



# 6<sup>TH</sup> APIMEDICA

# 5<sup>TH</sup> APIQUALITY



Istituto Zooprofilattico Sperimentale  
del Lazio e della Toscana M. Aleandri



INTERNATIONAL  
SYMPOSIUM  
*Final Programme*

ROME  
22-25 NOVEMBER 2016  
NOVOTEL - ROMA EUR

UNDER THE AUSPICES OF

**mipaaf**

Ministero delle  
politiche agricole  
alimentari e forestali



REGIONE  
LAZIO

REGIONE



TOSCANA



# 6<sup>TH</sup> APIMEDICA & 5<sup>TH</sup> APIQUALITY

*The Apiquality & Apimedica International Symposium 2016 is organized by the Apiculture Unit of the Istituto Zooprofilattico Sperimentale del Lazio e Toscana "M. Aleandri". This event aims to create direct connections between the world of scientific research and practical beekeeping through the exchange of information and knowledge.*

## **SCIENTIFIC COMMITTEE**

*Etienne Bruneau*

*Giovanni Formato*

*Cristina Mateescu*

INTERNATIONAL SYMPOSIUM  
ROME, 22-25 NOVEMBER 2016

### ***Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "Mariano Aleandri" (IZSLT)***

The IZSLT is an Italian government laboratory operating in the frame of the National Health Service with duties related to animal health and food safety. It provides services in the diagnosis of animal diseases and zoonoses, microbiological, chemical and physical controls over safety of foods and animal feed. In the same areas, it performs research, epidemiological surveillance, continuous training and international cooperation activities. In the frame of IZSLT activities, the Apiculture Unit's mission is to certify and guarantee the health and welfare of bees, the hygiene and safety of the hive products. Among the services offered we find the diagnosis of bee diseases and analyses on hive products, support to Governmental Institutions in drawing up legislation, research activity, collaboration with other laboratories or institutions, technical support and training for operators and pollution environmental monitoring using honey bees.

Contact person

**Dr. Giovanni Formato**  
*Head of Apiculture Unit*  
[giovanni.formato@izslt.it](mailto:giovanni.formato@izslt.it)

## SPEAKERS

### **Apimondia**

Apimondia, the International Federation of Beekeepers' Associations, was founded in 1895 and it is the world organisation representing the interests of apiculture. It aims to facilitate links between beekeepers, scientists and all involved with apiculture through the organization of international symposia and congresses. Apimondia is run on a basis of co-operation between beekeepers and scientists from many countries as well as international organizations. A special feature of Apimondia is the wide range of working languages used in publications and at meetings.

Contact person

#### **Riccardo Jannoni-Sebastianini**

Apimondia Secretary General  
apimondia@mclink.it

### **Apiquality**

Apiquality is the Apimondia event addressing issues related to the qualitative aspects of bee products, including their production, transformation, storage processes and food safety with a view of their application in the medical sector.

### **Apimedica**

Apimedica is the Apimondia event addressing topics on apitherapy a branch of alternative medicine that uses honey bees and honey bee products including honey, pollen, bee bread, propolis, royal jelly and bee venom to take care of human, animal and plant health.

#### **CRISTINA AOSAN**

Apitherapy Scientific Commission of  
Apimondia, Romania

#### **ETIENNE BRUNEAU**

President of the Apimondia Scientific  
Commission on Beekeeping  
Technology and Quality, Belgium

