulent practice of adulteration with pollen from other sources/origins, such as, for instance, pine pollen. As may be expected, studying the profile of a particular family of compounds in bee pollen has been proposed to specify/authenticate its origin as well as to evaluate its corresponding nutritional value. Therefore, the main goal of this study is to investigate the potential of minerals and three families of bioactive compounds scarcely investigated in this matrix (amino acids, betaines and glucosinolates) as bee pollen markers, by determining with different analytical techniques like high performance liquid chromatography (HPLC), gas chromatography (GC) and inductively coupled plasma (ICP) their respective content in bee pollen samples from experimental apiaries located within the same area (Marchamalo, Guadalajara, Spain). It should be mentioned that in most cases, new analytical methods which fulfilled some of the principles of green analytical chemistry were proposed, and they were fully validated according with current legislation. Results showed that bee pollen samples can be classified with more than 85% of accuracy by means of a canonical discriminant analysis (CDA) based on the content of the different compounds, according to the corresponding apiary of origin. Therefore, the potential of these compounds as bee pollen markers was demonstrated, and, in addition, new analytical tools to authenticate the origin of bee pollen were provided.

Keywords: bioactive compounds, bee pollen, chemometrics, chromatographic techniques, food authentication.

Authors thank financial support by the Spanish “Ministerio de Economía y Competitividad” and the “Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria” (Project number, RTA 2015-00013-C03-03).

Detection of saccharide adulteration in honey by ¹H NMR

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Abstract:

NMR has already a well established and accepted analytical technique in several studies applied to quality characterization and fraud detection in foods. Recent studies highlighted also capabilities in adulteration detection in complex matrices like honey. This illegal practice is attracting producers worldwide, mostly due to the relevant economic gain available and to the lack of an official analytical method able to detect adulterations, particularly saccharide syrups. In the present study the use of both targeted and untargeted NMR spectroscopy have been applied to reveal inulin, corn/malt syrup and invert sugar syrup addition. Untargeted NMR data of pure and artificially adulterated samples in the range of 10–40% were statistical analyzed and OPLS models were used to predict samples spiked with the saccharide adulterants, resulting in very good predictions of the adulteration percentage.

Targeted NMR data were concurrently evaluated, allowing to predict the adulteration percentage on the basis of calibration curves.

Keywords: NMR, saccharide syrups, honey, adulteration.

The effect of migratory beekeeping on the prevalence of viral pathogens in bee colonies

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Abstract:

Viruses are significant threats to the health and well-being of the honey bee. International efforts to assess honey bee health indicate that viruses are major stressors that undermine colony performance. Identifying factors affecting virus incidence, such as management practices, could help slow virus transmission. The effects of honeybee management, such as migratory beekeeping, are a subject of ongoing debate regarding the causes of colony health problems. Although comparisons of diseases and viral pathogens within differently managed bee colonies indicate some implications, the direct impact of migration practices on honey bee viral pathogens are poorly understood. In this study, to investigate impacts of managed migration on viral pathogens, honey bee colonies were examined with or without migratory movement. To understand the impact of migratory beekeeping on bee health, beekeeping enterprises in Denizli, Afyonkarahisar and Konya provinces, research was conducted in 24 apiaries (8 apiaries from each province). In study, a total of 361 bees were collected from 72 colonies (approximately 5-6 bees from each of colonies). Samples at different ages and life-history stages, taken at the beginning and end of the active season, were analyzed by real time reverse transcriptase-polymerase chain reaction (real time RT-PCR) method for viral pathogen loads. Bees exposed to migratory beekeeping had increased levels of the black queen cell virus (BQCV), deformed wing virus (DWV) and Sacbrood virus (SBV) infections. Specific primers were used for the genome of each virus in order to use for the molecular detection of these three viruses. Colonies from the migratory beekeeping history exhibited higher viral abundances for all viruses than colonies from the without migratory movement history. Our results provide evidence that migratory beekeeping history has persistent impacts on honeybee disease epidemiology. BQCV was detected in 19.44% (14/72) of colonies, and DWV in 29.16% (21/72), whereas SBV were not detected. The identification tests showed that many colonies were infected with one or two viruses.

Keywords: black queen cell virus, deformed wing virus, honey bee, Sacbrood virus, RT-PCR.

The effectiveness of biosecurity practices in the beekeeping sector in Turkey

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Abstract:

Biosecurity practices include measures to prevent the spread of diseases in the apiary or to reduce their impact. Biosecurity principles aim to prevent microorganism factors from entering and spreading in apiaries, in order to prevent bee health or the quality of honey produced by bees from being adversely affected. In this study, beekeepers were interviewed between March 2018 and May 2022 in order to provide information about the biosecurity practices necessary to prevent bee diseases, to prevent the spread of diseases and pests in and between the apiaries, and to obtain quality and reliable bee products. The data was a semi-structured questionnaire (SSQ), which consists of three parts investigating the operating characteristics, bee health and biosafety practices, and views on the practice. At the same time, 220 samples were collected from 75 apiaries by simple random sampling to evaluate viral pathogens (acute bee paralysis virus, black queen cell virus, deformed wing virus) while obtaining data from bee owners. Samples were analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR) method for viral pathogen loads. The general characteristics of the apiaries and sanitary information previous years was evaluated through questionnaires regarding, while the vegetation surrounding the apiaries sampled was assessed by logistic regression analysis. While viral bee diseases were observed in 21.33% (16/75) of apiaries, the Varroa problem was reported in 48.36% (37/75). Beekeepers' views on the most important problems in the beekeeping sector are the agricultural sprayings around the accommodation areas and apiaries, areas with weak flora and the inadequacy of honey forests. Beekeeping enterprises have high production costs and significant problems in bee health and marketing of bee products. Overall, 55 (73.33%) of the apiaries were visited by a bee specialist, but 10.66% (8/75) reported the biosecurity procedure for these visits. As a result, biosecurity practices in apiaries can prevent the spread of potential risk sources and provide feedback to the industry and industry stakeholders on effective biosecurity practices.

Keywords: Biosecurity, honey bee, viral pathogens, RT-PCR.

Importance of gut microflora on bee health and evolution

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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 Health, Host-Microbe interaction, Bee gut microflora

Oral Presentation

Antagonistic and Antibiofilm Activity of Potentially Probiotic Lactic Acid Bacteria Against Honeybee (*Apis mellifera* L.) Pathogens – An *In Vitro* Study[#]

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Abstract:

Introduction. Honeybees are exposed to many factors that adversely affect the health of entire colonies. Known honeybee bacterial pathogens include *Paenibacillus larvae*, and *Melissococcus plutonius*. Probiotics and postbiotics may be promising biocontrol agents for honeybee health used especially for prevention. The aim of the study was to assess the antagonistic and antibiofilm activity of LAB strains (or their metabolites) of various origins against several honeybee pathogens. Coaggregation tests of LAB with selected pathogens were also conducted. **Methods.** Antagonistic activity for alive LAB cells was evaluated with agar slab method, while for LAB metabolites (supernatants) with agar well diffusion method. Antibiofilm activity of LAB and coaggregation with pathogens was estimated spectrophotometrically. **Results.** The strongest antimicrobial activity against honeybee pathogens was displayed by cocci of the species *Pediococcus acidilactici* and *P. pentosaceus*. Strong antagonistic activity was demonstrated also by species *Lactiplantibacillus plantarum*. All these bacteria were mostly isolated from the honeybee environment (*e.g.* flowers, honey). Postbiotics inhibitory activity depended on the LAB and pathogen strain. Acidification of the environment was the basic mechanism of LAB antimicrobial activity. Co-aggregation was a strain-dependent feature and differed depending on the pathogen tested. All tested postbiotics showed a broad spectrum of anti-adhesive activity against the tested pathogens. The strongest inhibition of adhesion was observed for *Paenibacillus apiarius* DSM 5582 and *M. plutonius* DSM 29964. The low biofilm eradication shown by postbiotics suggests a weak effect of LAB metabolites on mature biofilms of pathogenic microorganisms. **Conclusions.** The metabolites of LAB display stronger anti-adhesive properties (*i.e.*, during the initial phase of biofilm formation) in comparison to the eradication of mature biofilms. The anti-adhesive activity demonstrated by LAB may contribute to the biocontrol of pathogens at very early stage of infection.

Keywords: probiotics, honeybee, lactic acid bacteria, postbiotics, *Paenibacillus larvae*, *Melissococcus plutonius*, honeybee pathogens

[#]The study supported by the project from the *Provincial Fund for Environmental Protection and Water Management in Lodz, Poland* (no. 729/BN/D/2019) entitled: "Selection of microorganisms for the construction of ecological protective preparation for honeybee (*Apis mellifera*)".

Probiotics in honeybee health and disease management

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Abstract:

Apis mellifera is one of the most important pollinating insects in the production of vegetables, fruits and crops. The honey bee microbiota is an ecosystem of microorganisms responsible for metabolic functions such as energy and vitamin management, satiety regulation, lipid homeostasis and adjustment of glucose levels. It stimulates the immune system against pathogens. Microbiota composition is unique to individuals within the same species. Gut dysbiosis refers to gut microbial imbalance leading to adverse host physiological and functional changes. Lactic acid bacteria (LAB), which are abundant in the microbiota, naturally live in the digestive tract of honey bees and stimulate the bee immune system, assimilate nutrients, fight pathogens and maintain microbiota homeostasis in the gut. Two of the most important genera found commensally in humans and animals and used as probiotics for both humans and animals are *Lactobacillus* and *Bifidobacterium*. LAB produce a variety of beneficial compounds for the host. Honey bee LABs are involved in food digestion, bee immune system, eliminating pathogens and maintaining microflora homeostasis in the honey bee gut. LAB are known to produce antimicrobial substances (antimicrobial peptide-AMP) that eliminate pathogenic microorganisms. Choosing the right probiotics for bee health is especially important for modulating the composition of the gut microbiota. It also has a positive effect on the immune response. In the prevention and treatment of many infections that cause significant economic losses in honey bees, it is very useful to give the right probiotic in the appropriate way and at the right doses. The fact that these probiotics are species isolated from honey bees will facilitate prevention and treatment and will not disturb the balance in the intestinal microbiome. In addition, giving probiotics with foods suitable for bee consumption such as pollen, bee bread and sherbet will increase success.

Keywords: Honey Bee, Probiotics, LAB

Synthesis of Iron oxide Nanoparticle using Propolis from Northern Cyprus and evaluation of its antibacterial, anticancer potential on MDA-MB 231 cells

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Abstract:

Research in nanotechnology has progressed in the last decades, and it is purposeful to explore the applications of biocompatible nanoparticles in general and their anticancer attribute specifically. This study is aimed at examining the cytotoxic effects of biocompatible Northern Cyprus propolis extract of iron oxide nanoparticles (PE-Fe₂O₃-NPs) on MDA-MB-231 epithelial human breast cancer cell line, in vitro. Propolis extract were used to obtain PE-Fe₂O₃-NPs and further characterized using several methods like ultraviolet-visible spectroscopy, Fourier-transform infrared spectroscopy, scanning electronic microscopy and X-ray diffraction analysis. The PE-Fe₂O₃-NPs has a distinctive Surface Plasmon Resonance band at 350 nm. The prepared PE-Fe₂O₃-NPs had a size of 108 nm by diameter with a zeta potential of +33.9 mV which indicates a good stability of the nanoparticles' while the size of Fe₂O₃-NPs was 89.40 nm by diameter. Antibacterial result showed that Fe₂O₃-NPs has highest Minimum Inhibitory Concentration and Minimum Bactericidal Concentration as compared to PE-Fe₂O₃-NPs with different concentration of propolis extract as regards the tested microorganisms. This indicates that PE-Fe₂O₃-NPs possesses a more effective threat to eliminating pathogenic bacteria at lesser dosages, be it Gram-ve or Gram+ve. PE-Fe₂O₃-NPs effectively halted the spread of MDA-MB-231 cancer cell lines and thus, prove to be commendable for anticancer biomedical uses.

Keywords: Propolis, Iron oxide, Nanoparticles, Anticancer, Antibacterial, X-ray diffraction, UV/Vis spectroscopy, FTIR

Honey Color Measurement Methods

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Abstract:

The color of honey is an important feature and may vary depending on its composition (sugars, fructo-oligosaccharides, amino acids, vitamins, minerals, enzymes, and color compounds) geographical location, climate, floral source, storage conditions, and honey-bee species. Although honey color is a valuable characteristic, there is currently no standardized method for its measurement. Various methods have been used to objectively measure and classify honey color including visual comparison scales, colorimetry, and spectrophotometry, have been employed to assess honey color, each with its own advantages and limitations. A commonly used method is the Pfund color classifier, which involves visually comparing a honey sample with an amber glass wedge and a wedge-shaped cell containing honey. Other visual methods for honey color analysis include the Lovibond and Jack's scales, which require visual comparison of honey samples with standard color references. However, these methods may have limitations in detecting subtle color differences and may be subjective depending on the judgment of the observer. Colorimetric and spectrophotometric techniques have also been used to measure honey color. These methods involve the determination of colorimetric parameters based on spectral information or color space. The commonly used color space in the honey color analysis is CIE L* a* b* (L*: whiteness-darkness, a*: redness-greenness, b*: yellowness-blueness). These techniques provide a more precise assessment of honey color and can detect smaller differences between samples.

Keywords: Honey, color, spectrophotometry

Influence of keeping conditions of worker honeybees on enzymatic activity and oxidative stress

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Abstract:

The aim of the experiments was to compare the enzymatic activity (phenoloxidase and lysozyme-like) and oxidative stress of worker honeybees kept in natural conditions (apiary) and in laboratory conditions. Honeybees were collected in 4 time variants (1, 10, 19, 28 days after emergence) in June and September from the laboratory and the local apiary located in Łuszczów Drugi, Poland. The level of phenoloxidase activity in hemolymph was checked by the spectrophotometric method using L-dihydroxyphenylalanine as a substrate and lysozyme-like activity by diffusion well assay on plates containing *Micrococcus lysodeikticus*. Changes in oxidative stress levels were measured by assaying total reactive oxygen species (ROS) and total reactive nitrogen species (RNS) activities using commercial kit in honeybees homogenates. Due to the high mortality of 28-day-old honeybees kept in the laboratory in June, it was not possible to collect hemolymph for lysozyme-type activity assays. The results of the experiments showed that the level of phenoloxidase in 10 and 28-day-old June honeybees kept in the laboratory was higher than in honeybees kept in the apiary, while in 10 and 19-day-old September honeybees the trend reversed. One-day-old June honeybees and 10-day-old September honeybees kept in an apiary had the highest levels of phenoloxidase. In the case of lysozyme-type activity, it was found to be higher in all time variants of June and September honeybees kept in the apiary compared to the laboratory. The highest level of lysozyme-type activity was observed in 19-day-old June honeybees and 10-day-old September honeybees kept in the apiary. Experiments have shown that in both 10, 19 and 28-day June and September honeybees, total ROS/RNS production is higher in the apiary compared to the laboratory. Additionally, September honeybees have a higher total ROS/RNS production than June honeybees. Based on the obtained results, it was found that the enzymatic activity and oxidative stress are influenced by the conditions of keeping, the age of honeybees and seasonality, i.e. the month when the honeybees were collected. The work was financially supported by grant number 2021/41/N/NZ6/00735 from the National Science Centre (Krakow, Poland).

Keywords: keeping conditions, phenoloxidase, reactive oxygen and nitrogen species, lysozyme-like activity

Role of Probiotics in Bee Health

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Abstract:

Honeybees are characterized as a crucial species that is critical to the existence of nearly all living things on Earth. However, colony collapse, which has resulted in catastrophic fatalities of honey bee populations worldwide, is a source of international worry. To avoid these losses, it is vital to develop innovative ways. According to the research, microorganisms with probiotic qualities in the bees' bodies play an active part in the bees' resistance to infections. In this context, in this study, it was aimed to examine the effect of probiotic bacteria obtained from the stomach of bees against pathogens that cause disease in bees and to use these bacteria to strengthen the immune system of bees. In this context, *Lactobacillus kunkeei* and *Bifidobacterium* sp. Bacteria were applied to adult and healthy bees and their effects on the immune system were examined. During the experiment, the control group was fed only with glucose syrup. Another group was given only probiotic bacteria. To cause disease in a group, *Nosema* sp. given. In the last group, both probiotic bacteria and *Nosema* sp. given. By creating 4 groups for each probiotic in total; 8 groups were formed. These were also tested by applying them in triplicate. When the results obtained are examined, it is seen that probiotic bacteria live more healthily than the control groups by affecting the immune system of the bees. When the results are evaluated, it is thought that the obtained isolates will be prepared in the future, will support the immune system of the bees, and as a result, resistant bees will be produced without resorting to antibiotics and chemical treatment methods.

Keywords: honey bee health, probiotics, bacteria, microbiology

The Effect Of Honey Types On The Production Of Hyaluronic Acid By *Streptococcus* spps.

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Abstract:

In this study, hyaluronic acid (HA) production amounts of spring honey and sunflower honey by microbial fermentation using *Streptococcus zooepidemicus* and *Streptococcus pyogenes* were compared with HA production amounts of glucose using the same species of bacteria.

Fermentation processes for production of HA was carried out in bioreactors with a working volume of 200 mL fermentation medium, and culture conditions were kept at 37 °C, pH 6.5 and mixing at 150 rpm. Three concentrations of each type honey was used: 15, 25 and 40 g/L to observe concentration dependent suppression of HA production. As control, 25 g/L glucose was used in each group. The dissolved oxygen levels were kept above 20% during the fermentation. HA concentrations in fermentation mediums at the end of 5 hour fermentation was measured by using carbazole method. The medium containing sunflower honey achieved higher concentrations of HA (*S. zooepidemicus*; 0.21 g/L, *S. pyogenes*; 0.11 g/L, P<0.05) when compared to the medium containing spring honey (*S. zooepidemicus*; 0.12 g/L, *S. pyogenes*; 0.05 g/L). The highest HA production (0.35 g/L) was obtained in the fermentation medium using glucose with *S. zooepidemicus*. We also observed anti-hyaluronidase in microbial fermentations using honeys from two different sources compared to the medium containing glucose as carbon source. It is possible that antimicrobial effects of honeys collected from different plants suppressed microbial fermentation process.

Keywords: Hyaluronic acid, Fermentation, Sunflower honey, Spring honey, *Streptococcus*

Effect of a common fungicide on the honeybees activites after chronic exposure

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Abstract:

Bees are critical pollinators boosting crop yield and maintaining biodiversity. Unfortunately, bee populations are declining worldwide due to several factors, including loss of habitat and resources, pests (viruses, parasites, etc.) and exposure to pesticides. The increase in the number of threats is causing major concern among scientists, governments, and citizens, especially as some may have hidden effects. The sublethal effects of pesticides on non-target species are understudied and probably underestimated. One commonly used pesticide, the Boscalid, is used to control crops and seed fungi by inhibiting their cellular respiration his mechanism targets a cellular process present in numerous species, and its effects have been observed in other insects. Unfortunately, the sublethal effects of Boscalid on bees remains unclear. Our objective is to evaluate the consequences of early and recurrent Boscalid exposure on the life cycle and behavior of honeybees (*Apis mellifera*). We assumed that the sublethal consequences of Boscalid can lead to a reduction in long-term capacity and longevity of honeybee workers, which is difficult to assess in the field. We conducted laboratory exposure of individuals during the larval stage and after emergence. Then we individually tagged workers with RFID (radio frequency identification) chips to follow them throughout their lives under natural conditions. This enabled the automated recording of their flight activity (number and duration of flights) over 3 sessions in 2021. Our observations revealed significant seasonal variations in the characteristics of their life cycle, such as the age of first exit and their lifespan. Furthermore, the exposure to Boscalid resulted in a decrease in the success rate of their initial flights and affected their foraging during specific sessions. The results of this study will provide a better understanding of the effect of exposure to Boscalid on the life cycle of worker bees and their foraging behavior. We will discuss these results in the context of global rise in pesticide consumption. The results will contribute to the comprehension of pesticide impacts on non-target organisms and inform recommendations for management practices that aim to conserve bees, promote pollination services, and safeguard biodiversity in agricultural landscapes.

Keywords: *Apis mellifera*, Larvae rearing; Flight activities; Boscalid

This work was financially supported by the French Plan 'Ecophyto II+'.

An investigation on immune gene expressions of honey bees (*Apis mellifera anatoliaca*) against *Varroa destructor* mite

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Abstract:

Honey bees face a multitude of biotic stressors throughout their life cycle, including exposure to various pathogens, parasites, and predators. In response to these challenges, honey bees have evolved a range of adaptation mechanisms to thrive in changing environments. Among these biotic stressors, the *Varroa destructor* stands out as one of the most dangerous external parasitic mites worldwide since Varroa mite infestation poses a serious threat to honey bee health and colony survival. Moreover, the Varroa mites act as vectors for several devastating viruses, including *Deformed Wing Virus* (DWV) and *Acute Bee Paralysis Virus* (ABPV). These viral infections further compromise the health and vitality of honey bee colonies, leading to increased mortality rates and decreased overall productivity. To address the urgent need for Varroa-resistant honey bee populations, this study focused on evaluating the expression levels of 17 critical immune-related genes associated with three major immune response pathways: Toll, Imd/JNK, and JAK/STAT. The colonies under investigation were specifically selected for Varroa-resistance or susceptibility over a span of 15 years on Marmara Island. Notably, some of these genes had not been previously studied, making this research particularly valuable. Through qRT-PCR experiments, we assessed the gene expression results and identified differential regulation in at least one resistant colony compared to the susceptible colony for genes such as Antdh, Dscam, Bgluc1, Futsch, Dorsal-1, Arrestin2, GB12154, GB12705, and GB19804. These findings suggest that these genes may play a crucial role in conferring Varroa-resistance in honey bees and merit further investigation. Additionally, the study investigated the prevalence of DWV and ABPV in both Varroa-susceptible and Varroa-resistant honey bee colonies. The knowledge gained from this research offers valuable insights into the molecular mechanisms underlying Varroa-resistance in honey bees. By understanding the specific genes and pathways involved in the immune response to Varroa mite infestation, scientists can potentially develop more effective strategies for breeding Varroa-resistant honey bee populations. These findings hold great promise for future breeding programs and provide essential guidance for beekeepers seeking to mitigate the devastating effects of varroosis on honey bee colonies.

Keywords: *Apis mellifera anatoliaca*, *Varroa destructor*, honey bee viruses, immune-related gene expression.

The corresponding author was granted financial support through the TUBITAK BİDEB 2218 program.