#### **ARISTIDE COLONNA**

President of the Italian apitherapy  
association, Italy

#### **ANTONIO COUTO**

Apitherapy practitioner, Portugal

#### **CARLO D'ASCENZI**

University of Pisa, Italy

#### **GIOVANNI FORMATO**

Apiculture Unit, IZSLT, Italy

#### **CHRISTINA KAST**

Agroscope, Switzerland

#### **MARÍA ALEJANDRA LÓPEZ PAZOS**

Veterinary Doctor; Apitherapist, MTCh  
(Traditional Chinese Medicine), Chile

#### **GIAN LUIGI MARCAZZAN**

CREA-API, Italy

#### **CRISTINA MATEESCU**

President of the Apitherapy Scientific  
Commission of Apimondia, Romania

#### **ALESSIA MENEGOTTO**

CG Scientific Director, DVM, Italy

#### **MARCELLA MILITO**

Apiculture Unit, IZSLT, Italy

#### **MICHELE MORTARINO**

University of Milan, Italy

#### **PEDRO PÉREZ GÓMEZ**

Professor at the UAH - Alcalá de Henares  
University, Spain

#### **MARCO PIETROPAOLI**

Apiculture Unit, IZSLT, Italy

22 NOVEMBER 2016  
APIQUALITY

07.45 Registration and set-up of poster exhibition

08.30 Welcome

*On. A. Olivero (ViceMinister MIPAAF)*

*P. Sacco (Head Policy Planning Unit, Foreign Affairs Ministry)*

SESSION

**APIQUALITY: BEST PRACTICES AND HONEY BEE HEALTH**

*Chair: E. Bruneau (Belgium)*

08.45 **Observation of nature, a key to produce apimedica quality products**

*E. Bruneau (Belgium)*

09.25 Seasonal strategies in honeybee pollen production

*P. Fontana (Italy)*

09.50 Training activities for the development of the apiculture sector

*A. Bozzano (Italy)*

10.15 Quality focused apisector development in Ethiopia

*N. Bekena (Ethiopia)*

10.40 **COFFEE BREAK**

SESSION

**RESIDUES IN HIVE PRODUCTS**

*Chairs: C. D'Ascenzi (Italy), M. Mortarino, (Italy)*

11.10 **Opportunities to reduce the in-hive impact of acaricide treatments**

*M. Mortarino (Italy)*

11.35 Honey and bee products characterization of the Majella

National Park

*G. Ciaschetti (Italy)*

22 NOVEMBER 2016  
APIQUALITY

11.50 Chemicals compounds in beeswax samples collected during the years 2013 - 2015

*S.M.R. Tulini (Italy)*

12.05 Residues of pesticides in honey

*S. Alapala Demirhan (Turkey)*

12.20 **Study on risk assessment: pesticides residues in bee pollen**

*C. D'Ascenzi (Italy)*

13.00 **LUNCH**

SESSION

**FRAUDS**

*Chair: E. Bruneau (Belgium)*

14.00 **Adulteration of bee products: current situation and perspectives**

*E. Bruneau (Belgium)*

14:25 Genuine manuka honey

*N. Beitlich (Germany)*

14:50 Beeswax adulteration issue: aspects of contamination and outcome

*L. Svecnjak (Croatia)*

SESSION

**ALLERGENS AND TOXINS**

*Chair: C. Kast*

15.05 **Pyrrolizidine alkaloids and other plant toxins in honey and pollen**

*C. Kast (Switzerland)*

15.30 Development and validation of a quechers method coupled to HRMS detection to determine pyrrolizidine and tropane alkaloids in honey

*M. Martinello (Italy)*

22 NOVEMBER 2016  
APIQUALITY

- 16.00 COFFEE BREAK
- 16.30 **Practical training**  
**Pre-clinic indicators as Good Beekeeping Practices: sampling methods and new traceability systems**  
- a check-list to verify best practices application  
- sampling methods and pre-clinic practices  
- innovative traceability systems”  
*G. Formato, M. Pietropaoli (Italy)*
- 17.00 *Discussion*

23 NOVEMBER 2016  
APIQUALITY

SESSION

STANDARDS AND CHARACTERIZATION OF BEE PRODUCTS

*Chairs: G.L. Marcazzan, M. Milito (Italy)*

- 09.00 **Geografical and botanical origin of Honeys.**  
**International Honey Commission proficiency test 2015-2016**  
*M. Milito (Italy)*
- 09.25 **Studies for italian propolis characterization**  
*G.L. Marcazzan (Italy)*
- 09.50 New approaches for determination of honey quality in relation to its biological activity  
*J. Majtan (Slovakia)*
- 10.15 Antioxidant content, free radical scavenging activity and identification of phenolic/flavonoid compounds in pollen of fourteen plants using hplc-dad  
*B. Lyoussi (Morocco)*
- 10.40 COFFEE BREAK
- 11.05 *Crataegus monogyna* vs. *Salix* sp. Bee pollen: nutritive value and biological activity  
*R. Margaoan (Romania)*
- 11.30 Application of hs-spme/gc-ms for characterisation of satsuma mandarin (*Citrus unshiu* Marc.) Honey: nectar / honey-sac / honey headspace volatiles  
*I. Jerkovic (Croatia)*
- 11.55 Palynological Analyses of Honeys Produced in Honey-flow Season in Oyo State, Nigeria  
*A.B. Oyerinde (Nigeria)*
- 12.20 **Discussion and conclusion of Apiquality Symposium**  
*Chair: E. Bruneau (Belgium)*
- 13.00 LUNCH

## 23 NOVEMBER 2016

### APIMEDICA

#### SESSION

##### NEW PERSPECTIVES

Chair: C. Mateescu (Romania)

- 14.00 **Apitherapy**  
**A new approach for honey and hive products**  
C. Mateescu (Romania)
- 14.30 Rojal jelly improves mental health  
N. Iegaki (Japan)
- 14.45 Essential and omega fatty acid content of bee bread and its effect on heart disease  
S. Silici (Turkey)
- 15.00 Bee bread: various usage of bee bread and clinical experiences  
P.S. Bhandari (India)
- 15.15 Practical api-nutrition - what happens when we introduce bee products in our daily diet  
A.F. Varadi (Romania)
- 15.30 "Beesabled project": beekeeping in reahabilitation  
A. D'Angeli (Italy)
- 15.45 Apiculture intervention for malaria - mitigation in Nigeria  
D.B.V. Musa (Nigeria)
- 16.00 COFFEE BREAK
- 16.30 **Practical training**  
**Apipuncture: practical demonstration and clinical evidences**  
P. Pérez Gómez (Spain)
- 17.30 Discussion

## 24 NOVEMBER 2016

### APIMEDICA

#### SESSION

##### PROPOLIS AND ROYAL JELLY

Chair: C. Mateescu (Romania)

- 09.00 **Apitherapy**  
**New medicines, supplements, cosmetics and new technologies**  
C. Mateescu (Romania)
- 09.30 Activity studies of characterized, standardized and highly purified propolis extract  
L. Crimaldi (Italy)
- 09.45 Influence of propolis and royal jelly on the antibacterial activity of honey against *Pseudomonas Aeruginosa*  
K. Rihar (Slovenia)
- 10.00 Propolis: is there a potential for the development of new and efficient antimicrobial agents?  
B. Lyoussi (Morocco)
- 10.15 Propolis intracellular meccanism of action to clarify anti-inflammatory and antioxidant activities  
V. Zaccaria (Italy)
- 10.30 Promising anti protozoal activities of bee propolis  
E.H. Abd Al Rahman (Egypt)

#### SESSION

##### BEE VENOM

Chair: A. Couto (Portugal)

- 10.45 **Bee Venom Teraphy: micro api-puncture application**  
A. Couto (Portugal)
- 11.15 Bee venom acupuncture multiple sclerosis  
L.I. Daker (Egypt)
- 11.30 COFFEE BREAK

## 24 NOVEMBER 2016

### APIMEDICA

#### SESSION

##### CLINICAL APPLICATIONS

*Chair: A. Colonna (Italy), C. Aosan (Romania)*

- 12.00 **New perspectives for apitherapy among integrated therapies. Hive products and their application in human medicine**  
*A. Colonna (Italy)*
- 12.15 **Apitherapy in the daily practice - Clinical applications**  
*C. Aosan (Romania)*
- 12.30 Evaluating the effectiveness of propolis in the non-surgical treatment of periodontal disease  
*C. Salvatori (Italy)*
- 12.45 Bee propolis in the treatment of helicobacter pylori: in the right way to clinical application  
*M. Biagi (Italy)*
- 13.00 Experiences and perspectives of infected ulcers and wounds treated with knitted viscose mesh  
*A. Miceli (Italy)*
- 13.15 Medicinal honey dressings for burns and other types of wounds: properties and clinical effects  
*A. Zbucea (Romania)*
- 13.30 LUNCH
- 14.30 **Selected study cases: 15 years of Apitherapy in Veterinary Medicine practice from Southern Chile**  
*M.A. López Pazos (Chile)*

## 24 NOVEMBER 2016

### APIMEDICA

#### SESSION

##### APITHERAPY IN VETERINARY MEDICINE

*Chair: M.A. López Pazos (Chile)*

- 15.00 Apitherapy in daily practice  
*M.V. Strant (Romania)*
- 15.15 **Premises for the application of apitherapy in veterinary medicine**  
*A. Menegotto (Italy)*
- 15.30 **Discussion and conclusion of the Apimedica Symposium**  
*C. Mateescu (Romania)*
- 16.00 COFFEE BREAK

# 6<sup>TH</sup> APIMEDICA & 5<sup>TH</sup> APIQUALITY

INTERNATIONAL SYMPOSIUM  
ROME, 22-25 NOVEMBER 2016

## GENERAL INFORMATION

### CONGRESS VENUE

Novotel Roma Eur - Viale dell'Oceano Pacifico, 153 - 00144 Rome

### HOW TO GET TO THE CONGRESS VENUE

From Fiumicino Airport

Toward Rome, follow the signs for the Grande Raccordo Anulare ring road (GRA), toward Pontina/EUR/Laurentina; continue along the GRA until exit 26 EUR, toward the center.

From Ciampino Airport

Take Via Appia toward Rome, then take the GRA ring road toward Pontina/EUR/Laurentina, exit 26 EUR, toward the center.

From Termini train station

Take Metro Line B, Eur Palasport stop, then take the 777 bus line for 3 stops.

### CONGRESS LANGUAGES

The languages of the Symposium 2016 are English and Italian.

Simultaneous translation will be provided.

### COFFEE BREAKS & LUNCHESES

Coffee breaks are included in the registration fee for the congress participant. Lunch is at participant's expenses and can be purchased directly at the bar and restaurant of the hotel. Outside the hotel you can also find other places where you can have lunch: Ennio Restaurant and the shopping center Euroma2, both 10 minutes walking distance from the hotel

### NAME BADGE

Upon arrival, all participants will receive a name badge, which must be worn visibly for the entire duration of the congress.

## SCIENTIFIC INFORMATION

### AUDIOVISUAL INSTRUCTIONS

Presentations can be downloaded directly in the meeting room at least 30 minutes before the presentation. Please bring the presentation on a Pen Drive, Hard Disk USB, CD-Rom and DVD-Rom. Video presentations need to have the following format .avi, .wmv, .mpeg. If Speakers have their presentation using a system different from MS Windows (such OS MAC or Linux), they are kindly requested to come earlier at the slide centre, in order to have enough time to solve possible compatibility issue. Speakers using their own MAC are kindly requested to bring also their Apple/VGA adapter.

## REGISTRATION

### REGISTRATION FEES (VAT INCLUDED)

	AFTER 30 <sup>TH</sup> SEPTEMBER 2016
<b>APIQUALITY</b> <i>22<sup>nd</sup> and morning of 23<sup>rd</sup> of November</i>	<b>€ 140,00</b>
<b>APIMEDICA</b> <i>Afternoon of 23<sup>rd</sup> and 24<sup>th</sup> November</i>	<b>€ 140,00</b>
<b>APIQUALITY + APIMEDICA</b> <i>The 3 days</i>	<b>€ 220,00</b>
MEMBER APIMONDIA (DISCOUNT 10%)	
	AFTER 30 <sup>TH</sup> SEPTEMBER 2016
<b>APIQUALITY</b> <i>22<sup>nd</sup> and morning of 23<sup>rd</sup> of November</i>	<b>€ 126,00</b>
<b>APIMEDICA</b> <i>Afternoon of 23<sup>rd</sup> and 24<sup>th</sup> November</i>	<b>€ 126,00</b>
<b>APIQUALITY + APIMEDICA</b> <i>The 3 days</i>	<b>€ 198,00</b>

The registration fee includes: congress kit, congress material, coffee breaks.  
The fees don't include lunches and the excursion to the apiary near Rome.