Fast And Direct Molecular Detection Of *Nosema Ceranae*, A Parasitic Microsporidium Of Bees, Using LAMP

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Abstract: *Nosema ceranae* is a microsporidian parasite infecting the gut of the western honeybee (*Apis mellifera*) causing diarrhea and malnutrition, with high prevalence around the world. As there are no effective treatments available, preventive diagnosis and epidemiological surveys are essential for disease management. Traditional microscopy can be applied to detect *N. ceranae* spores in bee gut homogenates but it does not allow to distinguish *N. ceranae* from *N. apis*, an additional comparatively less prevalent and pathogenic bee pathogen. *Nosema* spp. differentiation is possible using molecular techniques based on PCR, which is considered the current gold standard. Emerging technologies such as loop-mediated isothermal amplification (LAMP) are an attractive alternative to PCR due to their high sensitivity, fastness, and simplicity. We have developed a LAMP technique based on the gene encoding the microsporidian polar tube protein 3 (PTP3). Using isolated genomic DNA (gDNA) from partially purified spores, LAMP showed to be up to 10 times more sensitive than a gold standard PCR, allowing to detect as low as 1 spore per bee. The reaction showed no cross-reactivity with gDNA from *N. apis* or *N. bombi* spores and can be performed in 30 min. To evaluate the performance of the developed LAMP, a set of honey bee field samples (n=70) from four different geographic regions of Argentina, positive by microscopy for *Nosema* spp. spores, were tested by LAMP and PCR. A slightly higher efficiency of detection was observed by LAMP (98.6 vs 95.5% for LAMP and PCR, respectively), with an agreement of 94.3% between techniques. In addition, a “direct-LAMP” format using purified spores was tested, bypassing the time-consuming and expensive DNA extraction step. Importantly, all *N. ceranae* samples that previously tested positive by LAMP, were consistently amplified by direct LAMP. Current research is devoted to further improve the direct-LAMP protocol to achieve pathogen detection in less time and with fewer resources. In conclusion, we have developed a rapid and efficient technique for the detection of *N. ceranae* that can be easily applied in rudimentary laboratories to facilitate the monitoring of this ubiquitous pathogen.

Keywords: *Nosema ceranae*, *Nosema apis*, molecular diagnostics, LAMP, *Apis mellifera*,

#financed by PEi069, INTA and 80020220100013UM, UM

Nosemosis development induces necrotic changes in the honeybee intestine

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Abstract:

Honeybee nosemosis attack the midgut cells of bees, where it multiply and produce infectious spores. The middle intestine of a honeybee is the focal point of nutrient absorption. Still, many aspects of this microsporidian disease are unknown among others the impact of nosemosis on the viability of honeybee intestine cells. The intestines were isolated from healthy and infected bees on 21th day after infection. The isolated intestine fragments were stained using two dyes: SYTO 9 and propidium iodide. The number of live and dead cells in infected and healthy bees met the requirements of normal distribution for the Kolmogorov–Smirnov test with the Lileforse correction. The mean value of live cells in healthy and infected honeybee intestine was 78.32% (SD = 6.37%) and 41.05% (SD = 6.28%), respectively. The difference in the number of live and dead intestinal cells of healthy and infected bees was statistically significant ($p < 0.01$, $n = 800$). During the infection the spots of dead intestinal cells were noticed. The damage of intestine during severe infection may explain the presence of pathogenic DNA, not only in the intestine, where it multiplies, but also in other tissues, such as the hypopharyngeal glands, salivary glands, Malpighian tubules, and fat body. Earlier studies indicated that apoptosis was inhibited during *Nosema* spp. infection, therefore the observed spots of dead cells were connected with necrotic changes of ventricular epithelial cells. In conclusion, this research demonstrated for the first time that a heavy microsporidian infection can lead to the interruption of the host's intestinal continuity via cellular necrosis.

Keywords: intestine cells, necrosis, intestinal leaking, live/dead tests

A highly effective bee product against American foulbrood pathogen: Anatolian bee venom

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Abstract:

American foulbrood (AFB) is a larvae disease that occurs as a result of a severe bacterial infection in honey bees and causes significant colony losses. In order to control the bacteria (*Paenibacillus larvae*) that cause the disease, it has become necessary to search for new drugs with different mechanisms of action against the development of resistance resulting from the use of inappropriate chemicals. Therefore, the aim of the study is to determine the content of Anatolian honey bee venom (HBV) and to test the antimicrobial activity of this alternative natural bee product against two different strains of *P. larvae*. HPLC method was used to determine the chemical profile of Anatolian HBV. According to HPLC analysis, separation of 2.61% Apamin, 10.83% Phospholipase A2 and 46.85% Melittin was obtained. Antimicrobial activity tests were performed using agar well diffusion and then minimal inhibition concentration (MIC) assays against *P. larvae* ATCC 9545 (ERIC I) and *P. larvae* DSM 25430 (ERIC II) strains. The inhibition zones obtained from agar well diffusion method was 28 mm for both *P. bacillus* strains. The MIC doses, on the other hand was at a very low concentration with a value of 3.125 µg for both strains. As a result Anatolian HBV has a good potential to inhibit AFB destruction in colonies and the obtained MIC values were evaluated as an important result showing that these natural products have the potential to be used in the control of AFB disease.

Keywords: Anatolian bee venom, *Paenibacillus larvae*, American Foulbrood, HPLC, antimicrobial activity.

Is Single-factor or Multi-criteria Assessment the Better Choice? - Illustrated by the Example of Pollen-enriched Biscuits

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Abstract:

In our research we analyse the nutritional parameters of pollen-enriched biscuits. The control biscuit was prepared according to the AACC-approved method. For the preparation of the pollen-enriched biscuits, the same recipe was used, but 2, 5 or 10% of the wheat flour was substituted with hot-air dried, ground, monofloral bee pollens of rapeseed, phacelia or sunflower. The most nutritionally important macronutrients (moisture, carbohydrate, crude protein, ash) and the results of antioxidant capacity methods (TPC, FRAP, DPPH, TEAC) were analysed. The evaluation was first performed by nutritional parameters (Kruskal-Wallis nonparametric test, Dunn's pairwise procedure with Bonferroni correction). The advantage of single-factor analyses is that cause-and-effect relationships can be identified, so that the effect of pollen enrichment on specific nutrient parameters can be investigated. In nutritional research, however, there are many situations where, in addition to univariate analyses, it is advisable to carry out a complex assessment of products together, since all the nutritional parameters of a product have a combined effect on the individual when consumed. The sum of ranking differences (SRD) method was used to rank the biscuits by considering the content values together (from best to worst): 1. phacelia 10%, 2. rapeseed 10%, phacelia 5%, sunflower 10%, 3. rapeseed 5%, sunflower 2%, sunflower 5%, 4. rapeseed 2%, phacelia 2%, 5. control. Overall, it can be concluded that increasing the amount of pollen added to biscuits improves the nutritional biological value. Single-factor and multi-criteria statistical evaluation have different logics and can complement or confirm each other's results. Naturally, we choose our methods according to our objectives, but for complex evaluations, we recommend using both methods and comparing their results.

Keywords: in vitro antioxidant capacity; Kruskal–Wallis nonparametric test, sum of ranking difference (SRD), bee pollen.

This research was funded by National Research, Development and Innovation Office, OTKA, contracts number 135700. This research was supported by the Ph.D. School of Food Science of Hungarian University of Agriculture and Life Sciences.

Post grafting time significantly influences royal jelly yield and chemical composition

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Abstract:

Royal jelly (RJ) is commercially harvested after the 4th day of queen larval age. Here RJ was harvested after 24, 48, and 72 hrs of grafting to study the effect of harvest time on the yield of RJ/queen cell and the nutritional components in RJ harvested at different times post-grafting. Also, changes in macro and trace elements associated with harvesting timepoint was investigated. The highest yield of RJ/queen cell was harvested after 72 hrs of grafting. The lowest moisture content was found in RJ harvested after 72 hrs of grafting. The maximum concentrations of crude protein, ash, fructose, and glucose were found in RJ harvested after 72 hrs of grafting. The highest P and Zn contents were obtained from RJ harvested 24 hrs after grafting. Royal jelly harvested 48 hrs after grafting had the highest concentrations of Mg, Ca, K, Na, Fe, and Mn. Conversely, the highest lipid content was obtained from RJ harvested after 24 hrs of grafting. The pH value decreased and acidity increased over time after grafting. It was concluded that RJ can be harvested after 72 hrs of grafting to obtain maximum yield. The nutritional components in RJ may be changed over time after grafting, and it could differentiate between RJ harvested at different times post-grafting.

Keywords: Post grafting, royal jelly, chemical composition

Development of a Highly Sensitive Biosensor for the Detection of 2,3-Pentanedione in Honey

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Abstract:

The adulteration of honey with synthetic compounds poses a significant threat to consumer safety and the reputation of the honey industry. Among the various adulterants, 2,3-pentanedione has gained attention due to its widespread use as a flavoring agent and its potential health risks. This study presents the development of a novel biosensor for the rapid and sensitive detection of 2,3-pentanedione in honey. The biosensor utilizes a biorecognition element composed of immobilized enzymes specifically engineered to selectively bind and catalytically convert 2,3-pentanedione. The enzymatic conversion leads to the generation of an electrochemical signal, which is proportional to the concentration of the target analyte. The biosensor was fabricated using state-of-the-art nanomaterials and microfabrication techniques to achieve enhanced sensitivity and selectivity. Extensive optimization and characterization of the biosensor were performed to ensure its reliable and reproducible performance. The effects of various parameters, including temperature, pH, and immobilization conditions, were systematically investigated. The biosensor exhibited a wide linear dynamic range, from low trace levels to high concentrations of 2,3-pentanedione, making it suitable for both quality control and regulatory purposes. Validation experiments were conducted using spiked honey samples with known concentrations of 2,3-pentanedione. The biosensor demonstrated excellent sensitivity, with a limit of detection below the permissible levels defined by relevant food safety regulations. Moreover, the biosensor showed excellent selectivity, exhibiting minimal interference from common honey components. The developed biosensor holds great promise as a rapid, reliable, and cost-effective tool for the detection of 2,3-pentanedione adulteration in honey. Its simplicity and portability make it suitable for on-site analysis, ensuring consumer safety and maintaining the integrity of the honey industry. Further studies are warranted to evaluate the biosensor's performance with a broader range of honey samples and to explore its potential applications in other food matrices.

Keywords: 2,3-pentanedione, Honey, Biosensor.

Botanical origin identification and detection of adulteration of honey by Laser Induced Breakdown Spectroscopy (LIBS)

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Abstract:

Honey's botanical origin and purity are among the key factors that influence its production, price and its retailing in general. In particular, the certification of the botanical origin of honey can result to important added value to the product while the detection of adulteration in honey and its products, not only protects the consumers, e.g., from health risks, but also secures their trust on producers and honey, as a premium product, itself. Honey is a rather complex substance, and its several constituents support its unique identity. Consequently, for the efficient characterization of honey, the use of several methods is usually necessary. Nonetheless, the standard chemical techniques require specialized personnel, laboratory conditions, while they are time-consuming and often of high cost. In addition, in most cases, they can provide reliable results only in combination with other chemical techniques. Several of these limitations can be overcome by modern photonic technologies, as e.g., some newly developed laser-based techniques that can operate in-situ and on-line, without requiring any sample preparation, thus providing results in real time. Such a laser-based technique has been developed in our laboratory during the last years, the Laser Induced Breakdown Spectroscopy (LIBS). According to this technique, a pulsed laser beam is focused on the surface of a few milligrams honey sample inducing a spark, i.e., a micro-plasma, which emits radiation containing valuable information about the elemental composition of the sample. Thus, the plasma emission can serve as a fingerprint of the sample. The collected emission spectra are then used for the training of some machine-learning algorithms which then become capable of recognizing and classifying unknown spectra in real time and with high accuracy. In this work, LIBS aided by machine learning is used for the identification of the botanical origin of several pure Greek honeys and also for the detection of adulteration with various syrups (as e.g., glucose, fructose, etc.).

Keywords: LIBS, machine learning, honey, adulteration, food analysis

Support from Greek national funds through the Public Investments Program (PIP) of General Secretariat for Research and Technology (GSRT), under the Emblematic Action "The bee routes" (project code: 2018ΣΕ01300000) is greatly acknowledged. The members of the network of the Emblematic Action "The Bee Routes" are also acknowledged for making available the pollen analysis characterizations of the honey samples. D.S. acknowledges support from the «Andreas Mentzelopoulos Foundation».

Green Extraction of Propolis with Different Oils and Their Chemical and Oxidative Properties

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Abstract:

Propolis is one of the natural and functional beekeeping products produced by bees and used in different areas such as food, pharmaceuticals, and cosmetics because of its antimicrobial, antioxidant, antitumor, anti-inflammatory, and antimutagenic effects. It contains chemical compounds mainly apolar characteristics like plant resin, wax, and essential and aromatic oils. Since raw propolis is difficult to use, propolis extracts are produced, and its extraction is mostly performed with alcoholic solutions. Besides, propolis extraction with olive, hazelnut, canola, coconut, and soybean oils has been reported in previous studies as alternative natural extraction solvents. Herein, we focus on the extraction of propolis with commercially available vegetable oils such as corn, olive, and sunflower, and the determination of phenolic, flavonoid, antioxidant, and oxidative properties of obtained extracts. The phenolic contents of propolis extracts with corn, olive, and sunflower oils were determined as 93.56, 93.40, and 74.71 mg GAE/100 g, respectively. The phenolic and flavonoid extraction yields were between 19.16-24.85% and 11.19-13.93%, respectively. The corn oil-propolis extract showed the highest yield results than the olive and sunflower oil extracts. According to Schaal oven storage stability test results, peroxide and conjugated diene values of oils and propolis-oil extracts increased during 10 days of storage. The peroxide results for corn, olive, and sunflower oils were 95.34, 20.05, and 135.21 meq O₂/kg, whereas they were detected as 21.53, 12.51, and 115.90 meq O₂/kg in propolis oil extracts, respectively. Regarding conjugated diene results, the corn oil-propolis extracts were more stable against oxidation than the other samples. Overall, natural vegetable oils also have promising potential used in propolis extraction in addition to common solvents, and corn oil-propolis extracts could also be produced in addition to the existing commercial olive oil-propolis extracts as it has both extraction efficiency of phenolic compounds and oxidation stability.

Keywords: propolis phenolic, inhibition, oxidation, conjugated diene

Characterization and evaluation of probiotic lactic acid bacteria for potential use in food production

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Abstract:

The objectives of this study was to investigate probiotic characteristics and fermentation profile of selected lactic acid bacteria isolated from honeybees' stomach and mid-gut bacteria. Physiological properties, cell surface properties (hydrophobicity, autoaggregation, co-aggregation, adhesion to Caco-2 cell), acid and bile tolerance, exopolysaccharide (EPS) production, hemolytic and tolerance to sodium chloride, resistance toward lysozyme and heat, and fermentation profile (pH and growth) were examined. All LAB isolates showed auto-aggregation ability, good hydrophobicity ability against different organic solvents, high co-aggregation, moderate antimicrobial activity and EPS production. The survival percentages of simulated gastric and intestinal juice conditions of LAB varied greatly. Among the isolates, *Pediococcus pentosaceus* HBMSS2 and *Lactobacillus plantarum* HBMSS3 exhibited remarkable tolerance to sodium chloride and good resistance toward lysozyme and heat. Similarly, *Pediococcus pentosaceus* HBMSS2 and *Lactobacillus plantarum* HBMSS3 showed very promising fermentation profiles. The antioxidant effects of Intact cells, intracellular and cell-free extracts of LAB strains were assessed by several antioxidant assays, including DPPH and ABTS radical scavenging. The results showed that the bacterial lysate and cell-free supernatant (CFS) of *Pediococcus pentosaceus* HBMSS2 and *Lactobacillus plantarum* HBMSS3 possessed excellent antioxidant capacities. The current study demonstrated for the first time the high capacity of these strains to have antioxidant effects by several antioxidant assays. This may be a promising finding for future functional foods applications using natural probiotics.

Keywords:

Characterization of honeydew honey from *Quercus pyrenaica* forests

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Abstract:

Honeydew honey is produced by honey bees from exudates of living parts of plants or excretions of sucking insects living parts of plants. This study aimed to characterize the honeydew honey produced from *Quercus pyrenaica* forests. For that, 47 samples harvest in 2021 and 35 from 2022, where collected in different apiaries of *Apis mellifera iberiensis*, located in Montesinho Natural Park, Bragança, Portugal. Several key physicochemical parameters were measured, such as colour, electrical conductivity, moisture content, acidity, proline content, 5-hydroxymethylfurfural (5-HMF) content and diastase. The melissopalynological characteristics were also access. The results of the study revealed no significant variations in the physicochemical properties of honeydew honey between the two years. The results of the physicochemical parameters analysis showed that the colour presented by the samples was a dark amber, in both years, while the moisture ranged from 14.4 to 18.5 % in 2021 and from 13.4 to 16.8% in 2022. The electrical conductivity varied from 0.93 to 1.4 mS.cm⁻¹ in 2021 and from 0.99 to 1.49 mS.cm⁻¹ in 2022. The amount of 5-HMF range from 0 to 24 mg.kg⁻¹ in 2021 and from 0 to 8.66 mg.kg⁻¹, while the diastase index varied from 9 to 33 DN in 2021 and from 15.28 to 22.25 DN in 2022. Concerning the melissopalynological analysis, the honeydew honey samples showed a high pollen diversity, with a total of 52 pollen types. These belonged to 32 families corresponding to the flora of interest to beekeepers in the region. However, the most representative pollen types in the 100% of the samples were *Castanea* (\bar{x} = 87%) and *Rubus* (\bar{x} = 9%). The pollen content of the honeydew honey samples had a mean value of 16341 pollen grains/g and 952 HDE/g of honey, which originates a value of HDE/P= 0.13. although honeydew honeys contain fewer pollen grains and a higher content of HDE than nectar honeys, the honeys studied presented higher values of pollen content and lower HDE content than honeydew honeys from other sources. In conclusion, this study provides valuable insights into the physicochemical parameters of honeydew honey produced from oak forests.

Keywords: *Apis mellifera*, honeydew honey, black oak, physicochemical, pollen, HDE

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES (PIDDAC) to CIMO (UIDB/00690/2020 and UIDP/00690/2020) and SusTEC (LA/P/0007/2021), for the support through the project ACORNDEW (MTS/SAS/0099/2020). National funding by FCT, Foundation for Science and Technology, through the individual scientific employment program-contract with Soraia I. Falcão. Finally, this work is funded by the European Regional Development Fund (ERDF) through the Regional Operational Program North 2020, within the scope of Project GreenHealth - Digital strategies in biological assets to improve well-being and promote green health, Norte-01-0145-FEDER-000042.

Conservation of the gene pool of local honey bee populations

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Abstract:

Wild and feral honey bee populations receive little attention despite being in danger. Introduced honey bee subspecies have the potential to have a severe negative impact on native honey bee wild population. Introgressive hybridization and exposure to new pests and pathogens are both favored when wild and feral bee populations coexist. Because of the spread of the ectoparasitic mite *Varroa destructor*, wild and feral populations of *A. mellifera* in Europe saw a rapid decline that brought them dangerously close to extinction. Through time, populations of honey bees in wild and feral honey bees developed resistance to the *V. destructor* mite but drastically lost their genetic diversity. Because of their higher genetic variety than other bee populations in their natural habitat, wild and feral honey bee populations are more resilient to changing climatic circumstances and new diseases. By means of hybridization, the interaction of a bee population with a wild or feral population can alter the genetic diversity of both groups. According to previous studies, honey bee populations in the wild and feral states are genetically distinct from honey bee populations and serve as a source of genetic variety and advantageous adaptations for bee populations. Nowadays, populations in hives and the wild are mainly mixed but not identical. Different selection pressures are applied to these populations. In apiary populations, artificial selection is used to maintain pure subspecies, but in wild populations, natural selection is used to favor features that improve fitness and survival. In our research, we discovered genetic diversity of the Bashkir bee population on the territory of the Toratau Geopark", Ural, Russia.

Keywords: *Apis mellifera*, honey bee, gene pool, population, conservation.

#This research received funding from the grant of the head of the Republic of Bashkortostan Rady Khabirov "Study of the Bashkir bee population on the territory of the Toratau Geopark" and from the IDB RAS Government basic research program in 2023 No 0088-2021-0019.

Principle Molecular Mechanism of Propolis on Neuroinflammation Regulated by Pro- and Anti-inflammatory Cytokines

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Abstract:

Propolis is a natural product produced by honeybees and widely used in traditional medicine. It consists of plant resins, waxes, essential and aromatic oils, pollen, and other organic substances. In other words, it is rich in bioactive compounds such as quercetin and its derivatives, kaempferol and related compounds, pinocembrin, apigenin, caffeic acid, caffeic acid phenethyl ester, naringenin, vanillin, myricetin, and lupeol. These compounds exert beneficial effects for human health through various pharmacological properties, especially their antioxidant, antiallergic, anticonvulsant, antitumor, neuroprotective, and anti-inflammatory properties. The progression of neurodegenerative diseases is associated with the production of proinflammatory mediators and cytokines, as well as the generation of reactive oxygen species (ROS). Propolis exerts neuroprotective properties by modulating several signaling pathways, including the nuclear factor-kappa B (NF- κ B), mitogen-activated protein kinase (MAPK), and Janus Kinase (JAK)/Signal transducer and activator of transcription (STAT) pathways, which are involved in the production and release of proinflammatory cytokines. Propolis also interacts with immune receptors to inhibit the release of proinflammatory cytokines, leading to a decrease in neuroinflammation. In addition, propolis has been demonstrated to modulate immune cell function and regulate the balance between pro- and anti-inflammatory cytokines. This immunomodulatory effect may contribute to its anti-inflammatory properties and further promote the reduction of neuroinflammation by lowering levels of proinflammatory cytokines. Propolis has significant therapeutic potential for neurological diseases and offers a promising neuroprotective agent for the pharmaceutical industry. Accordingly, this work is a comprehensive review that highlights the use of propolis in neuroinflammation by identifying the specific molecular mechanisms underlying its effects on neuroinflammation promoted by proinflammatory cytokines.