# 6<sup>TH</sup> APIMEDICA & 5<sup>TH</sup> APIQUALITY

IN COLLABORATION WITH



**BNATURAL**

PROPOLIS AND BEEKEEPING DERIVATIVES



# 5<sup>TH</sup> APIQUALITY

ABSTRACTS BOOK

### KEYNOTE SPEAKERS

1. E. Bruneau - Observation of nature, a key to produce apimedica quality products
2. M. Mortarino - Opportunities to reduce the impact of acaricide treatments on the hive
3. C. D'Ascenzi - Study on food risk assessment of pesticide residues in bee pollen
4. C. Kast - Pyrrolizidine alkaloids and other plant toxins in honey and pollen
5. G. Formato - M. Pietropaoli - Pre-clinic indicators as Good Beekeeping Practices: sampling methods and new traceability systems
6. M. Milito - Geographical and botanic origin of honey. International Honey Commission Proficiency tests 2015-2016
7. G.L. Marazzan - Studies for Italian propolis characterization

### ORAL PRESENTATIONS

1. P. Fontana - Seasonal strategies in honeybee pollen production
2. A. Bozzano - Training activities for the the development of the Apiculture sector
3. N. Bekena - Quality focused apisector development in Ethiopia
4. G. Ciaschetti - Honey and bee products characterization of the Majella National Park
5. S.M.R. Tulini - Chemicals compounds in beeswax samples collected during the years 2013 - 2015
6. S. Alapala - Residues of pesticides in honey
7. N. Beitlich - Genuine manuka honey
8. L. Svecnjak - Beeswax adulteration issue: aspects of contamination and outcome
9. M. Martinello - Development and validation of a quechers method coupled to HRMS detection to determine pyrrolizidine and tropane alkaloids in honey
10. J. Majtan - New approaches for determination of honey quality in relation to its biological activity
11. B. Lyoussi - Antioxidant content, free radical scavenging activity and identification of phenolic/flavonoid compounds in pollen of fourteen plants using HPLC-DAD
12. R. Margaoan - Crataegus monogyna vs. Salix sp. bee pollen : nutritive value and biological activity
13. I. Jerkovic - Application of HS-SPME/GC-MS for characterisation of Satsuma mandarin (Citrus unshiu marc.) honey: nectar / honey-sac / honey headspace volatiles
14. A.A. Oyerinde - Palynological analyses of honeys produced in honey-flow season in Oyo state, Nigeria

### POSTERS

1. B. Lyoussi - Comparison of the protective effect of moroccan propolis and Thymus vulgaris against oxidation of cosmetic emulsions
2. A. Owayss - Quality analysis of egyptian pollens collected by honey bee, Apis mellifera L., Colonies
3. G. Formato - A project to improve and characterize South African honeys: the Eden Honey Bee Project
4. M. Milito - Organoleptic and melissopalynological analysis of honeys from Western Cape province in South Africa
5. M. Milito - Bacteria associated to different honey bees' matrices
6. J. Rivera-Gomis - The Scientific Veterinary Medical Association for Apiculture (SVETAP)
7. G. Ragona - Microbiological quality and food safety evaluation of bee pollen: a preliminary study in Tuscany
8. J. Rivera-Gomis - AFB and EFB treatment with oxytetracycline and shock swarming to guarantee honey bee survival and no detectable residues in jar honey - preliminary results
9. H. Boudegga - Feeding effect on the evolution of honeybees' swarms
10. M. Pietropaoli - Honeybee health status in the Western Cape Province of South Africa
11. I. Djimrevska - Botanical origin of honey from different agricultural regions of the Republic of Macedonia
12. S. Prdjun - Preliminary research on nectar secretion in two Citrus unshiu cultivars: honey production potentials
13. V. Ramajayam - A seasonal analysis of honey bee-collected pollen from Matelica (Marche, Italy): palynological origin, phenolic content and antioxidant properties
14. S. Rasic - The quality and safety evaluation of East Serbian honey- Djerdap honey
15. S. Silici - Amino acid and vitamin content of propolis collected by native caucasian honeybees
16. M. Pietropaoli - The Apiculture Laboratory of the Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri" to guarantee hive products quality
17. E. Messineo - Proficiency-testing scheme for pesticides in honey

### OBSERVATION OF NATURE, A KEY TO PRODUCE APIMEDICA QUALITY PRODUCTS

E. BRUNEAU

CARI asbl, Louvain-la-Neuve

The observation of bees in their natural conditions of life shows us how this social insect feed, reproduce and defends himself against pathogens (Varroa destructor...) without the use of drugs. Here we will see what are the tips that a beekeeper can draw from these observations to produce bee products for apitherapy. They focus on the environment of the hive, equipment use, maintenance, on hive management (fight against swarming ...) and harvesting products. The aim of this presentation is to have a deeper reflection on our current methods of work and provide ideas to improve our production methods in better compliance with bees and the products she shared with us.

### OPPORTUNITIES TO REDUCE THE IMPACT OF ACARICIDE TREATMENTS ON THE HIVE

M. MORTARINO - Dipartimento di Medicina Veterinaria, Università degli Studi di Milano

M. BLONDA - Apilombardia

S. ZANZANI - Dipartimento di Medicina Veterinaria, Università degli Studi di Milano

F. DELL'ORCO - Dipartimento di Medicina Veterinaria, Università degli Studi di Milano

E. FACCHINI - Dipartimento di Medicina Veterinaria, Università degli Studi di Milano

L. COLOMBARI - Apilombardia

R. RIZZI - Dipartimento di Medicina Veterinaria, Università degli Studi di Milano

In the hive, Varroa destructor can be counteracted through different types of intervention, including treatments with natural or synthetic pesticides, and integrated pest management methods. Repeated use of synthetic acaricides may lead both to the formation of Varroa strains carrying drug resistance, and increased possibility of residues in hive matrices and products. Consequently, in recent years there has been an increase of research activities for the development of acaricides of natural origin and their use in beekeeping practice. Among the acaricidal products of natural origin that are currently used in beekeeping practice, there are oxalic acid, formic acid and other essential oils such as thymol, menthol and eucalyptol. Moreover, in recent years the multiple properties of several other substances of natural origin have been investigated in laboratory and field conditions, with results not always consistent as to the acaricidal effect and tolerability for honeybees. These discrepancies may be due to several factors, including complex chemical composition, different content in active principles, and different preparations of the natural products. Other variability factors are represented by different doses, different test sites and variable application ways in in vitro tests and in the field.