Keywords: Neuroinflammation, propolis, bee products, cytokines, molecular pathways

COMMUNITY READINESS OF BALI, INDONESIA AND BICOL, PHILIPPINES TOWARDS MELITOURISM

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Abstract:

Melitourism integrates tourism components in meliponiculture, a technology of domesticating stingless bees to produce valuable bee products and by-products. This form of fascination can bring people to a level of guided experience, allowing melitourism in rural communities where bee forage sources are still abundant and diverse. This paper aimed to determine the level of readiness of the community concerning the developed components: 1) knowledge & responsible practice; 2) stakeholders' involvement; 3) community empowerment; 4) access to finance; and, 5) cultural preservation. The research used a quantitative approach to determine the readiness of the different beekeeping communities using 42 components gathered from the previous study and related literature, later reduced to 35. The study calculated the composite mean based on eight major components: access to finance, market skills, social networks, environmental preparedness, technology awareness, ethics, tourism participation, and cultural consciousness. The study adopted the Principal Component Analysis, where five components were generated to develop a framework for community readiness and measure Bali, Indonesia, and Bicol, Philippines' readiness and potential towards melitourism. These components include knowledge and responsible practice, stakeholders' involvement, community empowerment, access to finance, and cultural appreciation. Generally, Bali is more ready than Bicol to establish a melitourism industry.

Keywords: melitourism, meliponiculture, stingless bees, *Tetragonula biroi*, *Tetragonula laeviceps*, beekeeping communities, community readiness

Challenges facing beekeepers in Turkey and proposed solutions

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Abstract:

Beekeeping is a sector that ensures the continuity of nature through its contribution to pollination and provides people with products of high nutritional value, whose value is increasing every day in the world and in our country. There has been an increase in the number of bee-keepers and the number of hives in the country from year to year, but the production target has not been reached. There are many reasons for the low productivity of beekeepers. The aim of this study is to examine the problems faced by beekeepers. Beekeepers play an important role in agricultural production in our country. For the study, an online questionnaire was performed. The questionnaire was sent to beekeepers. The survey questions are aimed at determining the structure of beekeeping enterprises and the current situation of the sector, and include the level of education, experience, number of colonies, type of beekeeping and problems faced by beekeepers in beekeeping. As a result of the study, 71.71% of the beekeepers participating in the survey are itinerant beekeepers, 28.29% are permanent beekeepers, 35.06% have primary school education, 13.81% have secondary school education, 27.22% have high school education, 23.90% of them are university graduates. Taking into account the length of experience of the beekeepers participating in the survey, it was found that 36.65% of the enterprises had been in beekeeping between 1 and 10 years, 36.65% of the enterprises had been in beekeeping between 10 and 20 years and 26.69% of the enterprises had been in beekeeping for 20 years or more. According to a questionnaire in which beekeepers could make multiple choices, the main problems encountered in beekeeping were marketing (70.12%), lack of confidence due to fake honey (69.32%) and drugs used in herbal production (58.96%).

Keywords: Beekeeping, Honey bee, Beekeeper problems

The Antimicrobial Effects of Krgyz Honey

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Abstract:

Bee products, including honey and its derivatives such as propolis, royal jelly, and bee pollen, have long captivated the attention of humans due to their unique flavors, nutritional profiles, and potential health benefits. Honey, often referred to as nature's liquid gold, is a sweet and versatile substance produced through the intricate relationship between bees and flowers. Beyond its delightful taste, honey is a rich source of antioxidants, enzymes, vitamins, and minerals, making it a valuable addition to our diet and a potential supporter of overall well-being. In addition to being an important food substance, honey possesses antibacterial properties, which have rendered this valuable bee product a significant antimicrobial agent. Due to its geographical location encompassing the Tien Shan and Altai mountain ranges, Kyrgyzstan has become one of the prominent apiculture regions of Central Asia, benefiting from the rich flora and natural environment of these mountains. However, there has been a lack of sufficient research on honey produced in Kyrgyzstan. This study was conducted to determine the antibacterial activity of honeys sold in the Chuy region of Kyrgyzstan. For this purpose, monofloral honeys such as White honey and Buckwheat honey, as well as three different strains of filtered multifloral honey, were utilized. Pathogenic bacteria including Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* were used. Collected honey samples (at 500 mL⁻¹ dilution) were prepared and inoculated with 40 microliters of 0.5 McFarland pathogen using disk diffusion method on Mueller-Hinton Agar. The honey samples were also tested using the well method. In conclusion, it has been observed that different types of honey have varying degrees of antimicrobial effects. The zones formed using the well method were more clearly visible, and this method provided more sensitive results compared to the disk diffusion method. No zone formations were observed for both pathogens using the disk diffusion method with monofloral White Honey samples. This study has demonstrated the influence of Kyrgyzstan's natural environment on the antimicrobial properties of honey. However, further comprehensive research is needed on honeys produced with the unique geographical location and natural flora of Kyrgyzstan.

Keywords: Antimicrobial effect, Bee, Honey, Kyrgyz honeys

Monitoring Pesticides Residues in Bee Products and the Future of food security in Egypt

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Absract:

Use and application of pesticides in Egypt vary in quantity and type based on location to control various plant pests and disease pathogens. Pesticides that are used include insecticides (as a major group), followed by fungicides, and finally, limited amounts of herbicides. Current study monitored pesticide residues in bee products (honey, pollen, and beeswax) collected from newly reclaimed agricultural communities in Egypt. Gas chromatography coupled with a mass spectrometry system was used to analyze pesticides in samples after being extracted and cleaned up using the QuEChERS protocol. Results showed that pesticides detected were identified as acaricides, insecticides, nematicides, fungicides, and herbicides, with limits of quantification ranging from 10 to 50 ng/g. Samples collected from El-Noubariya, El-Beheira (North of Egypt), had the greatest content of pesticide residues, followed by samples from Ismailia (East of Egypt). In contrast, Toshka (South of Egypt) samples had the least content of pesticides. Therefore, honey and honey products from Toshka were clean of pesticides compared to samples from El-Noubariya and Ismailia.

Keywords: Bee products, food security, pesticides residues

Methodology of honey bee feed quality evaluation

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Abstract:

There are numerous supplements and substitutes for honey bee colonies feeding currently available on the worldwide market, with a highly variable and sometimes even undefined composition, claiming a set of actions at the level of brood stimulation, energy supplementation, queen rearing support, reduction of varroa reproduction levels, improvement of the intestinal microflora of bees, nosema prevention, improvement of the health of hives infested by American foulbrood, among others. To address this issue, the members of COLOSS (Honey Bee Research Association), NUTRITION Task Force, for the first time propose an action on honey bee feed control and monitoring, setting the four main objectives: 1) Elaborate methodologies to study bee aliments (protocols, good laboratory practices), and to implicate different stakeholders to clarify the type of analyses depending on their needs (e.g. organic or legal framework); 2) Create and coordinate a network of laboratories able to use the proposed methodologies (ring tests, evolution of the methods with new technologies); 3) Apply the methodologies to a large set of bee aliments at a worldwide scale; and 4) Elaborate guidelines to support and assist food companies and regulators to proceed the effective control of the quality and safety of supplements and substitutes for honey bees. Globally, this action will allow more information to the stakeholders via monitoring and set the basis for regulation of products to reach the minimum standards for quality, effectiveness, and economy of honey bee feed, and finally guaranteeing the quality of the bee products.

Keywords: honey bee, nutrition, supplements, bee feed, bee health.

Bioactive phenolic profile and antioxidant activity of functional foods based on honey and dry fruits

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Abstract:

In the recent years, an increasing interest in the study of honeys and the other bee products has been observed due to their therapeutic potential. Current tendency of consuming natural products with therapeutic potential beyond their nutritional roles (functional foods) increased the interest on honey supplemented with natural sources such as fruits, plants and other apiary products rich in bioactive substances, among the most important are phenolic compounds. This work aims to evaluate the influence of supplementation of rape honey with dry fruits (goji berries, black currant and sea buckthorn) on the phenolic compound content and on antioxidant activity of resulted honey functional foods. Supplementation of honey with dry fruits resulted in significant increase of phenolic acids (chlorogenic, syringic, p-coumaric, ferulic and cafeic acids), flavan-3-ols (catechin and epicatechin) and flavonoids (guercetin and rutin) contents, improving the antioxidant activity of a honey and providing significant amounts of bioactive compounds into the human body. Among the studied products, honey supplemented with dry goji and black currant fruits were the richest in phenolic compounds, the antioxidant activity of these functional foods being significantly higher compared with the simple rape honey, due to the introduction of numerous phenolic acids and flavonoid from the fruits.

Keywords: phenolic compounds, honey, fruits, functional foods

Acknowledgement: This research was supported by project *Establishment and operationalization of a Competence Center for Soil Health and Food Safety* – CeSoH, Contract no.: 760005/2022, specific project no.5, with the title: *Improving soil conservation and resilience by boosting biodiversity and functional security of organic food products*, Code 2, financed through PNRR-III-C9-2022 – 15 (PNRR-National Recovery and Resilience Plan, C9 Support for the private sector, research, development and innovation, 15 Establishment and operationalization of Competence Centers).

Honey quality on the Slovak market

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Abstract:

Quality of honey from producers that applied for the "Slovak honey" trademark, from grocery shops and from the self-control of honey producers, importers or sellers was monitored in the period from 2013 to 2021. We have analyzed 692 honeys that applied for the "Slovak honey" trademark, of which 50 did not meet legislation criteria, which represents 7.22%. Employees of the State Veterinary and Food Administration of the Slovak Republic took 1,267 samples of honey of different packers from shops, of which 88 honey samples did not meet the quality criteria, which represents 6.95%. The quality of honey as part of the self-control of honey producers or sellers was carried out on 1712 honey samples sent by the producers or packers by themselves. Out of 1712 honey samples, 174 did not comply with criteria, which represents 10.15%. Since nowadays it is not possible to detect the adulteration of honey with a single method, it was necessary to monitor several parameters that could indicate type of honey derogation. We monitored the following parameters: fructose and glucose content, sucrose content, water content, electrical conductivity, diastatic activity, hydroxymethylfurfural content, acidity, optical rotation, presence of ammonia sulfite cooler E150d, foreign enzymes activity, B-fructofuranosidase, B/gamma amylase and C3/C4 sugars.

The results of the work can be beneficial for the consumer public, as well as for beekeepers and packers to improve proper management practices.

Keywords: honey, honey quality, trademark "Slovak honey, hydroxymethylfurfural, honey adulteration.

Oral presentation

Study of antimicrobial activity of different varieties of honey from the region of Medea (Algeria)

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Abstract

Honey is one of the hive products containing several natural antibiotic substances. The present work is a contribution to the evaluation of the antibacterial effect of 3 different varieties of honey (Jujube, Mountain and Euphorbia) harvested in the region of Medea (Algeria), as well as their mixtures, against a few bacterial strains positive and negative Gram. The experimental approach is based on the evaluation of this antibacterial power through different conventional techniques. The results obtained clearly demonstrate the impact of these products on bacterial sensitivity, this inhibitory effect was observed for most of the samples tested with a certain variability from one sample to another and from one strain to another. The diffusion well method shows that the three honeys have an antibacterial effect on certain Gram positive and Gram negative bacteria, the reading of the chromatograms demonstrate that all the samples have an inhibitory effect, and also all the samples have a bactericidal effect against tested the strains.

Keywords: Medea, honey, antimicrobial, sensitivity, natural substances

POSTER PRESENTATIONS

Braulid (Diptera: Braulidae Egger, 1853) infestations on honey bees (*Apis* Linnaeus, 1758) in Türkiye, and in the world

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Abstract:

Braulids, also called bee-louse, are a group of parasitic Diptera with a wide-ranging geographical distribution. Up to now, the family of Braulidae includes seven described species in two genera associated with honey bees: *Braula* (*B. coeca*, *B. kohli*, *B. orientalis*, *B. pretoriensis*, and *B. schmitzi*) and *Megabraula* (*M. antecessor* and *M. onerosus*). *Braula* larvae cause physical damage to wax cappings on honeycomb by opening tunnels. Adults of *Braula* consume by stealing the queen bee's food, causing malnutrition and reducing their ability to lay eggs (it settles on regions of the head and thorax and then feeds on nectar or pollen from the host's mouth). However, its parasitic effects, vectorial potential, and economic effects on honey bees are not known enough. Acute Bee Paralysis Virus (ABPV) was detected molecularly in *B. schmitzi*, but more comprehensive studies are needed to understand whether it has vectorial potential. The scoped Braulid studies on honey bees are generally old. Unfortunately, nowadays or recently, studies are insufficient and generally narrow in scope. The Braulid species presence in Türkiye was reported only in two studies published previously but not reported afterwards. The infrequency of reports of Braulid infestations in the world and Türkiye may be because the chemical pesticide control against *Varroa* mites also eliminates Braulid species or that beekeepers do not sufficiently recognize Braulid species due to *Varroa* similarity. Morphological and biological characteristics can eliminate the second possibility: Braulid flies have three pairs of legs, while *Varroa* mites have four; viewed dorsally, Braulids can be distinguished as having a head, thorax, and abdomen, while *Varroa* species are in one piece (idiosoma + gnathosoma); mouth structure is licking-sucking in Braulids, it is piercing-sucking in *Varroa* mites; Braulids lay their eggs on wax cappings of honeycomb, while *Varroa* species lay them in honeycomb cells with bee larvae. More comprehensive studies are needed to reveal Braulids' parasitic and economic effects on honey bees. Beekeepers and experts should also be aware of Braulid flies' effects. This paper aims to draw attention to Braulid infestations on honey bees.

Keywords: bee lice, bee louse, bee fly, diseases of honey bee.



Therapeutic use of honey in veterinary medicine

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Abstract:

Honeybees produce products with pharmaceutical properties and the best known of these is honey. In this study, we searched the scientific literature for *in vivo* studies conducted on the honey medical properties. Google Scholar, Medline/PubMed, LILACS, SciELO, EMBASE and SCOPUS databases were used for the search and selection of studies. Honey among its constituents has numerous bioactive substances, including flavonoids, phenolic acids, and polyphenols, give it antithrombotic, anti-ischemic, antioxidant and vasorelaxant properties, essential for reducing the onset of coronary heart disease. Three mechanisms are involved: enhancement of coronary vasodilation, reduction of platelet coagulation capacity, and prevention of LDL oxidation. The flavonoid acacetin, at dosages of 5 mg/kg, would lengthen the effective atrial refractory period in sedated dogs without extending the corrected QT interval. At doses of 2.5 mg/kg and 5 mg/kg, acacetin reduced the onset of atrial fibrillation, by 50% and 85.7%. *In vivo* studies in rats and rabbits have demonstrated antidiabetic effects. The blood glucose and fructosamine levels of animals with induced diabetes were lowered by a honey-enriched diet. Other studies in dogs have shown that small amounts of fructose increase hepatic glucose uptake and glycogen storage while lowering blood sugar and insulin levels. Honey also has protective effects on the gastrointestinal systems. In *Helicobacter pylori* infection, honey reduces acid secretion and promotes healing of gastric ulcers. Experiments in murine models have also demonstrated an anticancer action of honey, which inhibits the onset, growth, and development of cancer, promoting apoptosis, cell cycle arrest, modulation of oxidative stress, mitochondrial membrane permeabilization external and the inhibition of angiogenesis. Another use is related to anti-inflammatory, antibacterial and antifungal properties (60 bacteria species Gram⁺ and Gram⁻, yeasts and fungi *Aspergillus*, *Penicillium spp.* and common dermatophyte are inhibited by honey). It can promote the development of granulation and accelerate the proliferation of epithelial tissue. Topical use has promoted the healing of open wounds and necrosis in dogs, rabbits, and equines. In conclusion, honey would represent a valid therapeutic aid in the veterinary field. However, to be used in clinical practice, more *in vivo* studies should be conducted to better identify dosages and administration timing.

Keywords: Honey, cardiovascular modulation, antidiabetic activity, anti-cancer effects, anti-inflammatory and antimicrobial activity, wound healing.

Honey Botanical Origin and Honey-Specific Protein Pattern: Characterization of Some European Kinds of Honey #

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Abstract:

Honey adulteration generates low-quality products on the market. The study aimed to find a simple, specific, and less time-consuming method than standard melissopalynology only, for monitoring honey botanical and geographical origin. In this study, 42 honey samples from different sources were examined for their botanical origin by using melissopalynology and their specific protein patterns by electrophoresis on SDS polyacrylamide gels (SDS-PAGE). The melissopalynological analysis consisted of counting all melliferous pollen, non-melliferous pollen and honeydew elements to identify the species of pollen present in each sample. The honey samples had predominant pollen from Apiaceae, Boraginaceae, Brassicaceae, Compositae H, Fabaceae (*Trifolium* sp.) and Fagaceae family. From the Fagaceae family, the most important species was *Castanea sativa* Mill., while the Boraginaceae was represented by *Echium* sp. and *Myosotis* sp. SDS-PAGE showed that the different origin honey shared protein bands between 45 and 85 kDa (animal origin proteins, i.e. major royal jelly proteins, enzymes) and that specific proteins (presumably plant origin proteins) can be attributed to individual honey types. This study shows that the combination of melissopalynology and SDS-PAGE is a useful tool for modern discrimination between different kinds of honey, even without performing specific protein identification.

Keywords: honey, melissopalynology, SDS-PAGE, adulteration, multivariate analysis

This work was supported by a grant of the Romanian Ministry of Education and Research, CNCS - UEFISCDI, project number PN-III-P1- 1.1-PD-2019-0670, within PNCDI III, and from the Internal Project 26.526/07.12.2017

Antiradical and Antitumor Activity of Portuguese Bee Pollen Through the Differentiation of Bioactive Compounds

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Abstract:

Bee pollen is frequently characterized as a natural source of bioactive compounds such as phenolic compounds responsible for its therapeutic potential along with nutritional properties. However, there is quite limited work on whether the presence of phenylamide and volatile compounds, as well as phenolic compounds in bee pollen, has considerable action on antioxidant activity and human cancer cell lines. Herein, we evaluated the bioactive compound contents of monofloral bee pollen specimens by spectroscopic and chromatographic methods and establish links with their antioxidant and antitumor activity. The findings demonstrated that the botanical origin of bee pollen has a remarkable impact on their phenolic (3–36 mg GAE/g) and flavonoid (1–5 mg QE/g) content. Liquid chromatography-mass spectrometry analysis revealed the presence of 74 compounds, including phenolic and phenylamide compounds in bee pollen, while gas chromatography-mass spectrometry showed its richness in volatile compounds such as hydrocarbons, fatty acids, alcohols, ketones, etc. The concentration of bioactive compounds in each sample resulted in a substantial distinction in their antioxidant activity, DPPH (EC₅₀: 0.07–0.75 mg/mL), ABTS (1–1.6 mM Trolox/mg), and reducing power (0.02–0.09 mg GAE/g), with the most bioactive pollens being the monofloral specimens from Cistaceae, *Rubus* sp. and *Castanea* sp. Complementary, several specimens revealed a strong (GI₅₀: 17 µg/mL) and moderate (GI₅₀: 746, and 814 µg/mL) effect on breast adenocarcinoma cell line, what may be associated to compounds such as kaempferol, methyl herbacetin-*O*-dihexoside, and quercetin-3-*O*-glucoside present in pollens from *Carduus* sp., *Rubus* sp., and *Castanea* sp., respectively. Overall, the results highlighted the potentiality of bee pollen to serve health-promoting formulations in the future.

Keywords: Bee pollen; Phenolics; Phenylamides; Antioxidants; Antitumor activity

Funding:

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 and UIDP/00690/2020) and SusTEC (LA/P/0007/2021). Thanks to the project GreenHealth, Norte-01-0145-FEDER-000042, and BeeLand PRR-C05-i03-I-000081. Thanks to national funding, FCT, through the individual and institutional scientific employment program contract with Soraia I. Falcão and the Ph.D. research grant (2021.07764.BD) for Volkan Aylanc.

AI-Technology based on 2D-CNN Algorithm: Determination of Indian Honey Adulteration and Authentication

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Abstract:

The market and aesthetic value of honey rely on the source of nectar collected by bee from a specific flower, and the authenticity of honey based on botanical origin is of prime concern in the market. Moreover, the economical motivated adulteration in honey has been increasing since many decades. Besides the adulteration detection methods has such sophistications which make them out of the reach of small bee farmers. Therefore, A deep learning framework based on the 2D-CNN model was used for the botanical authentication as well as detection of adulteration in Indian Unifloral honey varieties. An inexpensive and robust analysis methodology based on computer vision (CV) was fabricated to determine the botanical authentication of honey varieties. The .mp4 videos were recorded using camera fixed on stand with adjustable distance. The developed model was trained using images which were extracted from captured .mp4. The extracted data set of images for classification was fed to a developed 2D-CNN which was further validated using various performance metrics, namely, accuracy, precision, specificity, F1-score, and AUC-ROC. The value of AUC-ROC was more than 0.98 for most classes of unifloral honey varieties used for classification, and for detection of honey adulteration with cane sugar showed the average accuracy of 0.96. The obtained results proclaimed that this experimental approach, in amalgamation with the developed 2D-CNN model, outperforms in contrast to the existing algorithms used for evaluating food quality attributes. Henceforth, this novel approach would positively influence the honey industry and the honey consumer regarding honey adulteration detection and authentication—moreover, it encourages researchers to exploit this application of hybridized technology in food quality assurance and control.

Keywords: Computer Vision, Neural Networks, Authentication, Adulteration, AI-Technology, Food quality, Food safety

Exploring the Therapeutic Potential of Bee Products in Dermatology and Skincare.

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Abstract:

Due to their variety of bioactive components and potential therapeutic qualities, bee products have drawn much interest in dermatology and skincare. This review analyses scientific research on using bee products in dermatology and cosmetics. Honey, beeswax, propolis, royal jelly, and bee venom are all bees' products and have antibacterial, anti-inflammatory, antioxidant, wound-healing, and immunomodulatory properties. They are suitable for controlling dermatological diseases like acne, psoriasis, eczema, dermatitis, and wound healing because of their qualities. Honey is an effective solution for wound care because it creates a moist environment, encourages tissue regeneration, and has anti-inflammatory properties. Beeswax functions as an occlusive and emollient agent, creating a barrier of defense. Skin infections and inflammatory diseases can be helped by the antibacterial, antifungal, and anti-inflammatory properties of propolis, which is abundant in flavonoids and phenolic compounds. Royal jelly increases skin suppleness, promotes collagen formation, and enhances overall skin health. The ability of bee venom to treat wounds, lessen swelling, and possess antibacterial characteristics seems promising. More investigation is needed to strengthen formulations, establish ideal dosages, and assess long-term safety. Standardized methodologies and clinical trials are required to confirm their efficacy and safety characteristics. The therapeutic effects of the bioactive substances found in bee products can be used to treat dermatological disorders and enhance skin health. Novel, efficient, and sustainable skincare treatments may result from further research and development.