In order to further promote the use of natural miticide products with low or no residual impact on the hive, new studies and experiments should be performed aimed to standardize preparations of the substances of interest under comparable conditions and with repeatable results. In particular, a thorough examination of the possible ways of action against mites, and synergy with suitable beekeeping methods, should be investigated in order to maximize such effects and make them compatible with practical needs in the field activity.

### STUDY ON FOOD RISK ASSESSMENT OF PESTICIDE RESIDUES IN BEE POLLEN

C. D'ASCENZI<sup>1</sup>, G. FORMATO<sup>2</sup>, G. MANNAIONI<sup>1</sup>, E. CARNESECCHI<sup>3</sup>, I. CARACCILO<sup>1</sup>

<sup>1</sup>University of Pisa, Department of Veterinary Sciences, V.le delle Piagge 2, 56124 Pisa, Italy

<sup>2</sup>Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana- Apiculture Laboratory, Italy

<sup>3</sup>European Food Safety Authority (EFSA), Via Carlo Magno 1A, 43126 Parma, Italy

This study applies the methodology of the food risk assessment to the pollen contamination by pesticide residues processing data collected from long-term monitoring conducted in Italy and Europe.

The contamination of pollen by pesticide residues can take place with different schemes:

contamination of the flowers from which the pollen originates;

contamination of the foraging bees during the flights in the surrounding territory of the beehive (flight range of about 3 kilometres)

use of active substances in therapeutic or preventive treatments of bee's parasitic diseases (e.g. acaricidal treatments targeted at Varroa destructor)

The fungicide are the category of active substances more involved in the contamination of pollen (47,9%), followed only by insecticides (23,9%).

The evaluation of the food risk exposure has revealed a prevalence of insecticides. Chlorpyrifos represents the active substance with the higher exposure to the chronic risk while Chlorfenvinphos represents the active substance with the higher exposure to the acute risk.

The underlined values of risk indicate that, considering the intrinsic toxicity of the involved substances, the content of the detected residues and the amount of daily intake of bee pollen, although the exposure to contamination was widespread, the food risk related to the consumption of bee pollen is relatively small. Between the two categories of potential consumers (adults and children), children have shown to present reduced safety margins.

The applied assessment model proves to be essential in defining the food safety objectives, the priorities in the prevention management to be given to the operators of this food sector as well as objectives and methods to implement specific official control programmes for the competent authorities.

### PYRROLIZIDINE ALKALOIDS AND OTHER PLANT TOXINS IN HONEY AND POLLEN

C. KAST<sup>1</sup>

<sup>1</sup>Agroscope, Swiss Bee Research Centre, Schwarzenburgstrasse 161, 3003 Bern, Switzerland

Many plant species worldwide produce natural toxic substances as a chemical defence against herbivores. While some toxins are the active components of medicinal plants, others are undesired components in medicinal products. Toxic substances occasionally also get into foods of plant origin, such as for example tea, spices or bee products. Acute intoxication of humans through honey from some Rhododendron-species nectar date back to ancient time. Several plants of the Ericaceae family, especially Rhododendron ponticum, produce grayanotoxins, which are poisonous for humans. Recently, various studies have shown that honey and pollen may contain toxic pyrrolizidine alkaloids (PAs), causing chronic toxicity affecting the liver function (Ref.1). So far, legal limits exist only for medicinal products of plant origin, but not for foods. However, the German Federal Institute for Risk Assessment (BfR) recommends a maximal uptake of 0.007 µg of 1,2-unsaturated PAs/day/kg body weight. This corresponds to 21 µg PAs in 1kg of honey, as calculated for a person of 60 kg body weight consuming 20 g of honey daily. When bees collect nectar and pollen from PA-containing plants, they transfer the PAs into honey or pollen. We investigated the level of contamination in honey and pollen produced in Switzerland and studied whether PAs in honey originate from floral nectar or plant pollen. Furthermore, we correlated the flowering period of PA-containing plants with the time point when bees bring pollen with PAs into the hives in order to assess the plants responsible for PAs in bee products. Our results suggest that Swiss honey and pollen can contain PAs, but usually at concentrations

that pose no risk for consumers. Nectar contributes to a more significant extent to PA contamination in honey than pollen does. Echium vulgare is currently the most relevant plant for PA contamination of Swiss honey and pollen, followed by Eupatorium cannabinum and Senecio sp. Therefore, beekeepers should pay close attention when many PA-containing plants are blooming around their apiaries, since PAs can get into honey and pollen, especially when no other attractive nectar and pollen plants are blooming simultaneously. Recognizing problematic plants around the apiaries is essential in order to produce good quality honey and pollen for medical use or as food.

Ref 1: German Federal Institute for Risk Assessment (BfR): Stellungnahme Nr. 030/2016, 28. September 2016

### PRE-CLINIC INDICATORS AS GOOD BEEKEEPING PRACTICES: SAMPLING METHODS AND NEW TRACEABILITY SYSTEMS

G. FORMATO<sup>1</sup>, M. PIETROPAOLI<sup>1</sup>

<sup>1</sup> Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana- Apiculture Laboratory, ITALY

Nowadays, the standardization of disease control methods at apiary level and the objective evaluation of the beekeeper's management skills are crucial to conform the field inspections realized by the control bodies. In the presentation it will be described a new approach for Good Beekeeping Practices (GBP) able to prevent bee diseases like varroa, viruses, American foulbrood, European foulbrood and noseosis. In fact, pre-clinic diagnosis is possible starting using innovative matrixes like hive debris and icing sugar (e.g. used to check the varroa mite infestation level of the colonies).

Moreover, it will be described an innovative traceability system based on QRCode/RFID (radio frequency identification) technology applied to the entire honeybee food chain, from hive to jar, finalized to record the apiary management data (e.g. colony strength, queen's performances and to record the pre-clinic GBP at the apiary level) for the beekeepers and to illustrate to consumers the hive products characteristics.

### GEOGRAPHICAL AND BOTANIC ORIGIN OF HONEY. INTERNATIONAL HONEY COMMISSION PROFICIENCY TESTS 2015-2016

M. MILITO<sup>1</sup>, M. DIMOU<sup>2</sup>, P. GOTSIOU<sup>3</sup>

<sup>1</sup> Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri"

<sup>2</sup> Laboratory of Apiculture & Sericulture, Faculty of Agriculture, Aristotle University of Thessaloniki, Greece

<sup>3</sup> CIHEAM-Mediterranean Agronomic Institute of Chania, Greece.

The quality assurance of the bee products is very important as it offers to the consumers an unexceptionable product regarding its nutritional value, the hygienic and sanitary effect, and even more in case of therapeutic applications. In the case of honey, the determination of the botanical and geographical origin helps to verify the quality of the product as well as its marketing value. The laboratories that perform analysis regarding the botanical and geographical origin of honey, such as pollen analysis, are often verifying their methodologies and results through international ring tests. The Working Group for the Geographical and Botanical Origin of the International Honey Commission (IHC) is working on the development and improvement of proper analytical methods for honey and other bee products and it helps in their dissemination among its members. In this regard, the leaders of the group are organizing annually proficiency testing schemes for honey pollen analysis. The participant members/laboratories (distributed both in Europe and in Western Asia) receive annually one honey sample which they analyse for a) pollen identification b) pollen classification between nectariferous and not nectariferous pollens; c) the proper interpretation of the results concerning the

botanical and / or geographical origin; as well as some physicochemical analysis. The honey is dispatched from a member of the group (i.e. in 2015 the samples was sent by M.C. Seijo Coello of the Faculty of Science of the University of Vigo (Spain) and in 2016 by Paolo Antonio Russo Almeida from the Laboratorio Apícola of UTAD from the Universidade de Trás os Montes e Alto Douro (Portugal)); while the test (organization, statistical analysis, report) is coordinated by the leaders of the group.

In the honey sample used for the Proficiency Test in 2015 the following pollen types were to identified and counted: Cytisus/ Ulex type, Castanea, Eucalyptus, Rubus. In the honey sample used for the Proficiency Test of 2016 the pollens asked for identification and counting were: Echium spp., Cytisus spp., Lavandula pedunculata, Anarrhinum bellidifolium. In 2015, the great majority of the participants identified the honey sample as a blossom honey; in 2016 most of the participants characterized the sample as lavender honey.

### STUDIES FOR ITALIAN PROPOLIS CHARACTERIZATION

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The chemical composition of propolis and its biological properties is strictly dependent on its geographic and climatic origin. However when using a product for medical purposes it is of essential importance that the product is standardized.

With our study we conducted a preliminary survey to test the different compositions of propolis from different Italian areas with the aim of laying the foundation of its geographical characterization and to contribute to future standardization.

The first step was to calculate the percentages of resin, wax and mechanical impurities in forty-three samples of propolis collected from all over Italy.

The resinous extract from the sample was then analyzed and evaluated for its polyphenolic profile. This was performed by using liquid chromatography coupled with a triple quadrupole mass spectrometer (HPLC/TQMS). About twenty molecules of phenolic acids and flavonoids were quantified with the external standard method.

Determination of the phenol and flavonoid contents was also performed using three spectrophotometric methods.

The antioxidant activity of resinous extract was also studied using reductive power assay, desoxyribose assay, free-radical scavenging effect (DPPH assay) and chelating activity assay. The data were then processed by statistical analysis to identify groups and connections with the geographic and climatic zones of origin.

### SEASONAL STRATEGIES IN HONEYBEE POLLEN PRODUCTION

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Since the primordial of humanity, pollen has been considered a good source of nutrients and energy. Recently the interest of pollen collected by honeybees increased, and many authors stressed on the properties of pollen and its importance in both human and honeybee diet. Botanical origin as well contamination are the most relevant aspects in evaluating pollen. This highlights the need of palynological and multi-residue analysis in order to identify suitable areas and seasons to collect honeybee pollen of high quality. Up to date, there are no regulations concerning pollen, so according to the EU, the Maximum Residual Level should be considered equal to 10.0 ng g<sup>-1</sup>, as established for unregulated substances and products (EC-396/2005). In this work, we analyzed honeybee pollen collected in North Italy (Trentino district) by honeybees during spring and summer of 2015 and 2016. Pollen samples were analyzed by GC-MS/MS e UHPLC-MS/MS to detect contaminants. Palynological analysis were carried out to assess the botanical origin of the pollen. Preliminary data outline how pollen collected during early spring as well in late summer is more interesting for honeybee and human consumption. In these periods honeybee pollen results to be rich in nutrients, pleasant in taste and with low contamination level even in intensively cultivated areas. In prevision of a large-scale production of honeybee pollen, a qualitative monitoring both from a spatial and temporal point of view is, therefore, fundamental.