Keywords: bee products, honey, beeswax, propolis, royal jelly, dermatology, skincare.



Green Extraction Of Poplar Type Propolis: Ultrasonic Extraction Parameters And Optimization Via Response Surface Methodology

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Abstract:

Propolis (bee glue) is a beehive product consisting of plant resins and beeswax. It is recognized as a remedy for the treatment of various ailments. Poplar propolis, propolis with botanical source *Populus* spp., is one of the most well studied and widely distributed types of propolis. Due to its richness in bioactive compounds (phenolic acids, phenolic acid esters and flavonoids), this propolis type is commonly used in various pharmaceutical and cosmetic products, mainly as alcoholic extracts. In the recent years, however, the science and industry have focused their attention on using so-called “green” solvents as an alternative to conventional organic ones. Recently, we have tested several natural deep eutectic solvents (NADESs) as an extraction media for poplar type propolis, and citric acid:1,2-propanediol 1:4 was shown to possess high extraction capacity. In this study, we proceeded with optimization of the ultrasonic extraction process via response surface methodology. As a result, the optimal extraction conditions for maximum total phenolic content (TPC), total flavonoid content (TFC) and radical scavenging activity (RSA) were: solvent-to-solid ratio 30; 65 °C extraction temperature and extraction time 39 min. Under these conditions, the predicted values of TPC and TFC were 290.35 and 89.48 mg/g, respectively, and the RSA was 31.89%; the average recovery was in the range of 95-101%, presenting very good agreement between predicted and experimental values. GC-MS analysis of the optimized extract revealed the presence of the full combination of biologically active compounds.

Keywords: Green extraction, poplar type propolis, ultrasonic extraction

Acknowledgments:

This research was funded by the Bulgarian National Science Fund (Grant DH 19/4).

Determination of microbiological bee pollen composition using MALDI-TOF

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Abstract:

Bee pollen is a natural food product collected by honeybees from flowering plants. It is considered a superfood due to its high nutritional value and their several therapeutic activities, such as antioxidant, antibacterial and anti-carcinogenic¹. Bee pollen is very hygroscopic, so it easily promotes the growth of various microorganisms (both beneficial and pathogenic); nevertheless, there is no specific legislation for this product in the European Union. Microbiological contamination is one of the most important criteria to determine bee pollen quality, and several environmental factors may influence it such as botanical and geographical origins. Due to the absence of specific methodologies to determine the microbiological composition of bee pollen, this study investigates different culture medium and extraction methods to isolate bacteria and fungi from bee pollen for after identified using a Biotyper matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) system. Several non-specific culture mediums were tested to obtain bacterial and fungal communities. Potato dextrose agar medium (PDA) and lysogeny broth (LB) was selected for fungal and bacteria growth, respectively. In the case of bacteria, to obtain a protein extract a lysis process with formic acid 70% (v/v) was used. For fungal colonies, it was necessary a physical disruption process that involved three steps: i) lysis (formic acid 70% and acetonitrile); ii) ultrasonication with ceramic homogenizers; iii) centrifugation. Final extracts were analyzed using MALDI-TOF as reliable tool for the identification of microorganisms through comparison of protein profiles. The sample approaches proposed were applied to analyze thirty-four samples collected from local markets and experimental apiaries. In the case of commercial samples, the presence of beneficial bacterial species predominated, like *Bacillus simplex*, *Bacillus licheniformis* and *Methylobacterium organophilum*, among others. On the contrary, in samples from experimental apiaries, pathogen fungal species predominated such as *Aspergillus fumigatus*. Rapid and reproducible protein extraction methods of bee pollen samples were developed by using MALDI-TOF for species-level identification.

Keywords: bee pollen, culture medium, microorganisms, mass spectrometry, MALDI-TOF

Assessment of Sunflower Honey Quality and Stability During Storage[#]

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Abstract:

Sunflower honey is a traditional honey with exceptional healing properties and nutritional value. Sunflower is cultivated in the southern regions, where there are a lot of suns and a favorable climate for growing this plant. In Serbia, honey production is well-developed thanks to a suitable climate and geographic location. Sunflower honey is one of the most common kinds of honey produced in Serbia, but there is not enough information in the literature about the changes that can occur during the honey storage period. The aim of this study was to monitor sunflower samples (n=12) from several localities in Vojvodina, Republic of Serbia, during eighteen months of storage at room temperature ($22 \pm 2^\circ\text{C}$), regarding physicochemical parameters (moisture, electrical conductivity (EC), pH, diastase activity and 5-hydroxymethylfurfural (HMF)). The average values of the physicochemical parameters of fresh sunflower honey were as follows: moisture $17.40 \pm 0.89\%$, EC 0.34 ± 0.08 mS/cm, pH 3.62 ± 0.10 , diastase activity 18.92 ± 3.64 DN and HMF 2.22 ± 0.84 mg/kg. The initial test results indicated that Serbian sunflower honey is characterized by good quality since the examined parameters closely related to the quality of honey had values in agreement with legal limits established by national and European legislation. Eighteen months of storage at room temperature reduced diastase activity (8.21 ± 1.86 DN), increased HMF (33.79 ± 5.14 mg/kg) content, and decreased pH value (3.56 ± 0.11) of honey. Storage did not affect sunflower honey's moisture content ($16.25 \pm 1.43\%$). In general, 18 months of storage at room temperature affects the quality of sunflower honey. Therefore, further research on the effects of storage time and storage temperature is necessary, not only to predict the shelf life of sunflower honey but to preserve the natural properties of honey as long as possible.

Keywords: sunflower honey, physicochemical parameters, storage

Acknowledgments: This research was funded by Ministry of Science, Technological Development and Innovation of Republic of Serbia by the Contract of implementation and funding of research work of NIV-NS in 2023, Contract No: 451-03-47/2023-01/200031.

Different Analytical Methods For Determining Pesticides In Bee Products

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Abstract:

Pesticides (herbicides, acaricides, and insecticides) that are used both in agriculture and livestock can reach the beehives and subsequently their related products, like honey, through the pollination process. In addition, other pesticides, especially those employed to fight against *Varroa destructor* like the acaricides coumaphos and τ -fluvalinate, can produce direct contamination to the beehives because they are applied directly to the honeycombs. Different options for the use of acaricides, highly conditioned by the form of application, the climatic conditions, as well as the health status of the beehive, also compromise their effectiveness. This leads to the frequent application of doses higher than those recommended, and there is a high probability that acaricide residues appear in the different beehive products. Indeed, acaricide residues have been found in honeys from different countries, and maximum residue levels have been established. Therefore, the development of specific and sensitive methodologies for the determination of acaricides in bee products is justified. The main goal of this research was to propose alternative methods for determining simultaneously seven pesticides (atrazine, chlorpyrifos, chlorfenvinphos, α -endosulfan, bromopropylate, coumaphos, and τ -fluvalinate) in beeswax, bee pollen and honey by using gas chromatography-mass spectrometry (GC-MS). We developed a fast GC-MS method (< 21 min.) that was applied with slight modifications to all bee-related matrices. After an optimization study, the sample treatment selected for bee pollen and beeswax consisted of a modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method employing a solvent extraction, and a mixture of salts for the clean-up step, while it was only employed a solvent extraction for honey analysis. The methods were validated in terms of selectivity, limits of detection and quantification, linearity, matrix effect, trueness, and precision. The proposed methods were applied to analyze commercial samples, and some of the target pesticides (α -endosulfan, chlorfenvinphos, chlorpyrifos, coumaphos, and τ -fluvalinate) were detected. Finally, this study highlighted some of the advantages of developing specific methods rather than multi-residue approaches; these include the absence of a significant matrix effect, extraction efficiency, and precision.

Keywords: acaricides, bee products, GC-MS, sample treatment, method validation.

Authors thank financial support by the Spanish “Ministerio de Economía y Competitividad” and the “Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria” (Project number, RTA2017-00004-C02-02). Adrián Fuente-Ballesteros thanks the University of Valladolid for his PhD gran

Efficacy of Four Synthetic and Natural Substances in Control of *Nosema ceranae* in *Apis mellifera* Apiaries from Lebanon

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Abstract:

Nosema Ceranae is an obligate microsporidian intracellular parasite infectious to honey bees; it can cause bee mortality and has been correlated with colony losses. *N. Ceranae* is also associated with morbid physiological impairments, including immune function, foraging behavior, and pheromone production. The only registered product for its control is the antibiotic fumagillin. Despite the short lifespan in the hive, fumagilin degradation residues can persist and pose a potential risk to human health. On the other hand, fumagillin efficacy against *N. Ceranae* is uncertain. Sulfamethoxazole is a bacteriostatic sulfonamide antibiotic that interferes with folic acid synthesis in susceptible bacteria. It is generally given in combination with trimethoprim and is suggested by certain groups of beekeepers to treat *N. Ceranae*. Thymol is a natural monoterpene phenol, existing in several essential-oil mixtures and mainly in *Thymus vulgaris* essential oils. Chamomile extract is formed by a mixture of natural products produced in the daisy-like plants, mainly the common species *Matricaria chamomilla* and *Chamaemelum nobile*. The aim of this study is to investigate (using an optical microscope) the effect of the four mentioned products on the spore loads in bees infected with *N. Ceranae* as well as parameters of oxidative stress and bee survival. The results reveal mostly positive effects on the bees treated with thymol and chamomile extract, decreasing the *Nosema* spore loads. Fumagilin and sulfamethaxazole gave discouraging results when applied to *N. Ceranae*-infected bees. Even though the study hasn't finished yet, our finding suggests that among the screened compounds, thymol, along with the chamomile extract, has potential effective antifungal activities against *N. ceranae* in a real field.

Keywords: *Nosema Ceranae*, Honey Bees, Thymol, Fumagilin, Sulfamethaxazole, Chamomile extract.

The Role of Propolis in Cancer Treatment

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Abstract:

The aim of this study is to examine the therapeutic effects of propolis on cancer in the light of present literature information. Published reviews and randomized controlled trials involving the words "propolis", "cancer therapy" and "propolis and cancer" between 2019 and 2023 were reviewed in PUBMED. In recent years, cancer cases have been encountered commonly. Various and innovative research work are being conducted on treatment methods of cancer, which poses risk to public health and creates a burden for health expenditures. Propolis, one of the most important bee products, is a natural substance produced by honeybees (*Apis mellifera*) from various botanical sources. The therapeutic activity of propolis, including antibacterial, antifungal and anti-inflammatory effects, has been known since ancient times. Due to these beneficial properties, it is used in the production of functional foods, in cosmetic and pharmaceutical industries as well as in apitherapy, which is a supportive treatment procedure. Although propolis is one of the most important substances for human health in developing alternative medicine, studies have shown that it is effective against many cancer types such as head and neck, brain and spinal cord, blood, skin, breast, pancreas, liver, colon, prostate, kidney and bladder cancers. Recent studies show that propolis can inhibit proliferation, angiogenesis and metastasis of cancer cells and stimulate apoptosis. Propolis is thought to be a powerful alternative for cancer treatment.

Keywords: propolis, cancer, health

Medicines Used In The Fight Against Bee Diseases In Turkey And Europe

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Abstract:

Beekeeping is a widespread agricultural activity all over the world today. Bee products and the contribution of bees to pollination constitute an important economic income in our country and in the world. There are many diseases in bees as in other living organisms. The buying and selling of bee products contaminated with diseases, itinerant beekeeping, transfer of bees from one hive to another hive, careless behavior of beekeepers in some issues have caused the rapid spread of bee diseases. Diseases seen in bees can be classified as bacterial (European and American Foulbrood, Septicemia), viral (Tulumpy Foulbrood, Bee Paralysis), fungal (Lime and Stone disease), protozoan (Nosema, Amoeba) and parasitic (Varroa destructor, Acarapis woodi). Especially among these pathogens, Varroa destructor is a dangerous parasite that causes losses of bee products and bees all over the world. Most of the licensed drugs used against bee diseases in Turkey and Europe are drugs used to combat Varroa destructor. Chemical drugs used in Varroa control include drugs with active ingredients such as Flumetrin, Amitraz, Taufluvanilate, Kaumofos. In recent years, due to the development of resistance to these acaricidal drugs and residue problems, herbal extracts with organic acid and phytobiotic properties have become more preferred. Formic acid, oxalic acid and lactic acid are the most commonly used organic acids. Thymol, camphor, eucalyptol, menthol and herbal extracts such as thyme, clove, tobacco, canola, mint, bay leaf are the factors in the drugs used in varroa control. In addition, there are also licensed drugs to combat other disease agents. The use of licensed medicines enables effective control of disease-causing agents in bees. The use of licensed products as recommended minimizes the amount of residue in bee products.

Keywords: honeybee, drug, disease.

Amitraz and Cypermethrin used in beekeeping and agriculture have a pronounced toxic effect on the model test system *Allium cepa*

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Abstract:

The importance of honey bees for plant pollination is enormous, but the last few decades it has seen a decline in the number of bee colonies all over the world. Despite the complex nature of the problem, it is largely related to pesticide exposure. Their Improper use has a negative effect on the bee health. In the present study, by applying the *Allium* test, the individual and the combined toxic potential of the pesticides amitraz and cypermethrin were analyzed. Although banned for use in many European countries, residual amounts of amitraz (0.0015 – 0.0054 mg/kg) have been detected in honey bee samples collected from apiaries with high mortality in 2021 in Bulgaria. This study aims to investigate the potential toxic effect of amitraz and cipermetrin by usage of *Allium cepa* as a model test-object. One-year-old onion seeds were used for the purpose of the study. Temporary microscopic squash preparations stained with acetocarmine have been prepared from the grown roots. The cytotoxic potency of pesticide solutions at different concentrations has been determined by calculating a common mitotic index and phase indexes. The results show that all tested concentrations of amitraz and cypermethrin exhibit cytostatic effect by inhibiting mitotic activity of onion apical meristem cells. The decrease in the mitotic index correlates significantly with the increasing concentration of the amitraz. The lowest proliferative activity in the samples treated with the maximum tested concentration of this pesticide has been recorded. The insecticide cypermethrin also inhibits cell division. Amitraz has a higher cytostatic potential than cypermethrin. The calculated phase indices show an influence on the duration of the individual phases. The mitosodepressive effect is more pronounced with the separate exposure of the two pesticides compared to their combined effect. As conclusions: The investigated pesticides amitraz and cypermethrin, applied separately and in combination, exhibit a general toxic effect expressed in the suppression of germination of *Allium cepa* seeds. A more significant cytotoxic potential of amitraz compared to cypermethrin has been found. The complex application of investigated pesticides influences the intensity of cell proliferation in *Allium cepa* but demonstrate lower cytotoxic activity in comparison with their separate use.

Keywords: amitraz, cypermethrin, toxicity, beekeeping, agriculture.

#This study was supported by the National Research Fund of Bulgaria by 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".

Phytochemical Composition and Antioxidant Properties of Monofloral Beebread Samples From Serbia

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Abstract:

Beebread is a valuable product composed of fermented bee-collected pollen. It has a high nutritional value and provides the necessary food for bees. Phytochemical content and antioxidant activity depend on the botanical origin, which also affects beebread consumption by bees. In order to find the differences, two monofloral beebread samples, *Amorpha fruticosa*, and *Sophora* sp., from the same locations in Serbia, were analyzed. The phytochemical composition (expressed as total phenolic content (TPC), total flavonoid content (TFC), and derivatives of dihydroxy cinnamic acid (HCAs)) and antioxidant properties (expressed as ferric reducing power (FRP), cupric ion reducing antioxidant capacity (CUPRAC), 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH[•]) and 2,20-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid radical cation scavenging activity (ABTS^{•+})) of extractable (I) and alkaline hydrolyzable (II) methanolic fractions of two beebread samples, were compared. Results showed significantly higher values for I fractions, indicating that phenolics are presented mainly as free forms in beebread samples. Secondly, differences between the two samples were noted mostly by the results obtained for I fractions, where *Sophora* stood out with increased phytochemicals content and antioxidant properties. Contrary, the obtained results for II fractions for two beebread samples were significantly different only for ABTS^{•+} scavenging activity, in favor of for *Sophora* (183.35 μg/g TE) in contrast to the *Amorpha fruticosa* (16.32 μg/g TE). This study confirmed the dependence of phytochemical content and antioxidant activity of beebread on the botanical origin. In addition, this was mainly noticeable for the free phenolic fraction, which was shown to be dominant in the beebread samples. *Sophora* beebread sample could be a more valuable source of phytochemicals with expressed antioxidant properties than *Amorpha fruticosa*. Further research into the chemical constituents of beebread samples, obtained with modern chromatographic techniques, would increase the understanding of this bee product.

Keywords: beebread, phenolic content, antioxidant activity.

For the financially support, we would like to thank the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract Nos: 451-03-47/2023-01/200051 (M.K.); 451-03-47/2023-01/200168 (Ž.T.)).

Poster Presentation



Analysis of Endocrine Disruptors-Hormone Chemicals In Bee Products

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Abstract:

Currently, there is a significant amount of apprehension regarding the correlation between nutrition and its impact on bodily functions and disease processes. As a result, there is a growing demand for organic food, particularly those derived from beehives such as honey or bee pollen, owing to their numerous beneficial health properties. Nonetheless, the global issue of plastic contamination poses a significant threat to pollinators like bees, as they are exposed to various sources of pollution from multiple sectors, including microplastics (MPs). MPs exhibit high persistence, prevalence and can be transmitted through the food chain, leading to the accumulation of environmental pollutants and the release of additives that induce toxic effects. Bisphenols, which are plastic components used in combination with other chemicals during plastic production, are among these contaminants. Some of these compounds are classified as Endocrine Disrupting Chemicals (EDCs) due to their ability to mimic estrogenic activity. These contaminants can also be found in beehives and their derived food products. Therefore, it is essential to establish methods for controlling these pollutants and ensuring food safety for both consumers and pollinators. Consequently, the primary objective of this research is to investigate the presence of hormone-disrupting compounds, particularly bisphenols, by developing suitable chromatographic analytical methods. Several stationary phases were examined to optimize various chromatographic and detector-related parameters. Additionally, different sample treatments were employed to achieve the highest recoveries and minimize matrix effects for two distinct bee products, honey, and bee pollen.

Keywords: analytical methods, bisphenols, microplastics, additives, bee products.

#The authors thank the Women's Institute belong to the Spanish Ministry of Equality for funding through the project n°10-2-ID22.

The use of spontaneous and controlled fermentation to convert bee pollen to bee bread substitute in laboratory conditions[#]

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Abstract:

Bee bread is considered one of the most valuable among the bee products used in apitherapy, due to its nutritional, antioxidant and immunomodulatory properties. The scale of obtaining natural bee bread is not too high, therefore an effective method of reproducing this product in laboratory conditions is searched. The research was aimed at evaluating the possibility of spontaneous fermentation of bee pollen caused by the natural pollen microflora and with the use of a bacterial starter culture (*Lactobacillus rhamnosus* GG). The prepared mixtures consisting of crushed pollen loads with the addition of multifloral honey (10%) were incubated for 3 days at 32°C and then left for 4 weeks at 25°C. After fermentation, the obtained product was dried and evaluated for physicochemical (pH, acidity, water activity) and antioxidant properties. The obtained results were compared to natural bee bread (5 samples from south-eastern Poland) and to the initial bee pollen. There were no significant differences between the product obtained with the starter culture of lactic acid bacteria and those fermented spontaneously, which suggests the dominant participation of the native pollen microflora in the fermentation process. Partial fermentation of pollen was confirmed based on changes in pH (started from 5.0 to 4.5-4.6) and acidity (from 2.49% lactic acid for initial pollen to 3.1-3.7%). The obtained product showed intermediate properties between the converted bee pollen and natural bee bread, which showed lower pH (average 4.08) and higher acidity (average 4.24% lactic acid). As a result of fermentation, an increase in the reducing power (by the FRAP method) and a decrease in the antiradical activity (DPPH) of the bee bread substitute compared to initial pollen were noted. Obtained product has also been shown to have a protective effect against hydrogen peroxide-induced oxidative stress in a yeast model. The conducted experiment confirms that obtaining "artificial bee bread" in laboratory conditions is possible, but further research is needed to optimize the fermentation process and the bioactivity of this type of products.

Keywords: bee bread, bee pollen, fermentation

[#] The research was supported by the project financed under the program of the Minister of Science and Higher Education of Poland entitled "Regional Initiative of Excellence" in 2019–2023 (project no. 026/RID/2018/19).

Sensory Characteristics of Honeys from Serbia

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Abstract:

Serbia has a very long tradition of beekeeping, because of its good geographical conditions and a variety of botanical species. Honey acceptability is mainly determined by its sensory characteristics. The purpose of this study was sensory characterization of fifty Serbian honeys with a descriptive and hedonic scale approach. Types of honey included in this study were acacia honey (34%), meadow honey (26%), multifloral honey (20%), linden honey (12%) and forest honey (8%). To evaluate the color, texture, odor, and taste a 9-point hedonic scale was performed by an experienced and trained panel of five assessors. The average results of color, texture, odor, and taste were 6.78 ± 1.80 , 6.55 ± 1.94 , 6.00 ± 2.24 , and 5.55 ± 2.50 , respectively. Higher values of the color and texture parameters were determined in the linden honeys, while higher values of the odor and taste parameters were determined in the acacia honeys. Three meadow honeys and one acacia honey received the maximum score. Additionally, the sensory characteristics of the five types of Serbian honey were described. The descriptive sensory analysis shows wide variability among analyzed honey types. The colors of the honeys were noticeably different, and varied from almost colorless to dark brown, while the texture varied between almost watery to a finely granular mass. The odor and taste were characteristic for each individual honey type. The meadow and multifloral honeys were most diverse, as their sensory characteristics depend on the types of flowers from where the bees gathered the nectar. Overall, the study shows variations among same and different honey types, and assists the beekeepers to understand the sensory quality of their products.

Keywords: unifloral honeys, multifloral honeys, sensory descriptive analysis, hedonic scale.

This study was funded by Ministry of Science, Technological Development and Innovation of Republic of Serbia by the Contract of implementation and funding of research work of NIV-NS in 2023, Contract No: 451-03-47/2023-01/200031.

Regional aspects in the problem with honey bee colony losses for Bulgaria

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Abstract:

During past years the Bulgarian beekeeping is under great danger. During the period 2017 – 2022 honey bee colony losses have increased from 2.04% to over 21%. Two main reasons could be mentioned for that: 1) the uncontrolled imports of honey bee queens with foreign origin, although the Bulgarian law forbids it and 2) the usage of different pesticides in the agricultural activities which seem to be in relation to the reported high mortality rate of bee colonies in some regions in Bulgaria. The rate and the reasons for honey bee colony losses were investigated among all regions of Bulgaria by the standardized international COLOSS questionnaire. Through the survey in 2022, data were summarized for 46 apiaries with 5444 bee colonies from 36 locations throughout the country. More than 21% (1159 bee colonies) were reported as dead. The most significant losses were reported in apiaries located in the regions of Sliven, Plovdiv, Lovech, Stara Zagora, Vratsa, Pleven, Kardjali. Apiaries in Blagoevgrad, Varna, Veliko Tarnovo were with minimal losses. Of the total dead colonies the following losses due to objective reason were detected: problems with the queen bee – 14.8%, natural disaster – 0.8% and mortality in the hive or a sharp decrease in the number of bees with the family to several hundred – 85.4%. The most significant losses were found for apiaries located near agricultural areas with sunflower, rapeseed, corn, orchards and autumn fodder crops. Oxalic acid, coumaphos and amitraz and less frequently – flumethrin, thymol and lactic acid were the most frequently applied against varroaosis among the beekeepers surveyed. Residual amounts of 27 pesticides were found in the areas with the highest mortality. Among the group of the pesticides detected were insecticides (including acaricides), fungicides, herbicides and growth regulators. The presented and analyzed data should be considered when developing activities to protect the honey bee health status in Bulgaria.

Keywords: *Apis mellifera*, regional honey bee colony losses, reasons

Acknowledgments: This study was supported by the National Research Fund of Bulgaria by 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".

Sperm comet assay reveals deterioration of DNA integrity in honey bee (*Apis mellifera* L.) populations with high mortality rates

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Abstract:

One of the most alarming global phenomena is the worldwide loss of honey bee colonies. Various pesticides widely used in agricultural practices are considered to be one of the main causes of high honey bee mortality worldwide. The honey bee *Apis mellifera* L. is a suitable biomonitor species for various biomonitoring studies due to its high sensitivity to environmental pollution. DNA integrity in drone sperm is an important factor for the survival of bee populations. Therefore, the focus of this study was to assess the possible genotoxic potential of pesticides by comet assay in sperm cells of honey bee (*Apis mellifera*) populations with a known high mortality rate. The study included four populations from the territory of Bulgaria – three with increased honey bee mortality over than the average (21.28%) – Plovdiv (26.7%), Vinitsa (53.3%), Dimovtsi (65%) and one as a control – Krasново (0.8%). In contrast to the control, pesticide residues were detected by chromatographic analysis in samples of bees and food in the hives. The statistically significant differences in the mean values of the comet assay parameters Tail Intensity (TI%), Tail Length (TL) and Olive Tail Moment (OTM, μm) in the investigated localities compared to the control prove the *in vivo* effect of pesticides as genotoxic agents in the impact areas. The results reflect the genetic damage that occurred during spermatogenesis in honey bees living in Plovdiv, Vinitsa and Dimovtsi. While the comet assay with somatic cells gives an idea of short-term DNA damage (single- and double-strand breaks and alkali-labile sites that can be repaired), the study of male germ cells assesses the long-term effects that can affect offspring as a result of DNA breaks that cannot be repaired. This study presents, for the first time, an assessment of DNA damage in *A. mellifera* spermatozoa and should be considered as a warning of the potential impact of pesticides on the reproductive capacity of honey bee populations.

Keywords: *Apis mellifera* spermatozoa, DNA damage, comet assay, pesticides, colony losses.

Acknowledgements: 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".

Selenium content in honey: An example from Serbia

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Abstract:

Honey bees and bee products (honey, bee bread, pollen, royal jelly, propolis) can be used as bioindicators of environmental pollution, and to determine pesticides, radionuclides and heavy metals since bees travel considerable distances for the foraging flights (usually up to 2 km per flight). During harvest time, bees are exposed to different sources of contaminants through their pollen and nectar that can contain heavy metals of natural and/or anthropogenic origin. Some of these elements are bioaccumulative and can be measured in honey, and can for instance be used to determine the botanical and geographical origin of different honeys. Selenium is an essential trace element. The cellular processes that require identified selenoproteins include the biosynthesis of deoxyribonucleotide triphosphate, removal of peroxides, reduction of oxidized proteins and membranes, metabolism of thyroid hormones, transport and storage of selenium and intervention in protein. Data on the selenium levels of honeys is very limited, but some studies from Serbia show that the selenium content is below limit of detection (<0.2 mg/kg). Similarly, it has been shown that the botanical origin of the honeys can have an influence on their selenium content. Other studies show that selenium content of the studied honey samples ranged from <1.0 to 2.91 mg/kg. This low selenium contents are not surprising due to the low protein and mineral content of honeys. The different honeys (multifloral and monofloral) contained different selenium levels and the level depends on the floral species, the amount present in soils and availability, which depends on the soil acidity, salinity and CaCO₃ content.

Keywords: selenium, honey, biological activity, safety, quality.

#This study was supported by the Ministry of Science, Technological Development and Innovation, Republic of Serbia, Grant No. 451-03-47/2023-01/200050 from 03.02.2023 and the German Federal Ministry of Education and Research (BMBF) (Bridge2ERA2021: 100579052).

The impact of spermidine dietary supplementation of honey bees on their putrescine, spermidine and spermine levels during ageing

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Abstract:

As a prominent global pollinator, the honey bee (*Apis mellifera* L.) plays a crucial role in maintaining biodiversity. Unfortunately, a steady decline in colony numbers has been observed in the past few decades. This phenomenon may be a consequence of negative human activities on the environment. A potential way to lessen the impact of such activities is the use of chemicals which improve health and consequently, longevity. Polyamines, which include putrescine, spermidine and spermine, are a class of positively charged molecules, the levels of which decline with age. These molecules are involved in a wide variety of processes, including cell proliferation, growth, and development, but also promote longevity and overall health. The aim of this study was to examine whether oral supplementation of honey bees with spermidine leads to an increase in the levels of all three individual polyamines. Two experimental groups were formed: control (C), which was fed with 50% (w/v) sucrose solution, and S_{0.01} group named after the spermidine concentration in mM. The duration of the experiment was 10 and 17 days. The content of individual polyamines was determined in the whole body of bees by HPLC analysis. The levels of all three polyamines declined with time in the control group. When comparing the levels of all three individual polyamines after supplementation with their respective control, a significant increase was observed after 17 days. These results highlight the fact that spermidine supplementation has a positive effect in maintaining steady levels of polyamines during ageing. These findings could be the basis for the implementation of polyamine supplementation in beekeeping practice.

Keywords: honey bee, polyamines, supplementation, ageing

The authors acknowledge financial support from the Science Fund of the Republic of Serbia (Program IDEAS, Grant No. 7721972, project title: Implication of dietary and endogenous polyamines for the health and longevity of honey bees B-HEALTH) and Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grant No. 451-03-47/2023-01/200125).

The content of natural polyamines in honey bee products

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Abstract:

Due to increasing concern about the overconsumption of synthetic foods and overuse of food additives, there is an increasing demand for natural products in human nutrition. In this context, a prominent number of studies aimed at finding functional natural products/supplements with high nutritional value, rich in bioactive substances with a positive effect on health and longevity. Bee products elegantly fulfill these requirements. The aim of our pioneering study was to determine the biological origin and presence of three major polyamines (PAs) – putrescine, spermidine and spermine, in bee bread, pollen loads, royal jelly and honey. Samples of different bee products were collected directly from different markets, during the 2022 season, which practically means that the bee products originated from foraging bees in 2021. Results show that pollen from plant families Araliaceae, Brassicaceae, Tiliaceae and Asteraceae dominated in analyzed bee products, whereas the biological origin did not affect the total content or proportion of individual PAs which was always representative for a specific bee product. The total content of PAs was the highest in pollen (125.34–711.57 mg/kg), slightly lower in bee bread (287.75–602.37 mg/kg), much lower in royal jelly (68.94–121.14 mg/kg) and negligibly small in honey samples (ranged between 2.80 and 10.84 mg/kg). When examining the representation of individual PAs in total PA profile of bee products, spermidine dominated in pollen (69.73–478.04 mg/kg), bee bread (131.49–429.31 mg/kg) and honey (1.66–3.93 mg/kg). Royal jelly differs from the other analyzed bee products because the dominant PA was putrescine (43.91–85.16 mg/kg), making up about 60% of the total PA content. Spermine was the least abundant in royal jelly, bee bread and honey. In conclusion, this study highlights a significant content of PAs, especially spermidine, in bee products, such as pollen loads and bee bread. These PA rich products could find a place in the field of health and longevity promoting foods, considering that during the aging process, cellular spermidine levels tend to decrease. Maintaining spermidine levels by consuming foods or supplements abundant in spermidine should be a priority for improving health and longevity.

Keywords: honeybee product, polyamines, health, longevity

We acknowledge financial support from the Science Fund of the Republic of Serbia (Program IDEAS, Grant No. 7721972, project title: Implication of dietary and endogenous polyamines for the health and longevity of honey bees B-HEALTH) and Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grant No. 451-03-47/2023-01/200125).

Histopathological Changes In Reproductive System Of *Apis Melifera* L. Drones Exposed To Pesticides

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Abstract:

The assessment of the histopathological changes could be successfully applied for biomarkers of the environmental contamination with pesticides. Histopathological methods include determination of factors causing a negative effect on bioindicator species. Histopathological biomarkers could be also applied in environmental monitoring programs, as well as, to clarify the effects between organism and the concentration of the toxic substances in the environment. In the present study three test apiaries were determined (Plovdiv, Vinitza and Dimovtsi) as *in situ* locations and one – as a control (Krasново). The selection of the test apiaries was based on the reported by beekeepers relatively high mortality of bee colonies observed in the winter of 2021-2022 – over than 21%. Chemical analyses of bees and food supplies (honey, wax and pollen) samples showed the presence of different priority organic substances such as neonicotinoid insecticides (imidacloprid, clothianidin, flonicamid), synthetic pyrethroids (cyhalothrin, tau-fluvalinate), organophosphates (chlorpyrifos (-ethyl), coumaphos, dimetoat) and carbamates (carbaril, fenoxycarb). After dissection of the experimental male individuals at different stages of their development, the gonads were separated and the hematoxylin-eosin (H&E) staining method was applied. The results showed histopathological changes concerning the seminiferous tubules and sperm cells located in the gonads. They were expressed mainly in the thinning of the epithelium covering the tubules and separation of the basal membrane and the seminiferous tubules. Moreover, separate areas with necrotic changes were observed. The mentioned above histopathological changes affected mainly the mature males. According to the obtained results, a tendency towards an increase in the observed degenerative changes in the experimental individuals from the city of Plovdiv was found compared to the other studied areas.

Additional histological and histopathological analyzes will contribute to the more complete study of the pesticide effect on the reproductive system of drones.

Keywords: pesticides, pollution, *Apis mellifera* drones, histology, reproductive system

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”.

Effect of Solvent on Protein, Total Phenolic and Reactive Amino Group Content in Different Honey Types

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Abstract:

Along with carbohydrates and water, honey also contains many components that, although present in small quantities, significantly contribute to its nutritional and therapeutic properties. Minor honey components were also evaluated as potential markers of botanical and/or geographical origin of honey. When performing analyses, the choice of solvent significantly affects the share and the type of components that will be determined. The aim of this study was to evaluate the effect of two polar solvents (water and methanol) on protein, total phenolic and reactive amino group (RAG) content in different honey types. A total of 131 honey samples (30 black locust, 30 multifloral, 20 honeydew, 18 chestnut, 13 winter savory, 11 lime and 9 sage honey samples) were collected in two production seasons. Prior to the spectrophotometric analyses, the botanical origin was confirmed based on the melissopalynological analysis, determination of selected physicochemical parameters and sensory analysis. The results showed that solvent type has a significant impact on analyzed parameters. Generally, the content of all analyzed components was higher when using water as solvent. The greatest difference was obtained for protein content where the results were approximately 6-fold higher for water honey solutions than methanolic solutions for the same honey sample. Average percentage of RAG content in methanolic solutions was 84 % of those detected in water honey solutions, indicating that majority of reactive amino groups present in honey can be attributed to the molecules soluble in methanol, such as free amino acids and small soluble peptides. The notable differences were also observed between honey types when using the same solvent. Light honeys, like black locust and lime honey, had the lower content of all analyzed parameters than darker honeys (e.g. chestnut and honeydew honey) when using water as solvent, but the same trend was not observed for protein content determination of methanolic solutions. Sage honey had several fold higher RAG content than other analyzed honey types regardless of solvent type, which could be potentially used as a fast method for identification of this specific honey type, but further research is needed to confirm these results in comparison to other honey types not included in this study.

Keywords: honey, protein content, total phenolic content, reactive amino group content, solvent

Drone brood steroid hormones bioaccessibility during *in vitro* digestion

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Abstract:

Bee drone brood is a little-known bee product, often treated as beekeeping waste or natural varroosis bait and its obtaining from beehive does not weaken the bee family. This product is frequently used as natural medicine in Eastern European countries. Among bee products, drone brood is characterized by an especially rich chemical composition, which makes it biologically active. It contains approximately 40% protein, 30% reducing sugars, but also sex steroid hormones. The aim of this study was to check the bioaccessibility of selected sex hormones (testosterone and estradiol) present in the drone brood. Three samples of drone brood (7-days old) were collected from one apiary in the south-eastern part of Poland (Podkarpackie Province) in June 2022. The material was immediately homogenized, frozen and then freeze-dried (Alpha 1-2 LG plus). Such a material was subjected to a hormones content as well as their accessibility during simulated *in vitro* digestion. An undigested fraction (control) and 2 fractions after digestion: gastric and intestinal were tested in terms of testosterone and estradiol accessibility. Hormones analysis was performed using an immunoenzymatic Elisa test (Abbexa). In freeze-dried drone brood the level of testosterone (0.29–0.31 nmol/100 g) and estradiol (431.2–847.90 nmol/100 g) were found. The bioaccessibility of steroid hormones at the end of digestion was 63.3 and 21.3% for testosterone and estradiol, respectively. The release of testosterone from the matrix was observed in the gastric and intestinal phases, respectively 23.8 and 93.3%, while estradiol was released only in the intestine (21.3%). It was found, that tested hormones occurred in freeze-dried drone brood, due to their hydrophobic nature, become accessible mainly after emulsification of the digested content, which occurs in contact with bile. Moreover, their bioaccessibility seems to be much higher compared to synthetically produced supplements containing hormones.

Keywords: drone brood, testosterone, estradiol, bioaccessibility

Insight into the Influence of Natural Deep Eutectic Solvents on the Extraction of Phenolic Compounds from Poplar Type Propolis: Composition and *In Vitro* Biological Activity

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Abstract:

Natural deep eutectic solvents (NADESs) have been considered promising to replace traditional volatile and toxic organic solvents for the extraction of biologically active substances from natural sources. This work applied an efficient and greener strategy for extraction of phenolic compounds from poplar type propolis using five NADESs (two choline chloride based, two lactic acid based and one betaine based). Their extractability was evaluated by measuring the amount of extracted total phenolics, and total flavones and flavonols. Then the chemical composition of the extracts obtained was studied by GC-MS analysis. It confirmed that all biologically active compounds extracted with 70% ethanol were found in the NADES extracts in similar ratios. To expand the knowledge about the role of the five selected NADESs in the poplar propolis extraction process, the *in vitro* antimicrobial, cytotoxic and genotoxic activity of both NADESs and extracts were evaluated. The results confirmed the potential of NADESs to be used for green extraction of bioactive components from poplar propolis and further suggest that some of them can improve the effects of bioactive extracts.

Keywords: natural deep eutectic solvents; poplar type propolis; green extraction; antimicrobial activity; cytotoxicity; genotoxicity.

Acknowledgment: The work was supported by the National Science Fund of Bulgaria, grant number DN 19/4-2017. In this investigation GC-MS equipment purchased by Project No BG05M2OP001-1.002-0012, Center of Competence "Sustainable utilization of bio-resources and waste from medicinal and aromatic plants for innovative bioactive products", funded by the Operational Program "Science and Education for Smart Growth" 2014-2020, co-financed by the European Union through the European Regional Development Fund, was used.

General phytochemical composition and antioxidant properties of bee-collected pollen originated from industrial hemp plant[#]

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Abstract:

Bee-collected pollen represents one of the best source of all nutrients as well as several bioactive metabolites important for health. Its chemical composition is strongly influenced by botanical origin. In this research bee-collected pollen was obtained from *Cannabis sativa* plants (during summer season 2020) in order to examine general phytochemical composition and accompanying antioxidant properties. For that purpose, two separated fractions were prepared: extractable (free) fraction (obtained through solvent extraction with 80% methanol) and alkaline hydrolysable (bound) fraction obtained after application of 4M NaOH solution. Both extracts were used for following parameter determination: total phenolic (TPC), flavonoid (TFC), and derivative of hydroxycinnamic acid (HCA) content. In addition, four antioxidant assays were applied: total antioxidant capacity (TAC) determined through *in vitro* phosphomolybdenum assay, ferric reducing power (FRP), cupric reducing antioxidant capacity (CUPRAC) ABTS^{•+} and DPPH[•] quenching abilities. All results are expressed based on fresh sample weight. Free fraction contained significantly higher amount of different phenolics (range: 5.55 mg/g GAE fresh weight (TPC) – 11.88 mg/g QE (TFC)) compared to bound fraction (range: 1.11 mg/g QE fresh weight (TFC) – 2.59 mg/g CGAE fresh weight (HCA)). In accordance to this, extractable phenolic compounds exhibited higher antioxidant activity in all applied assays compared to compounds released after alkaline hydrolysis. Obtained values for extractable fraction were as follow: TAC (8.31 mg/g AAE), FRP (8.70 mg/g AAE), CUPRAC (45.04 mg/g AAE), ABTS^{•+} (14.76 mg/g Trolox), DPPH[•] (1.66 mg/g Trolox). In case of bound fraction the following values were found: TAC (1.17 mg/g AAE), FRP (0.60 mg/g AAE), CUPRAC (12.30 mg/g AAE), ABTS^{•+} (1.10 mg/g Trolox), DPPH[•] (0.20 mg/g Trolox). Further statistical analysis should confirm any positive relation between determined phytochemical composition and observed antioxidant properties. Based on determined parameters both fractions can enhance biological activity of this pollen in case of application in some area/industry.

Keywords: bee-collected pollen, *Cannabis sativa*, phytochemical composition, antioxidant properties.

[#]The pollen sample was obtained by courtesy of JarmaHemp d.o.o (Pilot, Serbia). This work was supported by the Ministry of Science, Technological Development, and Innovation of the Republic of Serbia Grant No. 451-03-47/2023-01/200116.

Flavored creamed honeys as a new valuable offer of beekeeping industry[#]

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Abstract:

Creamed honeys with various additives, such as fruits, herbs and spices, are becoming more and more popular among consumers. The introduction of a plant additive to honey, besides shaping more favorable sensory properties, i.e. color, taste or smell, significantly increases its bioactivity, resulting mainly from the increase of the polyphenols and vitamins content. Moreover, using sweet honeys as matrix allows to mask bitter flavor of herbs which allows to introduce them to basic diet. Flavored creamed honeys with various additives (2 and 4%) were prepared and analyzed for the content of total polyphenols, antioxidant activity (FRAP, DPPH and ABTS) and biological activity using *in vitro* tests. As matrix rapeseed honey was used due to the best kinetic characteristics of crystallization process. As an additive dried micronized parts of various medicinal plants were used, including fruits (black raspberry, blackberry, raspberry, blackcurrant, wild rose hips, barberry, sea buckthorn, mulberry and chokeberry), flowers (elderberry and white sweet clover), and leaves (mulberry, white sweet clover, sea buckthorn, blackberry, raspberry). The significant dose-dependent increase in the content of antioxidant activity and total phenolics content in spiked honeys were found, from several to several dozen times. The observed multiplied content of polyphenols was not only for fruits abundant in anthocyanins but also for leaves and flowers rich in phenolic acids and flavonoids. Different bioactivities of enriched honey can be created or enhanced, depending on the plant additive used. It was found that honey with the addition of sweet clover flowers can inhibit platelet aggregation, honey with the addition of raspberry, aronia and blackberry fruits possess strong antibacterial and antiviral effects whereas honey flavored with sea buckthorn leaves and black raspberry fruits had a dose-dependent effect on inhibition of biofilm formation by *S. aureus* ATCC 6538. Beside of, all tested flavored creamed honeys can protect the body against oxidative stress.

Keywords: rapeseed honey, plant additives, enrichment, antioxidant activity, healing effect

[#] This research was funded by Polish Ministry of Education and Science research project within University of Rzeszów PB/ZChTZ/2023

Influence of spermidine supplementation on phenoloxidase system of the European honey bee (*Apis mellifera* L.)

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Abstract:

The health of bees has been an important and current topic in recent decades because, on the one hand, they are of great importance as pollinators, but on the other hand, they are vulnerable and at risk. Various environmental stressors can compromise the immune system of honey bees, making them more susceptible to parasites and pathogens. An important role in the immune response of insects is played by the phenoloxidase system, which is responsible for the melanization of pathogens and damaged tissue. The process of melanization depends on the activation of the enzyme phenoloxidase (PO), which is controlled by the prophenoloxidase (proPO) activation system, which consists of a cascade reaction of serine proteases. The aim of this study was to investigate whether immunity can be improved by dietary supplementation with spermidine, a naturally occurring polyamine with various metabolic functions. Three experimental groups were formed: C - control, fed with 50% (w/v) sucrose as a basic feed solution, S0.1 and S1 - whose diet was supplemented with 0.1 and 1 mM spermidine, respectively. Bees of the same age were fed for 17 days under controlled conditions. The activity of phenoloxidase in its zymogenic (proPO) and active (PO) forms, as well as the relative gene expression of prophenoloxidase (*ProPo*) and phenoloxidase-activating factor 2 (*Ppaf2*) were determined in the abdomen of honey bees. The results show that the addition of spermidine increased the expression of the immunity genes *ProPo* and *Ppaf2* in the abdomen of honey bees fed with 0.1 mM spermidine. Moreover, the measured PO and ProPO activity was significantly higher in both supplemented groups compared to the control. These results suggest a positive effect of spermidine supplementation on the immunity and resistance of honey bees. Further studies should clarify the exact mechanism of spermidine action.