### TRAINING ACTIVITIES FOR THE DEVELOPMENT OF THE APICULTURE SECTOR

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In this contribution are reported the training activities for the apiculture sector realized by the Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri" (IZSLT) in collaboration with the Apiculture Laboratory from 2003 to 2016.

Training activities for beekeepers, officers (Veterinary Services, rangers, Police, NAS, etc...) and all the stakeholders involved in the Apiculture sector in general (practitioners, veterinarians, technicians, researchers, agronomists, biologists, etc...) are crucial to disseminate innovations and to promote know-how in order to guarantee an improvement of the beehive products' quality.

Since 2003 IZSLT organized 61 events on specific aspects of apiculture, in collaboration with the Apiculture Laboratory. This the matters object of the training activities in apiculture: Good Beekeeping Practices (GBPs), sanitary legislation, registration, official controls, quality of the hive products, bee diseases and their diagnosis and treatments, HACCP system applied to the bee products, emerging issues such as the Colony Collapse Disorder (CCD), monitoring activities on the arrival in EU of the Small Hive Beetle (Aethina tumida) and Vespa velutina, new products to fight Varroa destructor, etc...

In the table below are summarized the number of training events organized by IZSLT from 2003 to 2016, and the number of participants for each year organized according their profession (officers, beekeepers or both).

Year	Number of events	Total number of participants	Officers only	Beekeepers only	Officers and Beekeepers
2003	1	55	YES (1)		
2004	1	30	YES (1)		
2005	3	110	YES (2)	YES (1)	
2006	4	320		YES (1)	YES (3)
2007	3	190	YES (1)	YES (2)	
2008	3	150		YES (2)	YES (1)
2009	4	200	YES (1)	YES (1)	YES (2)
2010	2	160	YES (1)		YES (1)
2011	4	280	YES (1)	YES (1)	YES (2)
2012	3	240		YES (2)	YES (1)
2013	9	685	YES (1)	YES (5)	YES (3)
2014	10	800		YES (3)	YES (7)
2015	11	880		YES (3)	YES (8)
2016	3	280			YES (2+1*)
<b>TOTAL</b>	<b>61</b>	<b>4'380</b>	<b>9</b>	<b>21</b>	<b>31</b>

\* = international event

Moreover, Apiculture Laboratory of IZSLT collaborated in training beekeepers in courses (both first level and advanced) organized by the beekeepers' associations of the two regions (Latium and Tuscany) of its competence.

Thanks to a specific link available on the IZSLT website for the Apiculture Laboratory (<http://www.izslt.it/apicoltura/>), all the documents (PPT presentations or pdf documents) of the training events realized were quickly shared among beekeepers and all the other stakeholders in order to develop their professionalism and skills.

### QUALITY FOCUSED APISECTOR DEVELOPMENT IN ETHIOPIA

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Ethiopia is the leading api-actor in Africa and among the top actors worldwide; a founding member of APITRADE AFRICA; member of the Regional Multi Stakeholder Platform; member of APIMONDIA. Ethiopia has been listed as a Third Country to export honey to the EU in 2008 and renewed the listing annually. Exports of organically and fair trade certified honey have been increasing from zero in 2008 to close to 1,000 tons.

The total national annual honey production of approx. 55,000 tons represents merely a tenth of the potential. A major focus of the sectors development - a main task of the Ethiopian Apiculture Board EAB - is to modernize the beekeeping activity, create a quality infrastructure and to put quality assurance systems in place, for local and international consumer satisfaction and protection.

Measures taken so far are:

- annual residue monitoring (no residues traced since 2008);
- quality task force establishment;
- establishment of traceability at producers and traders level;
- laboratory screening and upgrading with the intention of having internationally accredited facilities by end of 2016;
- characterization of Ethiopian monofloral honeys and development of markets for them;
- implementation of existing proclamation on apiculture resources development and protection;

- establishment of new proclamation and regulation on honey marketing;
- active presentation of the sector in national, regional and international fairs, for learning, experience sharing and market development.

### HONEY AND BEE PRODUCTS CHARACTERIZATION OF THE MAJELLA NATIONAL PARK

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Biodiversity and nature conservation is the primary mission of the Majella National Park (MNP). The intact environments, the abundance of floristic resources, the possibility to practice beekeeping in a sustainable way determine the possibility of obtaining bee products, from the plain even up to 1600 meters high, particularly appreciated for their purity, integrity and genuineness.

The poster will present the results of the activities realized in the MNP from 2010 to 2015 in collaboration with the Istituto Zooprofilattico Sperimentale del Lazio e della Toscana (IZSLT):

1. A monitoring activities of pollutants (heavy metals like Cr, Cd, Pb; radionuclides; polycyclic aromatic hydrocarbons - PAHs) was carried out, in collaboration with the University of Teramo from 2010 to 2015 in honey bees and hive products taken from hives placed in the MNP. The study showed the high quality of the Park bee products and the almost total absence of environmental pollution.
2. A monitoring activity of the honey bee queens subspecies present in the MNP by morphometrical analysis.
3. A monitoring activity of the amount of Nosemosis and virosis present in the colonies of MNP by nosema spores count and PCR analyses.
4. Maps of nectariferous and pollineferous areas in the MNP.
5. Characterization of honeys produced in the MNP through laboratory analyses: melissopalynological and organoleptic analysis for botanical characterization of different wildflower and monofloral honeys (e.g. Thyme honey, Sainfoin honey and Stachys honey); diastase index (indicator of honey freshness); humidity; identification of specific pollens as "markers" of the geographical origin using microscopy of biomolecular (PCR) techniques.
6. A procedural guidelines for honey production within the MNP and its proper labelling with a specific quality mark logo: a valuable help to beekeepers for the best use of land resources and improve their productions, both in terms of quantity and quality.