Keywords: dietary supplementation, polyamines, immunity

The authors acknowledge financial support from the Science Fund of the Republic of Serbia (Program IDEAS, Grant No. 7721972, project title: Implication of dietary and endogenous polyamines for the health and longevity of honey bees B-HEALTH) and Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grant No. 451-03-47/2023-01/200125).

Health Benefits Of Bee Products

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Abstract

The number of traditional medicine methods has increased with the development of science. One of these methods is Apitherapy. Apitherapy, which has become one of the traditional medicine methods, is gaining more and more importance day by day. The most used and researched product of apitherapy products is honey. In recent years, propolis and royal jelly have started to take place in daily nutrition thanks to the richness of their nutritional content and health benefits. Royal jelly is the most consumed bee product, which is secreted from the jaw and pharynx glands of young worker bees, contains many biological activities, has high nutritional properties, and is the most important food of the queen bee, which lives for about five years. larvae can consume in a limited way. Royal jelly, which is generally used as a functional food today, is produced in countries such as China, Korea, Taiwan, and Mexico and attracts attention in the cosmetics and medical sectors as well as the food sector. It is known that Royal jelly, which has been known for centuries, is used in religious rituals as well as to strengthen physical performance and memory, increase thinking capacity, aphrodisiac effect, and prolong life. Royal jelly, which is reported to contribute positively to many health problems with its components, is expensive and its use in foods is limited. In study, the effect of royal jelly, which is one of the bee products, on health was examined.

Keywords: Apitherapy, Bee, Royal Jelly, Bee Products

Botanical Origin of Honey Produced in Salto, Uruguay

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Abstract:

Honey bees *Apis mellifera* produce honey based on floral nectar, extrafloral secretions from different parts of plants, or insect excretions. These compounds can be mixed inside the hive, or stored pure if they are abundant. In this case they take the name of honeydew or honeydew honey. The characteristics of the honey are the result of the combination of several factors, such as the composition of the local flora, the presence of sweet juices, the flowering phenology, the selection of species by the collecting bees and the moment of the harvest. Knowing the botanical origin allows a better commercialization because in Uruguay 95% of the honey produced is exported. To authenticate the botanical origin of floral honey and honeydew in this region of Uruguay, a melissopalynological analysis was performed on 40 honey samples, produced in 4 different seasons (n=40). The results show the presence of multifloral honeys with 15 types of pollen that appeared in percentage depending on each season. It was possible to observe in this region, two times of the year where particular honeys were acquired that can be classified as monofloral honeys of *Citrus* sp., of *Eucalyptus* sp. and *Sorghum* sp. The important contribution of native Myrtaceae and *Echium plantagineum* as main or secondary pollen in some hives was also discovered. Studies that characterize the organoleptic and physicochemical properties are necessary to characterize this type of honey, which can be economically valued in a differential way.

Keywords: Honey, Honeydew, *Apis mellifera*, monofloral honey



Assessment of pesticides and antibiotic residues in samples of different honey types from Serbia

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Abstract:

Considering the long tradition of beekeeping, the rich variety of bee products and recognizable quality of honey from Serbia, the aim of this study was to verify that status by checking the presence of antibiotic and pesticide residues. Assessment the occurrence of pesticides and antibiotic residues contamination were done in 50 different honey samples from Serbia – 17 acacia (34%), 13 meadow (26%), 10 multifloral (20%), 6 linden (12%) and 4 forest honey samples (8%). The presence of antibiotic residues in honey was determined by microbiological screening method "Modified method 4 plates". Additional, detection of pesticides (sum of lindanes and endosulfanes) was performed using gas-mass chromatograph (GCMS) and detection of tetracyclines and sulfonamides was done on High Pressure Liquid Chromatograph (HPLC) with UV and FLD detector. All analyzed samples had zones of inhibition lower than 2 mm in width and concentration of sulfonamides, tetracyclines and pesticides were lower than limit of detection (<0,005ug/kg). The obtained results confirmed the status of honey from Serbia as high quality honey, i.e. free from antibiotic and pesticide residues.

Keywords: sulfonamides, tetracyclines, lindane, endosulfane, bee product

[#]This study was funded by Ministry of Science, Technological Development and Innovation of Republic of Serbia by the Contract of implementation and funding of research work of NIV-NS in 2023, Contract No: 451-03-47/2023-01/200031.

Vespa velutina nigrithorax intracolony microsatellite diversity: comparison between nests of the founder and leading edge populations in Portugal

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Abstract:

The invasive yellow-legged hornet, *Vespa velutina nigrithorax*, is a voracious predator of honeybees which has been causing important economic losses to apiculture across Europe. It was first seen in 2004 in France, and it rapidly spread throughout the European countries. In 2011, *V. v. nigrithorax* was first reported in Portugal, in the region of Viana do Castelo, and since then it has been spreading throughout the country, with Bragança representing the edge of its distributional range in northeastern Portugal. The aim of this study was to compare the intra-colony genetic diversity of nests collected Viana do Castelo and Bragança. A total of 120 workers (sterile females) were sampled from two nests from Viana do Castelo and two from Bragança (30 worker per nest). Total DNA was extracted from the thorax of each individual using the Nucleospin® Tissue (Macherey-Nagel). The individuals were genotyped using 16 microsatellite loci divided into three multiplex combinations. The lengths of the fragments were determined using GeneMapper 3.7 (Applied Biosystems). Genetic diversity statistics, which included observed number of alleles (N_a), effective number of alleles (N_e), observed heterozygosity (H_o), expected heterozygosity (H_e), and unbiased expected heterozygosity (uH_e) were estimated using GENALEX 6.5. This software was also implemented to assess genetic structure using a Principal Coordinate Analyses (PCoA). Our results show that both nests from both origins have low genetic diversity, with Bragança showing higher genetic diversity ($N_a=2$; $N_e=1.600$; $H_o=0.427$; $H_e=0.330$; $uH_e=0.333$) than Viana do Castelo ($N_a=1.750$; $N_e=1.482$; $H_o=0.363$; $H_e=0.258$; $uH_e=0.260$). The genetic structure analysis shows that the two nest origins form two distinct groups. These results suggest that yellow-legged hornet population from the region of Bragança may be an expansion from multiple source populations.

Keywords: yellow-legged hornet, population genetics, molecular markers, invasive species

Project “Plano Estratégico de Gestão Integrada da *Vespa velutina* para o Território das Terras de Trás-os-Montes”, com financiamento “POSEUR-03-2215-FC-000164 através do programa Sustentabilidade e Eficiência no Uso de Recursos (Aviso POSEUR-15-2021-02) e Fundo de Coesão”. FCT provided financial support by national funds (FCT/MCTES) to CIMO (UIDB/00690/2020 and UIDP/00690/2020) and SusTEC (LA/P/0007/2021).

Chemical Profile of Northeast Brazilian Propolis

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Abstract:

The interest in the potential of the bee products for treatment and prevention of various diseases is increasing. One of these products is propolis (bee glue), the bee product with highest concentration of specialized plant metabolites and valuable pharmacological activities. It is widely used in the traditional medicine and is available in drug stores as a part of many different products. Thus, elucidating the composition and activity of propolis from different geographical origins is important for its therapeutic efficacy, standardization and control, and is also a possibility to discover new biologically active compounds. The current study aimed to reveal the chemical composition of propolis from Northeastern Brazil in order to confirm *Mimosa tenuiflora* as its primary botanical source, as it is proposed by the local beekeepers. *M. tenuiflora* (Willd.) Poir. (family Fabaceae), also known as “jurema-preta”, is widely distributed in the Northeastern Brazilian Caatinga [2]. This plant species is rich in phenolic compounds (flavonoids and chalcones) and similar chemical profile was found for the analyzed propolis sample by GC-MS after derivatization. Several major compounds were isolated and characterized, among which phenolic compounds and triterpenes, by various chromatographic and spectral methods.

Keywords: Brazilian propolis, *Mimosa tenuiflora*, *Apis mellifera*, flavonoids, chalcones

Acknowledgments: This study is funded by the Ministry of Education and Science through the National Research Program "Young Scientists and Postdoctoral Students - 2", DCM 206 / 07.04.2022. In this investigation GC-MS equipment purchased by Project No BG05M2OP001-1.002-0012, Center of Competence "Sustainable utilization of bio-resources and waste from medicinal and aromatic plants for innovative bioactive products", funded by the Operational Program "Science and Education for Smart Growth" 2014-2020, co-financed by the European Union through the European Regional Development Fund, was used.

Stability evaluation of a bee bread and honey mixture

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Abstract:

Bee bread is a bee product prepared in honeycombs by fermenting bee pollen collected and partially processed by bees. The interest in bee bread is justified by the fact that it is a nutritionally rich food with potential biological activity, often being classified as a functional food. However, due to the difficulty normally associated with its production, beekeepers have not properly valued bee bread. Thus, the main objective of this work was to contribute to bee bread valorization through the formulation of an innovative product consisting of a mixture of bee bread and honey, what could be a good solution for its preservation. The study integrates the evaluation of the mixture stability over a storage period of three months. The combined product was characterized at the time of mixture (T0), after one month (T1) and three months (T3) of storage at room temperature. The analytical procedure focused on the analysis of the physicochemical (water activity, moisture content, pH, ash, protein, fat content and free acidity) and microbiological parameters (aerobic mesophiles, yeast and molds, lactic acid bacteria, spores of sulfite-reducing clostridium, coliforms, *Escherichia coli* and *Staphylococcus aureus*). Regarding the physicochemical characteristics, the results showed that there were no significant differences over time, except for water activity, which increased from 0,54 at T0 to 0,56 after 1 and 3 months of storage, respectively. Considering the low extent of this increase and also that the water activity values were lower than 0.60, it is plausible to assume that, although statistically significant, the increase registered in the water activity values will not have a relevant impact in the mixture stability. Regarding microbiological parameters, only the sulfite-reducing clostridium spore count showed a significant decrease from 0,82 at T0 to 0 after 1 and 3 months of storage. Overall, the results from the physicochemical and microbial analysis evidenced that the mixture of bee bread and honey mixture remained stable during a storage period of 3 months, making this innovative product a feasible approach for simultaneously preserve and add value to bee bread.

Keywords: Beeproducts, stability, preservation, physicochemical and microbiological analysis

Thanks to the Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES (PIDDAC) to CIMO (UIDB/00690/2020 and UIDP/00690/2020) and SusTEC (LA/P/0007/2021). Thanks also to the project GreenHealth, Norte-01-0145-FEDER-000042 and project BeeLand, PRR-C05-i03-I-000081.

The Role Of Royal Jelly In Preventing Kidney Toxicity

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Abstract:

Royal jelly is used worldwide as a cosmetic and dietary supplement. Royal jelly is made from the jaw glands and hypopharyngeal of worker honey bees as a milky secretion. Royal jelly contains proteins, monosaccharides, lipids, fatty acids, minerals, free amino acids and vitamins. Anti-tumor, anti-inflammatory, anti-oxidant, hypoglycemic and hypocholesterolemic effects are considered as the biological benefits of royal jelly. Recently, it has received special attention as a result of studies reporting that royal jelly is a highly effective antioxidant and has the capacity to capture free radicals. The high antioxidant capacity of royal jelly facilitates scavenging of free radicals, lowers nitric oxide levels, and subsequently reduces lipid oxidation and inhibits protein oxidation, as evidenced by the decrease in kidney function parameters. In addition, royal jelly plays an effective role in promoting normal cellular immunity. In addition, in vitro studies and animal experiments have shown that royal jelly inhibits cell proliferation and induces apoptosis in various malignant cells, and affects the production of various chemokines, antioxidants and growth factors and the expression of cancer-associated molecules in cancer patients. It is thought that royal jelly has anti-cancer effects on tumor growth and has protective functions against drug-induced toxicities. It has also been shown that royal jelly is beneficial in suppressing side effects, maintaining routine life comfort during treatment and improving prognosis in patients with malignancy. The nephrotoxic effect of some substances on the kidneys is known and this nephrotoxic effect causes treatment-limiting and restrictive effects. To understand the mechanisms of the beneficial effects of royal jelly, knowledge of the changes that royal jelly induces at the molecular level with respect to cell survival, inflammation, oxidative stress and other cancer-related factors is available. In addition, the effects of combination therapies of royal jelly and other anti-cancer agents or natural compounds have an important place in determining the future fate of royal jelly-based treatment strategies. In this study, studies on the use of royal jelly despite the nephrotoxic effect of some drugs used in the treatment of diseases were compiled.

Keywords: royal jelly; drugs; nephrotoxicity; kidney toxicity



Antioxidant activity of conventionally and organically produced honey

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Abstract:

Organically produced food has become a global trend. Conventionally produced food that includes the use of pesticides and artificial additives is increasingly being replaced by organic food. The aim of this study was to determine whether there are differences in the values of the degree of antioxidant activity between honey produced in a conventional way and by applying organic production. Four botanical types of honey produced in a conventional way and by applying organic production were selected: linden, acacia, chestnut and meadow honey. The antioxidant properties of honey samples were tested using three different tests: ferric reducing power assay (FRP-assay), DPPH radical scavenging activity (DPPH•-assay) and cupric ions (Cu^{2+}) reducing antioxidant capacity assay (CUPRAC-assay). The results obtained using the FRP and CUPRAC- assay are expressed as mg equivalent of ascorbic acid per g of dry sample (mg AAE/gDW), and with the DPPH•-assay, as mg Trolox equivalent per g of dry sample (mg Trolox/gDW). In the examined honey samples, organic meadow honey had the highest ability to reduce Fe^{3+} ions (FRP-assay) 194.4 μg AAE/gDW, while the lowest redox power was recorded in conventional acacia honey 105.1 μg AAE/gDW. Organic linden and chestnut honey had a lower reducing capacity compared to conventional honey of the same botanical species. DPPH•-assay values ranged from 14.6-77.1 μg Trolox/gDW. Organic honey of the tested botanical species, except chestnut honey, had a higher DPPH•- assay value compared to conventional honey. Organic meadow honey had the highest ability to neutralize DPPH• radicals in the tested honey samples (77.1 μg Trolox/gDW), while conventional acacia honey had the lowest ability to neutralize radicals in honey (14.6 μg Trolox/gDW). The antioxidant activity values of the tested honey samples determined by the CUPRAC method ranged from 136.1-217.5 μg AAE/gDW. The highest CUPRAC-assay value was conventional chestnut honey (217.5 μg AAE/gDW), while the lowest value was organic linden honey (136.1 μg AAE/gDW). The obtained results indicated that the organic production of honey can have a positive effect on the antioxidant characteristics of honey, but it was not decisive. The botanical species had a much greater influence on the antioxidant characteristics of the examined samples.

Keywords: FRP-assay, DPPH•- assay, CUPRAC- assay, botanical species of honey

Funding: This work was supported by the Ministry of Science, Technological Development and Innovation of Republic of Serbia Grant No. 451-03-47/2023-01/200116.

Pollen grains lysis: comparison between an Automated vs. a Manual Nucleic Acid Extraction Methods

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Abstract:

Honey is a food widely consumed and appreciated worldwide but also ranks as one of the products most prone to adulteration with mislabeling of botanical origin being one of the most frequent frauds. Botanical origin is still determined by the traditional technique of melissopalynology, which is laborious, requires expert knowledge, and frequently provides only family-level identification. Pollen identification using DNA-based methods would allow for a faster, easier, and more accurate determination. DNA-metabarcoding is currently considered as one of the most promising approaches for this purpose. However, the reliability of using the number of sequences reads to estimate pollen percentages and the accuracy of the qualitative findings still need further evaluation. To this aim, one of the most crucial steps relies in assuring an effective and representative DNA extraction from pollen grains. Therefore, the objective of this work was to compare the success of the lysis step of two extraction methods, namely the Macherey-Nagel NucleoSpin® Plant II Kit (manual method) with the Maxwell® RSC PureFood GMO and Authentication Kit using the Maxwell® RSC extractor (automated method). Thirteen pollen samples of different species selected by their different pollen size were extracted with both methods. On average, a 46x increase in DNA concentration was observed using the automatic extractor, with *Zea mays* pollen recording the largest increase (245x, from 1 ng dsDNA/μL to 249,4 ng dsDNA/μL). Moreover, *Zea mays* and *Fagus sylvatica* (large and small pollen, respectively) were individually observed under the microscope after the lysis step of both extraction methods, and the number of broken and whole pollen grains were counted. Results showed a 70% increase (from 26.9 to 97.3%) in pollen rupture using the automatic extraction kit in *Z. mays*, while *F. sylvatica* increased from 90% to 97.2% rupture. Overall, the Maxwell® RSC PureFood GMO and Authentication Kit proved to be a better option for DNA extraction of pollen samples as it increases pollen lysis allowing to obtain a higher concentration of DNA.

Keywords: pollen, DNA extraction, quantitative method

[#]This work was funded by the project “PRIMA, MEDIBEES: Monitoring the Mediterranean honeybee subspecies and their resilience to climate change for the improvement of sustainable agro-ecosystems.” and project “Mel I.D. - Autenticação da origem botânica do mel: metodologias inovadoras baseadas na análise de DNA do pólen e de compostos voláteis com vista à caracterização e valorização de méis portugueses” financed by Plano Apícola Nacional (PAN 2022-2023). The authors are also grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES (PIDDAC) to CIMO (UIDB/00690/2020 and UIDP/00690/2020) and SusTEC (LA/P/0007/2020), and M. Honrado and A. Quaresma acknowledge the PhD scholarship funded by the FCT (2021.08119.BD and DFA/BD/5155/2020, respectively).

Poster Presentation

Beekeeping and Honey Production in Krgzytan

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Abstract:

Beekeeping holds a central position due to its ecological equilibrium and economic importance. Bee products, serving as essential sources of nutrition worldwide, contribute to natural balance and agricultural success through pollination. Rural communities in developing nations reap the benefits of beekeeping, securing employment, income, and sustenance. Given its multifaceted advantages, beekeeping emerges as a vital component of agriculture. Kyrgyzstan, a landlocked nation bordered by Kazakhstan, Uzbekistan, Tajikistan, and China's Xinjiang region, features extensive high steppes and a mere 7% arable land, resulting in livestock farming overshadowing agriculture. Historical documents in languages such as Chagatai, Uighur, Karakhanid, Khwarazm, Kipchak, and more underscore the utilization of honey for sustenance and healing by Turkic tribes across Central Asia since antiquity. In the early 1990s, Kyrgyzstan boasted a substantial beekeeping presence with 12,000 beekeepers and 446,000 colonies, yielding an annual honey output of 12,000 tons. Once a leading honey and bee product supplier, the dissolution of the USSR presented challenges, leading to a decline in honey production. Presently, Kyrgyzstan maintains 1,000 beekeepers and 90,000 hives. Honey production reached 1,958 tons in 2018, escalated to 2,270 tons in 2019, and 2,422 tons in 2020, with projected production surpassing 3,000 tons by 2023. The "Kyrgyz Beekeepers Union," actively engaged in both national and international events, operates as a member of the global beekeepers' association "Apimondia." Key beekeeping areas encompass Toktogul, Issyk-Kul, At-Bashi, Suusamy, Chui, Talas, and Osh. Escalating demand for bee products drives heightened production of pollen, propolis, royal jelly, and bee bread (perga). Kyrgyzstan's beekeeping industry can thrive through a comprehensive strategy. This encompasses integrating beekeepers into training programs to enhance their knowledge and skills. Moreover, streamlined access to beekeeping supplies and equipment will bolster operational efficiency. Effective evaluation and strategic planning of honey and bee product marketing potential will harness market opportunities. Collaborative endeavors can flourish via the establishment of cooperatives and associations, encouraging the exchange of knowledge and collective growth. Ensuring bee colony health through technical support is imperative. Fostering research and development in beekeeping and bee products will foster innovation and progress. Ultimately, establishing quality standards and certification processes will cultivate consumer trust and ease market access, contributing to a flourishing beekeeping sector.

Keywords: Beekeeping, apiculture, honey, bee products, Kyrgyzstan.

INVITED SPEAKERS

Physicochemical spectra of *Tetragonisca angustula* (Latreille, 1811) honey from Brazil, Colombia, Costa Rica, Ecuador, Guatemala, and Venezuela: Proposal of quality standards

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Abstract:

Tetragonisca angustula is a small Neotropical stingless bee expanding from tropical Mexico to tropical Argentina. It is therefore the most widespread stingless bee and also known to adapt easily in urban landscapes. For these reasons stingless bee keepers have selected *T. angustula* and it is also kept by children, elderly and women because it is a gentle bee producing a delicious honey. In Venezuela it is known as Angelita and Jataí in Brazil. The Slow Food Foundation for Biodiversity Onlus showcases this honey in Arca del Gusto online. The moisture content of *T. angustula* honey is lower than the American *Melipona* and the Asian *Heterotrigona*, but until final adjustments are confirmed, a conservative approach is advised to protect the pot-honey limits. This honey was not included in the proposal of stingless bee honey standards made in Bee World, 2004 for *Melipona*, *Scaptotrigona* and *Trigona* from Guatemala, Mexico, and Venezuela. The objective of this research was to retrieve the quality factors studied for *T. angustula*, and propose preliminary quality standards. After reviewing the literature available for Brazil, Colombia, Costa Rica, Ecuador, Guatemala, and Venezuela, the following limits were proposed for seven quality factors of honey norms: Moisture maximum 30.0 g water/100 g honey, reducing sugars minimum 50.0 g/100 g honey, sucrose maximum 5.0 g/100 g honey, free acidity maximum 70.0 meq/100 g honey, ash maximum 0.5 g/100 g honey, hydroxymethylfurfural maximum 40.0 mg/kg honey, and diastase activity minimum 8.0 DN. Considering the social impact of this honey in Neotropical stingless bee keeping, would be important to provide its honey norm by regulatory authorities and departments of standards of the countries where *T. angustula* honey is produced, consumed and marketed.

Keywords: Neotropical, pot-honey, quality control, standards, *Tetragonisca angustula*

The Immunomodulatory Properties of Honey

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Abstract:

Honey is a natural gift for the human population and it has been used for various purposes in human being's lives. There are many known health benefits of the honey and its products. The main health benefits reported in the literature include anti-diabetic, anti-inflammatory, anti-oxidant and anti-bacterial agent. The main constituent of honey is carbohydrate (approx. 75 %). It is also rich in enzymes, vitamins, minerals, flavonoids and phenolics. Immunomodulation is required to maintain the homeostasis by stimulating or suppressing the immune system which is helpful in fighting against cancer, diabetes and other diseases. Honey and its components have been shown to activate or suppress the release of anti- and pro-inflammatory compounds like cytokines and reactive oxygen species. There are several reports which suggest the positive effect of honey on the activation of both type of immune cells. The polyphenolics present in honey shown to possess promising chemopreventive effect on in-vivo models. The formulations based on honey are also reported to have significant wound healing properties. However, there is significant variation in the immunomodulatory behaviour among honeys of different origins. These studies are still in the preliminary stages and further characterization of honey and its component will enable the use of honey and its components in immunomodulation properties for the treatment of diseases like cancer and diabetes.

Keywords: Flavonoids, Immunomodulation, Inflammation, Honey, Wound healing

Invited Oral Presentation

Bee Venom in the Treatment of OsteoarthritisAli Timucin Atayoglu^{1,2}¹Istanbul Medipol University, Department of Traditional & Complementary Medicine,
Istanbul, Turkiye²Turkish Apitherapy Association, Istanbul, Turkiye**Corresponding author:** atatayoglu@medipol.edu.tr**Abstract:**

Osteoarthritis is a prevalent condition characterized by joint inflammation and cartilage degeneration, posing significant challenges in medical practice. The primary objective of treatment is to alleviate pain, manage symptoms, and slow down the progression of joint damage. However, conventional treatment approaches often fall short in adequately addressing the symptoms and improving the overall well-being of osteoarthritis patients. As a result, complementary therapies, such as bee venom therapy, have gained attention. Bee venom contains bioactive compounds, including melittin and apamin, which have shown anti-inflammatory properties and potential benefits in reducing joint pain and inflammation associated with osteoarthritis. This presentation provides a comprehensive review of the potential benefits, challenges, and practical considerations of using bee venom therapy for osteoarthritis treatment. It also shares the experiences of clinics and research studies that have explored the efficacy, safety, and practical applications of bee venom therapy in managing osteoarthritis. By sharing clinical insights, patient outcomes, and research findings, this presentation aims to contribute to the existing knowledge base and shed light on the potential of bee venom therapy as a complementary therapeutic option for osteoarthritis. It serves as a valuable resource for healthcare professionals seeking to enhance their understanding and explore the use of bee venom therapy in the management of osteoarthritis. Research is warranted to elucidate the mechanisms of action and long-term effects of bee venom therapy, paving the way for advancements in osteoarthritis treatment options.

Keywords: Bee venom therapy, Osteoarthritis, Joint inflammation, Cartilage degeneration, Pain management

The efficacy of high-pressure processing on bee pollen conservation

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Abstract:

Bee Pollen is a natural food product with high nutritional value and several biological properties, namely antioxidant, anti-inflammatory, antibacterial, anti-cancer among others. Due to its nutritional composition, this matrix is widely used and appreciated, consumed alone, as food ingredient or mixed with many other foods. However, storage conditions and processing methods are essential to preserve its nutritional richness and properties. In order to assure the last point, the present work aims to evaluate the applicability of high-pressure processing and follow-up of the preservation of its individual constituents. For this purpose, *Cistus ladanifer* pollen was analysed for 30 and 60 days after being subjected to high-pressure treatment, pasteurisation and drying. To characterise the three conservation processes, pH, moisture content, total lipid content, total nitrogen protein content, ash content, fatty acids profile, total phenolic and flavonoid content, amino acid composition and microbiological determinations were evaluated.

The results showed that the amino acid profile and lipid content is lower most in pasteurised bee pollen, followed by the dried one. The best results for the different parameters analysed were obtained with high-pressure processing, including the microbiological properties and preservation during the studied storage period.

It can be concluded that high-pressure pasteurisation is the most advantageous process for the preservation of this product in order to maintain its nutritional value.

Keywords: Bee Pollen, high-pressure processing, storage, chemical composition.

Acknowledgement

This work is funded by National Funds through the FCT—Foundation for Science and Technology under the Projects: UIDB/00690/2020 (CIMO); UIDB/00239/2020 (CEF) and UIDB/00313/2020 (CQC). This work is also funded by NORTE-01-0247-FEDER-113540 - "Pharmapitox - Desenvolvimento de um coletor inovador e protocolo para purificação da apitoxina para uso nas indústria farmacêutica e cosmética".

Api-phyto-therapy, how does it work: from mechanism to practice

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Abstract:

Herbal medicines have been used since the dawn of civilization to maintain health and to treat diseases. Currently they represent a central component of all alternative medical systems and are becoming even more popular as people prefer the use natural remedies rather than synthetic drugs. To compete with the well-established and highly sophisticated pharmaceutical products, there is an urgency to utilize and scientifically validate and clinically tested herbal and bee products. Therefore, the study of pharmacological and biological effects of herbal-based medicines and apitherapy is becoming more competitive and complex. To compete with the growing pharmaceutical market, there is an urgency to utilize scientifically validated herbal and evidence based api-phytotherapy. Therefore, it is important to establish standardization and regulatory measures that regulate the quality, availability and preservation of these products. Apitherapy is part of the traditional medicine and the science and art of maintaining health based on bee product use. Since, oxidation/peroxidation involving reactive oxygen species (ROS) is an important contributor to the cause of major chronic diseases, there is emphasis on determination of antioxidant activity of bee products, and their protection against ROS-induced damage in human diseases. *In vivo, in vitro* studies of several biomarkers of disease and pharmacological targets reveal the synergistic interaction of the bioactive constituents of api-phytotherapy in favor of their overall protective, preventive, and therapeutic effects, principally as a result of their anti-oxidant/oxygen radical scavenging activity, mainly due to the presence of numerous types of phytochemicals, such as phenols at high concentration. In this presentation , we will provide a comprehensive overview on traditional herbal medicine in association with apitherapy. We will discuss the physiological basis for the use of api-phytotherapy, current clinical uses, as well as the use of Medicinal plants and bee products as good matrices for the search for new biomolecules. The present study focused on the evaluation of the hypoglycemic and the protective properties of two natural products widely used in complementary and alternative medicine (honey, olive oil, pomegranate, *Nigella sativa*). The results obtained showed that their combination protect against metabolic changes and the complications induced by diabetes, and their potential to treat and/or prevent urinary calculus, crystalluria and proteinuria, leading to their use in the management of liver and renal diseases by maintaining the activity of antioxidant defense system. Several studies including our own research provide a potential mechanistic basis for therapeutic effects of bee products, with herbal medicine which in part, are mediated by synergistic action of their bioactive components. Identifying their exact mechanism(s) of action will allow better understanding of their pharmacological properties and promote their wider translation into clinical practice. In conclusion, these data will open up a new perspective for the development of synergistic potential of bee products with herbal products in treating various pathologies and will promote api-phytotherapy in clinical practice, opening up new scenarios in green drug discovery, as well.

Keywords: Herbal medicine; medicinal plants, Diabetes, oxidative stress, api-phytotherapy

Conservation of pollinators for economic development and food security

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Abstract

The current agricultural transformation, once linked to apicultural operations, offers much scope for income generation through beekeeping. Pollinators provide pollination services that are crucial for the productivity of agricultural and natural ecosystems. Honeybees are the major pollinating agents of oilseeds, pulses, fruits and vegetable crops as well as leguminous fodders and several fibre crops. In order to achieve efficient levels of assured pollination input, beekeeping industry needs encouragement to utilize full potential in enhancing crop productivity. Besides, bees contribute to national health through nutritious honey, pollen and its other products. Bees and beekeeping also contribute to the upliftment of rural masses by way of employment generation and as a subsidiary source of income to the farmers. The population of honeybees and other species that pollinate plants life are declining at alarming rate which has threatened the existence of plant life and this downward trend could damage dozens of commercially important crops. Globally, there is a growing need to increase food production. By 2050, the global population is projected to reach approximately 15 billion people. A decline in pollinator populations is one form of global change that actually has credible potential to alter the shape and structure of terrestrial ecosystems. The decline in pollinator population and diversity presents serious threat to agricultural production and conservation and maintenance of biodiversity in many parts of the world. One indicator of the decline in natural insect pollinators is decreasing crop yields and quality despite necessary agronomic inputs. There is a need for discussion in the context of the world scenario on the causes of pollinator decline and future strategies to overcome the impending crisis. Strategies are required for Conservation of pollinators for economic stability and food security.

Keywords: pollinators, food security, economic development

Bio-economics of beekeeping and the supply of honey and pollination services

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Abstract:

The management of honey bees (*Apis Mellifera*), or beekeeping, is shaped by the forage resources and socio-economic context of where it is practiced. Beekeeping operations are very diverse, ranging from small sedentary operations of a handful of colonies to large migratory enterprises moving thousands of colonies over vast expanses. Drivers of beekeeping activity, which can be measured in colony numbers, honey produced, pollination services provided or other metrics, therefore vary widely across operations, locations and time. This heterogeneity in the causes of increases or decreases in populations of managed honey bees has posed a significant challenge for beekeeping research and will continue to do so in the foreseeable future. Yet, some regular patterns can be inferred from the many and increasingly abundant studies published on beekeeping and pollination. Here, I propose a template of beekeeping operation, or model, which is both applicable to many locations and beekeeping contexts and useful to capture the key tradeoffs that beekeepers face. This model incorporates the key beekeeping revenues: honey sales, pollination fees, other hive products (wax, propolis, bee bread, pollen), and stock sales (queens, packages, full or nuclei colonies). The model captures as well as key costs: labor, feed, fuel and equipment (both beekeeping specific such as hive components, and general such as transportation); but also constraints: forage access, weather and diseases. I illustrate how this model can bring insights in some well known case studies (e.g. US pollination markets, honey production in Canada) and discuss how it can be applied to other situations for policy analysis. Finally, I show how this model can be used to generate aggregated supply functions for pollination services and honey production in the case of heterogeneous beekeeping operations.

Keywords: beekeeping economics, representative operations, cost and return of beekeeping, pollination service supply, honey supply, forage.

Hydroxymethylfurfural in Honey Bee Diet

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Abstract:

"Hydroxymethylfurfural (HMF) concentrations in commercially available High-fructose corn syrup (HFCS) from different brands range between 3.1 and 28.7 ppm HMF. HMF is a chemical compound formed from carbohydrates, particularly fructose, under thermal and/or acid-catalyzed degradation conditions. Fructose is only stable to a limited extent in solution and gradually decomposes, forming HMF in a temperature-dependent manner. A temperature of 49°C can result in over 200 ppm HMF formation in 36 days, while exposure of HFCS samples to 69°C for the same duration can lead to HMF values exceeding 30,000 ppm. Honey exposed to 75°C for 24 hours resulted in HMF concentrations ranging from 43.4 to 226 ppm. In our laboratory experiment, we investigated the effects of different concentrations of HMF (100, 500, 1,000, and 1,500 ppm) on the longevity and midgut integrity of worker *Apis mellifera carnica*. We provided bees with standard diets containing these HMF concentrations and examined the impact of HMF on *Nosema ceranae* spore counts in infected honey bees. We observed changes at the cellular level through immunohistochemical analysis of the honey bee midgut. No correlation was found between the concentration of HMF and *N. ceranae* spore counts. Adverse effects of HMF on bees were not observed within the first 15 days of exposure. However, after 15 to 30 days of exposure, HMF caused the death of midgut cells and increased worker honey bee mortality across all treatment groups. Currently, there is no standardized limit for HMF in bee nourishment. Approximately 250 ppm HMF in the honey bee diet is considered toxic. High concentrations of HMF in stored honey could contribute to early bee deaths and the decline of honey bee colonies. Therefore, it is crucial to comprehend the potential adverse effects of elevated HMF doses on honey bees".

Keywords: honey bee, carbohydrates, nutrition, intoxication, toxicology.

This research was funded by the Slovenian Research Agency, Research core funding No. P4-164; Research for improvement of safe food and health and by the project CRISPR-B; number N4-0192; and Research Activity of the Republic of Slovenia under the Contract on Stable Financing of Scientific Research Activity for the Contract Period 2022- 2027, no. S-ZRD/22-27/552.

Can Bee Products Be Antivirals?

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Abstract:

Bee products, like honey, bee pollen, bee bread, royal jelly, and propolis, offer a vast richness of bioactive molecules with potent therapeutic potential, including antibacterial, antifungal, and antiviral activity. Flavonoids (e.g., rutin, naringin, luteolin, and artepillin C) are primarily responsible for the bioactivity of propolis and honey and were shown to inhibit important viral targets, viral-host interactions that trigger the inflammation and cytokine storm, and viral replication cycle. Additionally, various bee products have valuable nutritional potential due to their high sugar, protein, enzyme, amino acids, vitamins, and micronutrient content, making them potentially beneficial in the supportive treatment of viral infections. Moreover, natural honey and honey-containing medications have been used in folk medicine in many parts of the world to treat acute coughs during respiratory tract infections commonly caused by viruses. Antiviral activity of bee products was also confirmed in vitro against some viruses, including herpesviruses, influenza viruses, respiratory syncytial virus, dengue virus, and human immunodeficiency virus. Bee products are also undergoing several clinical studies to evaluate their effectiveness in preventing and treating viral infections. Also, the extracts and formulations of propolis have been studied as adjuvants for bacterial, viral, and parasitic vaccines. The work presented herein aims to critically evaluate the antiviral potential of various bee products, with special emphasis on honey, bee pollen and propolis.

Keywords: antiviral, bee products, virus inhibition, viral enzymes, replication cycle

Bee bread (Perga) from stingless bee: A potential supplement for liver health in obesity

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Abstract:

Obesity is a major risk factor for developing metabolic dysfunction-associated fatty liver disease which is linked with oxidative stress and inflammation. Bee bread has been reported to have antioxidant and anti-inflammatory properties. The objectives of this study, therefore, were to determine the effects of bee bread from stingless bee on body weight, serum lipid and liver profiles, oxidative stress and inflammatory parameters and liver histology in high-fat diet-induced obese rats. Twenty-eight male Sprague-Dawley rats were assigned into four groups (n = 7/group): normal control (NC), high-fat diet (HFD), bee bread (HFD + Bb, HFD + 0.5 g/kg/day bee bread) and orlistat (HFD + Or, HFD + 10 mg/kg/day orlistat) groups. After 12 weeks, the HFD group showed significantly higher body weight gain, serum levels of lipids and liver enzymes. Furthermore, the HFD group showed significantly decreased liver antioxidant enzyme activities, increased liver oxidative stress and inflammation and presence of non-alcoholic steatohepatitis and fibrosis. Bee bread significantly attenuated all these changes induced by HFD. In conclusion, our results suggest that bee bread has potential to be developed as a supplement to protect the liver health in obesity which needs clinical study in future as well as to evaluate its exact molecular mechanism.

Keywords: Bee bread; liver; obesity; oxidative stress; inflammation

Biological properties and nutritional values of bee products

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Abstract:

Bee products are often sold as nutritional supplements and/or health products, with potential anticancer, antimicrobial, antioxidant, anti-nociceptive, and anti-inflammatory activities. Bee products have played major roles in traditional medicine. Secondary metabolites from an immense diversity of bee products such as honey, pollen, propolis, royal jelly or bee venom represent a huge repository of chemical biodiversity, providing a renewable and rewarding source of therapeutic substances. Bee products especially polyphenols have neuroprotective actions via quenching the reactive oxygen species that cause neurotoxicity and aging as well as combating the pathological deposition of misfolded proteins. This presentation will highlight the impact of bee products and its ingredients against neurodegenerative diseases. Furthermore, we will demonstrate the importance of bee pollen and bee bread as vital products of health care. Bee bread is used mainly for their anti-cancer, anti-microbial, anti-fungal effect. Additionally, the antiviral properties of propolis will be discussed as potential therapeutic treatment for COVID-19 disease. 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. However, bee colonies are faced with many challenges that influence their growth, reproduction, and sustainability, particularly climate change. The talk will tackle the contribution of bees to crop pollination and the limitations facing the pollination.

Keywords: Bee products, pharmaceuticals, pollination, climate changes, economy

Apitherapeutic Potentials High Turkish Honeys

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Abstract

Turkey is one of the leading countries in the world in terms of honey production and honey diversity in terms of its geographical location and climate characteristics. Turkey is a bridge between Asia, Europe and Africa and has a rich flora. It also has a rich variety of bee products for reasons such as rich plant diversity, bee breeds and 9 months of the year being suitable for beekeeping activities. In this study, I will give you about apitherapeutic value high Turkish honeys. Honey is a functional food, and most of the bioactive components in its structure come from polyphenols. Our studies show that the higher the number of polyphenols in honey, the higher the antioxidant, antimicrobial and many other biologically active properties of honey. For this reason, in the studies we carried out in honeys belonging to different flora of Turkey, it was determined that the total phenolic substance amounts varied from 10 to 110 mg GAE/100 g. For this reason, it showed that the honeys with high TPC value in Turkey are chestnut honey, oak honey, pine honeys and black cumin honeys.

Keywords: Anatolia, honey, apitherapeutic

Needs for specifying the main reasons for the increasing honey bee colony losses in Bulgaria and grounds for conservation the local Bulgarian honey bee *A. m. rodopica*

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Abstract:

The *Apis mellifera* species has a high sensitivity to environmental pollution and is successfully used for biomonitoring studies in many areas of the world. In recent years the genetic richness of honey bee populations in Europe is threatened by the uncontrolled introduction of genes from other subspecies into the adapted local populations, the stress of a changing environment and the agrochemical pollution, the new pathogens and the global climate changes. By usage of complex approaches the general toxic, cytotoxic, genotoxic and histopathological effect of agrochemicals applied in beekeeping and agriculture and the possible synergistic interactions between them has been analyzed. The standardized international COLOSS questionnaire has been used to study the colony losses rate. The results reveal that for the period 2017 - 2022, the losses of bee colonies in Bulgaria increased from 2.04% to over 21%. During 2022 due to different reasons 1159 (21.28%) of the included in the study 5444 bee colonies have been lost as follows: problems with the queen bee - 172 (3.16%); natural disasters - 9 (0.17%); death or reducing in number to a few hundred bees in the hive - 990 (18.19%). Survey data show that in the areas with the greatest losses, sunflowers, rape, maize, orchards and autumn forage crops are predominantly grown on vast agricultural areas. Beekeepers report that different pesticides have been applied for plant protection in the areas of their apiaries, as well as that oxalic acid, coumaphos, amitraz, tau-fluvalinate are the most frequently applied against Varroaosis.

A toxic effect of the chemicals included in the study has been found both under laboratory and in situ conditions, and the following is generally reported: reduced cell proliferation; various chromosomal aberrations (bridges, acentric fragments, lagging chromosomes, micronuclei and others) in the plant model cells; DNA damage in spermatozoa and histopathological changes in the drone testes from regions in Bulgaria with established pesticide contamination and high level of bee colony losses. The results reveal the need to develop measures for conservation of the national genetic resource of *A. m. rodopica* and to conduct activities for further detailed studies of the risk factors for the honey bees health and viability in Bulgaria.

Keywords: *Apis mellifera*, colony losses, pesticides, cytotoxicity, genotoxicity

Acknowledgments: This study was supported by the National Research Fund of Bulgaria by 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".

Propolis as a multifunctional component and an effective ingredient in cosmetics

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Abstract

The use of natural products in cosmetic formulation as a prophylactic or therapeutic measure has attracted researcher interest. Topical use is the most common way to use natural products not only isolated and identified active compounds but crude extracts also. Propolis is a resinous material of vegetable origin collected by honeybees with recognized pharmacological properties widely used in pharmaceutical and food industries. However, few reports are focused on its potential use in cosmetic. We reported in the present presentation the potential uses and applications of propolis as a multifunctional component and an effective ingredient with special emphasis on its antioxidant, antibacterial, photoprotective and wound healing properties.

Keywords: propolis, cosmetic, multifunctional component, effective ingredient, pharmacological properties.

Melissopalynological spectrum of honey from native bees of Mexico

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Abstract:

The present work includes two systematic melissopalynological studies carried out on stingless bees from Mexico in order to analyze the pollen spectra of pot pollen and pot honey of *Plebeia* sp. in Chiapas, and the spectra of pot honey of *Scaptotrigona mexicana* in the state of Puebla. All samples were chemically processed according to Erdtman's technique. The first work was carried out in Unión Juárez (UJ) and Santa Teresita (ST), near the city of Tapachula, in the state of Chiapas, southeastern Mexico, 24 samples were collected in UJ and 18 in ST. In UJ, 118 pollen types belonging to 40 botanical families were found. On the other hand, a total of 92 pollen types belonging to 36 botanical families were found in ST. Among the families with the highest species richness in *Plebeia* samples, the following stand out: Arecaceae, Asteraceae, Euphorbiaceae, Fabaceae, Malpighiaceae, Moraceae, Rubiaceae, Solanaceae and Ulmaceae. In general, at both sites arboreal plants were very important, followed by shrubs and herbaceous plants. Although *Plebeia* is a polylectic bee, only one to four plant species became intensively exploited. In addition, this stingless bee is adapted to disturbed ecosystems, yet it incorporates a great diversity of native plants from secondary vegetation into its diet. The second work was focused on analyzing the pollen spectra of honey produced by *Scaptotrigona mexicana* inhabiting the secondary vegetation of the tropical evergreen and cloud forest of Cuetzalan del Progreso, Puebla, in order to know the plants of nectar or pollen interest, which probably confer curative properties, and the foraging strategies of this stingless bee are analyzed. Melissopalynological results show that native bees visit elements of the original or secondary vegetation, both in the low evergreen forest and in the montane cloud forest. The healing properties of stingless bee honeys may be due to multiple causes. The first one is related to the plant species they visit to produce honey, such as *Heliocarpus appendiculatus*, *Pimenta dioica*, *Bursera simaruba*, *Psidium guajava*, among others, that have curative properties. It is likely that the exploitation of different resources through an annual cycle is a factor that defines its curative potential as well as the microbiota that contains this type of honey and finally its particular physicochemical composition. It is important to note that in general the honey of native bees becomes very rich in pollen, due to the extraction methods, so it is convenient to talk about the pollen spectrum instead of the melissopalynological characterization.

Keywords: stingless bees, melissopalynology, *Plebeia*, *Scaptotrigona mexicana*, Mexico.

An effective product in the treatment of obesity: Perga

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Abstract:

Adequate and balanced nutrition constitutes one of the most important prevention strategies in the prevention of non-communicable diseases, which are among the leading causes of death all over the world. In addition, obesity is one of the protective factors that play a role in minimizing nutritional and health problems such as malnutrition, vitamin and mineral deficiencies. However, nutrition, which is necessary for the protection and development of the health of individuals and societies and for increasing the quality of life, is an action that must be done consciously. In order to provide adequate and balanced nutrition, the energy and nutrients needed; It should be met in the required amounts according to the age, gender, physiological characteristics, physical activity status and genetic structure of the individual. Therefore, nutrition is unique to the individual. In addition, a healthy diet should be based on food variety. While meeting the energy and nutritional needs, it is recommended to consume foods with different colors, types and contents instead of a monotonous diet. This study aims to summarize current scientific knowledge on bee bread and its effect on obesity.

Keywords: bee bread, perga, obesity, leptin, ghrelin

Influence of different storage conditions on antibacterial activity of propolis

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Abstract:

Propolis exhibits antibacterial activity against *Streptococcus mutans*, therefore, it may bring beneficial impact in dentistry and oral health management. Potentially, propolis may be used in the development of dosage forms recommended for oral cavity use. The research of propolis decomposition demonstrates the stability of this natural product which contains over 250 compounds. Thermal analyses are crucial for assessing the impact of physical conditions on biological activity of propolis. The aim of the study was to evaluate the antibacterial effect of Polish propolis against cariogenic bacteria and stable as well as susceptible of propolis to thermal degradation. Physical conditions are supposed to cause the deactivation of biologically active substances responsible for the antibacterial effect of propolis, therefore we evaluated antimicrobial analysis for EEP, as well as propolis after storage under stress conditions. In a conducted study *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus mitis*, and *Streptococcus oralis* were used. Determination of the Minimum Inhibitory Concentration (MIC), as well as the Minimum Bactericidal Concentration (MBC) of ethanolic extract of propolis, was evaluated. The thermal stability of propolis was determined by thermogravimetric analysis (TGA). Our findings demonstrate that, prepared ethanolic extract of propolis at range 25 - 100 µg/mL showed *in vitro* antibacterial activity for selected strains. The value of MIC was at range 5 µg/mL - 50 µg/mL EEP for tested strains, while MBC was 50 µg/mL - 100 µg/mL EEP for tested strains. The results of thermal decomposition behavior confirm that the mixture of organic components contained in the tested propolis is decomposed in three stages. The first stage began from 67°C and ended at 153 °C and was connected with weight loss of 3.14%. During this step we observed small weight, probably mass change was associated with loss of volatile compounds. The second step starts from 153°C and ends at 236 and was connected with small weight loss of 16.21%. The third has started from 236 and ends at 491°C exhibited maximum mass change about 50.30 %. The influence of different storage conditions on antibacterial effect of propolis is important to create the new formulation of propolis for the oral cavity.

Keywords: propolis, antibacterial activity, thermal degradation, thermal stability

Invited Oral Presentation

Effects of feed supplements on *Nosema* spp. infection level and gut microbiota of honeybees (*Apis mellifera*)

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Abstract:

Honeybee colonies (*Apis mellifera* L.) represent the most important social insects due to their crucial role in plant pollination and close link to global food production and natural biodiversity balance maintaining. Their specific nutrition physiology and flight behavior patterns show complex interactions with environmental ecosystems. Bacterial symbionts in the honeybee gut have important functions for host nutrition, food digestion, metabolism, development, weight gain, reproduction, behavioral physiology, and immunity through pathogen and insecticide resistance. Dysbiosis in honeybees is often defined as gut-intestinal microbial imbalance linked to a host deficiency such as deficient development, lower body mass, earlier workers mortality, and in general health, metabolism, and fitness status when the different environmental influences (e.g., immune response suppression caused by oxidative stress or *Nosema* spp. infections) could change the composition of gut bacterial phylotypes and remaining microbiome components, leading to the appearance of visible clinical signs of opportunistic diseases and colonies weakening. To facilitate full therapeutic success there is a need for the appliance of bio-inspired honeybee colony protection products in the form of feed supplements and novel technology designs, based on natural ingredients active against microsporidia pathogens. The other possibility is to trigger honeybees' immune defense responses. The aim of this study was to evaluate the therapeutic effects of EM for BEES as additional food for bees on *Nosema* spp. infection levels and relation to honeybee colony strength and the gut microbiota composition obtained using NGS analyses. If the changes in the gut microbiome are supported by implemented feed supplementation, it may be possible for this beekeeping management practice to reduce the negative effect of environmental xenobiotics on honeybees.

Keywords: honeybee colony; gut microbiota, *Nosema* spp.; EM for BEES; colony strength; NGS

Anticancer Activity of Bee Venom and Components against Brain Cancer

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Abstract:

Cancer continues to be one of the most important health problems threatening human health. Recently, methods such as surgical methods, chemotherapy, radiotherapy, immunotherapy, cancer vaccines are used in the treatment of cancer. However, the fact that these treatment methods, especially chemotherapy, cause some side effects, and thus the interest in the finding new natural products that can be applied in the cancer treatment has increased. Melittin, one of the peptide components of bee venom, is one of the most important active ingredients used in the cancer treatment. Glioblastoma (GBM) is a highly aggressive type of brain tumor arising from glial cells in the brain. Glioblastoma is usually treated with surgical resection, concurrent radiotherapy, and chemotherapy. While the disease tends to relapse after these treatments, it significantly reduces survival in patients. Therefore, new and natural approaches are needed. In response to this need, honeybee venom, mellitin and the two-dimensional (2D) nanomaterial (2D black phosphorus, phosphorene) were used in this study. The effective dose for bee venom, mellitine, apamin and their combination for the treatment of glioblastoma were determined, and the potential for mellitin combined with phosphorene therapy was revealed, as well as invitro efficiency tests were carried out. In the light of these findings, a therapy protocol using phosphorene in the treatment of glioblastoma was developed in the preclinical stage for the first time in our study, and also first time in the literature, a nanomaterial that can be combined with mellitine has been created and tested against to glioblastoma tumor cell. As a results, the anti-cancer efficacy of melittin combined to nanometarial was pre-clinically demonstrated

Keywords: Cancer, Bee Venom, Melittin, Nanotechnology

Invited Oral Presentation

Applications To Increase Productivity By Population Control In Honey Bee (*Apis mellifera* L.) Colonies

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Abstract:

Beekeeping, honeybee (*Apis mellifera* L.) colonies during the periods of abundant nectar flow, maximizing the adult bee populations and the production of bee products such as honey, beeswax, royal jelly, propolis, bee venom, queen bee, swarm and package bee. It is an activity carried out for the purpose of using and managing plants for pollination. Success in beekeeping is possible by knowing and fulfilling the technical beekeeping conditions. Technical beekeeping is a beekeeping model in which all the possibilities of modern technology are used and as a result, it provides the producer with as much profit as possible above the economic efficiency level. Factors that play a role in reaching this level; strong, healthy colonies of suitable bee breeds, modern equipment, rich nectar resources, availability of natural conditions and sufficient knowledge and experience in beekeeping. Population regulation should be done correctly and on time to increase productivity in colonies. For this, it is necessary to know the life cycle and life-related characteristics of the colony individuals well. Population control techniques present innovative solutions for enhancing honey bee colony productivity. By strategically managing worker bees, drones, and queen bees, beekeepers can optimize resource allocation, improve foraging efficiency, and promote colony health. These applications have the potential to address challenges faced by beekeepers, such as colony losses and reduced pollination services, contributing to the sustainability of both beekeeping and global agriculture. As honey bee populations continue to face threats, the exploration and refinement of population control strategies offer a valuable tool to support pollinator health and productivity.

Keywords: Honeybee, Queen bee, Colony Management Techniques, Population Regulation, Population Monitoring

The Effect of Apilarnil on Infertility

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Abstract:

For centuries, honeybee products such as honey, bee pollen, royal jelly, bee bread (Perga), and bee venom have been utilized in natural medicine due to their beneficial properties. A great deal of scientific research has been dedicated to exploring their physico-chemical properties and therapeutic effects. Despite this, drone larvae have not received as much attention from the scientific community. Within a honeybee colony, drones are responsible solely for fertilizing queen bee eggs and consuming food reserves collected by worker honeybees. As a result, beekeepers commonly remove excess drone brood from the hive, which is crucial for preventing and treating varroasis. Lyophilization is the most effective method for preserving drone larvae, and the physicochemical properties of fresh and lyophilized drone larvae were compared. The therapeutic effects of drone larvae, such as androgenic, hepatoprotective, immunostimulatory, and hypolipidemic effects in humans and experimental animals, were summarized. This study aims to summarize current scientific knowledge on drone larvae (apilarnil) and its effect on infertility.

Keywords: drone larvae, apilarnil, drone brood, infertility.

Honey Beeflora in Asia: A way forward to Explore potential plant Diversity

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Abstract

Asia is one of the important and richest hotspot of Honey Beeflora in the world. In this region Pakistan, China, India, Russia, Turkey hosted diverse ranges of plant diversity for honey production. This study confined to pollen, nectar and honey forage by diversity of bees around the Asia. Field trips were arranged to collect pollen samples of Beeflora. The samples were studied using light microscopy and SEM. The botanical origin of various honey samples were characterized to identify diversity of Beeflora. Monfloral and polyfloral Honey based on Beeflora were analysed. In total of 57 most common plants species were identified which belong to 30 flowering families. The most common wild species includes Acacia species, Zizyphus species, Asteraceous species, Prunus species etc. The honeybee pollen varies in shape and structure from psilate to echinate type, spheroidal to ovate, monoporate to polyporate types. It is stated that pollen attributes may provide evidences for correct identification of plants visited by honey bee. It is further suggested to extend this study for biochemical and biophysical analysis of honey bee samples to small scale industries for socio-economic development of the area.

Keywords: Asia, Pakistan, Turkey, China, Beeflora, Honey.

Invited Oral Presentation

Fluorescence spectroscopy - a promising tool for honey authentication and adulteration detection

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Fluorescence spectroscopy has great potential in analysis of food products which contain a variety of fluorescent components and presents several advantages compared to other techniques related to rapidity, sensitivity, accuracy, and selectivity. The technique is a non-destructive one and is relatively cheaper. In recent years, there is an increasing number of papers related to the use of fluorescence spectroscopy for the authentication of food or adulteration detection. One of the techniques used is the 3D fluorescence spectra, which appears as a landscape, which is an excitation-emission matrix (EEM) of the analyzed sample. The EEM is obtained by measuring the emission of the sample at different wavelengths by applying different excitation wavelengths. The EEM offers information regarding the fluorescence of a sample, which corresponds to the chemical composition of the sample and can uniquely characterize a specific kind of food. Honey presents a specific EEM according to the botanical origin, generated by the intrinsic fluorophores, which correspond to the aromatic amino acids, phenolic compounds or Maillard products. The addition of different adulteration agents may change the specific EEM profile of honey, and this technique may be used for the adulteration detection.

Keywords: Fluorescence spectroscopy, honey, authentication

Unveiling the Power of Pollen and Bee Bread. Exploring New Functionalities

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Abstract:

The beehive is an enormous source of natural products, most of them still underexplored. There is no doubt of the nutritional potential of honey, pollen, beebread or even bee larvae, and the benefits of apitoxin, propolis, or royal jelly to human health, but we may find many other potentialities of bee products outside these approaches. Pollen and bee bread, collected by bees when visiting flowering plants, are a great source of nutrients. Rich in proteins, amino acids, vitamins, and minerals, their consumption can enhance energy levels and support digestion. The enzymatic process occurring during bee bread production enhances its bioavailability and probiotic properties. This potential can be explored in today's food tendencies, particularly considering the consumer's appetite for functional foods. Here we show some possibilities such as the addition of those bee products towards traditional bread recipes or energetic bars and discuss the gain in the bioactivity of the enriched products. Another completely different approach is to explore pollen as a new biomaterial, taking advantage of the physical and chemical characteristics of the pollen shell. The Sporopollenin structure of the pollen shell has emerged as an alternative to synthetic capsules due to their low-cost, highly uniform size distribution, resistance to physical and chemical conditions, and renewable green source. Besides, the high diversity of structures that we can find among pollen from different plants, allows us to search for better's morphologic characteristics accordingly to the goal. As biocapsules, pollen shells can be engineered as selective molecular carriers, as functionalized protective structures and as guided carriers towards a specific target where the released molecule may fully be explored for its properties.

Keywords: Pollen; bee bread; functional food; biocapsules.

Thanks to the Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES (PIDDAC) to CIMO (UIDB/00690/2020 and UIDP/00690/2020) and SusTEC (LA/P/0007/2021). Thanks also to the project GreenHealth, Norte-01-0145-FEDER-000042

Invited Oral Presentation

Stingless bee products from the Soconusco, Chiapas, Mexico

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Abstract:

Stingless bees are highly appreciated since ancient times and also for new generations because of their ecological services and their nest products. Nowadays there are several studies related to elucidate the antimicrobial and antioxidant properties of stingless bee products, such as honey, pollen and propolis. In the Soconusco region we are performing stingless bee nest products studies in order to elucidate their characteristics and properties. Recent studies are focusing on their alternative uses in agriculture, such as pollinator attractants or as flower and fruit set promoters. In the Soconusco region, Chiapas Mexico, we are performing studies focused to protect the agroecosystem biodiversity by using a natural adherent such as *Melipona solani* honey. We presented a five year study advances in mango and rambutan crops, managed with *Allium sativum* aqueous extracts and *M. solani* honey as adherent; this aqueous extract promotes the emission of vegetative and floral shoots, increases flower development, works as an attractant for pollinators, promotes fruit set, stimulates fruit growth, acts as an insecticide to control thrips and mealybugs and stimulates the production of defense metabolites, such as polyphenol compounds. Therefore, the use of stingless bee honey as an adherent could be a key to potentiate the function of its components in leaves, panicles, flowers and fruits.

Keywords: Honey, propolis, pollen, stingless bee honey, aqueous extract, potentiate

Trehalulose as Biomarker for Stingless Bee Honey

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Absract:

Stingless bee honey has been used as traditional medicine, with records going up to hundreds of years in countries worldwide. Although there were many publications on the therapeutic properties, including anti-diabetic activity and pancreas-protective effects, only a few studies have identified the bioactive marker for these effects, and the description of the mechanisms involved is very scarce. The vast diversity of stingless bees worldwide has also given rise to unique byproduct properties, especially in honey and propolis bioactive compositions. Previously, phytochemical substances, including carbohydrates, phenolic compounds, organic acids and alkaloids; biomacromolecular substances, such as proteins and DNA; and melissopalynology methods have been used to characterize honey's origin, authenticity and biological activities. The discovery of trehalulose as one of the main sugars in stingless bee honey by Fletcher and colleagues (2020) has increased the interest in the biological effects of this low-glycemic index sugar and its function as a biomarker. However, the consistency of the trehalulose presence in stingless bee honey must be confirmed to elucidate the biological mechanisms further. Our study sampled honey from the stingless bee species *Heterotrigona itama* and *Geniotrigona thoracica* reared in Selangor, Malaysia, for three consecutive years. The species *G. thoracica* was found to consistently produce a higher amount of trehalulose than the species *H. itama* for all three years. However, the *H. itama* had a higher amount of total flavonoid, total phenolic and ferric-reducing antioxidant power than the *G. thoracica* species. We have also isolated the trehalulose using a patent-pending ion exchange chromatography technique to study the safety of the trehalulose further. No toxicity was observed in the in-vitro toxicity study of isolated trehalulose using HepG2 liver cells and L6 muscle cell lines at 24 hours, 48 hours and 72 hours of treatment time length. Using an in vivo study using white Sprague-Dawley rats, we have observed no acute oral toxicity effects of 15% trehalulose and the glycaemic response at 4g/kg body weight was found to have induced significantly lower glycaemic response compared to groups treated with sucrose or trehalose. Thus, initially, trehalulose was found to be a potential biomarker for improving glucose response, and more studies should be conducted to study its biological pathway for this biological response.

Keywords: Trehalulose, biomarker, stingless bee Honey

Microbiological Quality Of Honey Harvested From Hives With Use Of Natural Predators (Mites, Book Scorpions) In The Struggle Against *Varroa*

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Abstract:

Some beekeepers apply as part of natural beekeeping book scorpions (*Chelifer concroides*) and predatory mites (*Stratiolaelaps scimitus*) to control *Varroa destructor*. These scorpions and predatory mites are kept in compost below the beehive. There are potential hazards related to the use of compost so close to the bee hive for the microbiological quality and food safety of the honey like the possible presence of *Clostridium botulinum* (could cause infant botulism), *Clostridium perfringens* (could cause food poisoning), *Listeria monocytogenes* (causes the food-borne illness listeriosis), osmophilic yeasts and xenophilic moulds (spoilage of honey), and mycotoxins produced by *Aspergillus fumigatus* and other moulds. To study this issue, honey was sampled directly from a frame with a sterile spoon and collected in a sterile recipient. Samples were taken from 18 hives with compost ('natural' beekeeping) and from 10 hives with conventional beekeeping. The samples were analysed on total bacterial count, osmophilic yeasts, xerophilic moulds, *Listeria monocytogenes*, *Clostridium botulinum* and *C. perfringens* and a part of the samples also on mycotoxins. In one upon 18 samples ('natural' beekeeping) the total bacterial count was higher than the criterion of 3×10^6 cfu/g. Regarding osmophilic yeasts, in 8 upon 18 honey samples of 'natural' beekeeping and in 4 upon 10 honey samples of conventional beekeeping the tolerance of 300 cfu/g was exceeded. In two of these samples, both of 'natural' beekeeping, even the criterion of 3000 cfu/g at expiry date was exceeded. All honey samples ('natural' and conventional beekeeping) were in line with the tolerance for xerophilic moulds. All honey samples were negative for *Clostridium botulinum* en *Listeria monocytogenes*. All 18 'natural' beekeeping samples were also free from *Clostridium perfringens*. In 2 out of 10 samples from conventional beekeeping, presence of *Clostridium perfringens* was stated; in both cases it concerns an enterotoxinogenic type A strain. The 10 honey samples ('natural' beekeeping) were free from mycotoxins.

Keywords: honey, microbiological quality, natural beekeeping, food safety

Reference

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Importance of Bioactive Compounds Determination in Antioxidant and Antibacterial Activity of Honey and Propolis

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Abstract

It is known that the nutritive value of a food product is given by the content in sugars, lipids, proteins. With these parameters the nutritional or energetic value is calculated. The most important substances with biologically active value from bee products are by far the polyphenols and natural pigments. They are responsible in most part of the biological activity (antioxidant, antibacterial, anticarcinogenic, etc.). Honey is first a natural food product, with high nutritive values, but also is used as ingredient in different sectors, such as food industry, cosmetic industry, pharmaceuticals, as well as in medicine (apitherapy in special), being recognized since long time in history. Honey protects the cardiovascular system, the nervous system, the respiratory system and also the gastrointestinal system. How it is protected? By honey's the antioxidant, antidiabetic, anticarcinogenic and the most important, antiparasitic, antimicrobial and antiviral activities. Because Romania have a large diversity of landscape, vegetation, and flowers, different honey types are produced here, such as: rape, acacia, multifloral, linden, sun flower, raspberry, honeydew, buckwheat, heather and others. When we talk about honey benefits, we must refer to certain honey types. In Romania different types of honey possess very good bioactive properties, but the marketing and money for profound studies are almost missing. Few Romanian honeys with very good properties, such as antibacterial, antioxidant or antiviral, are sunflower honey (*Helianthus annuus*), heather honey (*Calluna vulgaris*), honeydew honey, buckwheat honey (*Fagopyrum esculentum*) and japanese knotweed honey (*Fallopia japonica*). Heather honey is produced in different parts of Europe, Great Britain also, and in Romania is characteristic to Apuseni Mountains from Transilvania. Heather honey has a specific characteristic, it has a gelling appearance, it does not flow, even if it is fluid, not crystallized. Honeydew honey is an extrafloral honey, which is made from different sweet substances that bees collect from the leaves of different trees, from fir needles, substances that are excreted by aphides. It is a dark colour honey, with high content of minerals and also other types of bioactive substances, very nutritive, but also with high antioxidant, antibacterial potential. A barely unknown honey type is japanese knotweed honey. This type of honey is unusual both for its dark, almost black color and for its special, caramel taste. The plant from which the bee produces this honey it is known to be invasive, blooming from mid-August until the first cooler temperatures (september-october). It grows, in particular, along the courses of rivers and valleys, in Romania mostly on western and northern part of the country. Knotweed is a plant that contains a concentrated source of resveratrol, a known antioxidant that has several health benefits. The dark color of honey is due to different pigments that are represented by antioxidants with beneficial effects for humans and that stimulate the immune system. In the literature it is mentioned this type of honey only in United States, mainly because in Europe there are many eradicating programs for this invasive plant, which is highly dangerous for infrastructure or buildings. Fallopia honey as well as honeydew honey possess very high amount of potassium and calcium compared to other honeys. For the determination of polyphenols using HPLC technique we identify the presence of different phenolic acids such as: caffeic, p-cumaric, ferulic acids and different flavonoids: pinocembrin, apigenin, kaemferol, crisin, galangin and others, found in different amounts in different honey types, showing that every honey is different, have different properties aside the nutritional properties. The antioxidant activity in vitro was determined for these honeys and higher percentages of inhibition were obtained. Also antibacterial activity against gram positive and gram negative bacteria was determined and very good results were obtained. Propolis have a more "simple" composition, compared to honey or other bee products. It is known as the most valuable natural antibiotic, have proved to have antioxidant, antitumoral, antifungal, antibacterial, anti-

inflammatory and the most important in the current situation, ANTIVIRAL properties. The chemical composition comprises of 30-50% wax and resins from different vegetal sources such as: poplar, fir, spruce, willow, oak. Other components are balms, resinous compounds, essential oils. Propolis is generally used as a tincture, which is made by soaking in alcohol of various concentrations. Depending on the botanical origin of the harvested resins, propolis has different colors. Studies on the chemical composition of propolis have shown the presence of quercetin in high amounts, but also other phenolic acids and flavonoids. The antimicrobial properties of propolis are evaluated *in vitro* towards both reference and clinical strains of *Staphylococcus aureus*, *Staphylococcus pseudintermedius*, *Bacillus cereus*, using the disc diffusion assay. In conclusion, higher bioactivity of honey is related to the darkness of the colour. In order to use honey for specific purposes, detailed chemical composition including phenolic profile and volatile profile is needed. Propolis is a rich source of bioactive compounds, being recognized as the most powerful natural antibiotic, antibacterial and antiviral product, due to different polyphenols from the chemical composition.

Keywords: honey, propolis, antioxidant and antibacterial activity, phenolics