### CHEMICALS COMPOUNDS IN BEESWAX SAMPLES COLLECTED DURING THE YEARS 2013 - 2015

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Beeswax finds important applications in food, cosmetic, pharmaceutical industries and in beekeeping for the production of comb foundations. Because of its lipophilic nature most of the chemicals used in agricultural and beekeeping activities, that are fat soluble, non-volatile and persistent, can accumulate easily in it. However, actually in Europe does not exist beeswax quality control and international standards, even if some countries have national ones. In Italy the Technical Guidelines RT 16 of 2009 is the document that establishes pesticides limits in beeswax (RT-16 rev.04, 2009). However it takes care of the organic

productions and set the limit only for 5 substances: amitraz, coumaphos, chlorfenvinphos, tau-fluvalinate and cymiazole. The present study was focused on the multiresidue analyses (247 chemicals) of the wax samples collected from 2013 to 2015. The higher pesticide frequencies were associated to: coumaphos (60.7 % of samples), tau-fluvalinate (50%) and chlorphenvifos (35.4 %), often with concentrations higher than the limits set by the RT 16. Moreover, the data reveal along the years, an increased frequency of new generation pyrethroids authorized in products for agricultural practices in these last years and a constant presence of dichlorodiphenyltrichloroethane (DDT) and its metabolites, banned in Europe from decades.

### RESIDUES OF PESTICIDES IN HONEY

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Honeys that have great contributions to human health have been experiencing serious residue problems in recent years. Bee products are contaminated with residues of the pesticides used to protect colonies against diseases, pests and contaminants stemming from environmental factors such as heavy metals, pesticides, organic pollutants, pathogenic bacteria and radioactive substances. In modern agriculture, use of pesticides has become an obligation. While using pesticides, protection of the product against diseases, pests and weeds and negative effects of these pesticides on human and environmental health should be taken into consideration together. Moreover, pesticides should be used before and after the harvest and producers should be informed about how to administer these pesticides. The current study intends to discuss the limits of residues of pesticides and veterinary drugs for the production of clean and healthy honey and to raise the awareness of importance of informed use of these pesticides and drugs.

### GENUINE MANUKA HONEY

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Manuka honey is one of the most adulterated monofloral honeys in the world since it is the major medical grade honey currently approved for clinical application, especially for wound healing. The antibacterial activity of manuka honey is mainly caused by methylglyoxal (MGO), aside of other as yet unknown compounds. This has led to more so called manuka honey being sold on the market than actually produced. For this reason the blending and adulteration of manuka honey has come into focus everywhere. Therefore, the New Zealand Government and the UMFHA have requested robust and clear parameters for the identification of genuine manuka honey.

In our study, more than 150 honey samples from monofloral manuka, kanuka, and other New Zealand honeys supplied by the UMFHA were characterized by SPE-UHPLC-PDA-MS/MS and HS-SPME-GC/MS. A classification system named HAHSUS (Honey Authentication by HS-SPME-GC/MS and UHPLC-PDA-MS/MS combined with Statistics) was developed which is capable of differentiating and classifying manuka honey from other honeys, especially from the pollen-identical kanuka honey. It is also possible to estimate the percentage of manuka honey in manuka-kanuka honey mixtures.

N. Beitlich et al., J. Agric. Food Chem. 2014, 62, 6435-6444.

### BEESWAX ADULTERATION ISSUE: ASPECTS OF CONTAMINATION AND OUTCOME

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Adulteration has been one of the main quality issues of beeswax production and represents a long-term and increasing problem worldwide. However, there are still no internationally standardized analytical methods or legislation regulating beeswax authenticity control. This problem is not referring only to the aspects of uncontrolled contamination via comb foundation trade but also to the fact that adulterants are being accumulated in the beekeeping technology through comb foundation production process. After developing an analytical procedure for routine beeswax adulteration detection using Fourier transform infrared spectroscopy coupled with attenuated total reflectance recording technique (FTIR-ATR), we have conducted a systematic monitoring of the situation on the comb foundation international market in the period from 2014 to 2016. The results revealed that more than 70% of analyzed samples were adulterated with paraffin (other adulterants were observed sporadically). An amount of paraffin that remains in the honeycomb built on adulterated foundations, after its full construction and melting was also assayed in order to determine residual quantities. It was observed that in average >50% of paraffin remains in the newly built and melted raw wax material. Such material is commercially being used for further comb foundation production and thus, re-enters the beekeeping technology by insertion in the hives. The results obtained in this study indicate "chronic" circulation of adulterants in the comb foundation production process and consequently a large-scale beeswax contamination problem.

### DEVELOPMENT AND VALIDATION OF A QUECHERS METHOD COUPLED TO HRMS DETECTION TO DETERMINE PYRROLIZIDINE AND TROPANE ALKALOIDS IN HONEY

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The presence of natural toxins as contaminants of food and feed is an open issue in the European Union due to their adverse effects to human and animal health. In February 2016, maximum levels of TAs in cereal-based foods for infants and young children have been regulated (Commission Regulation (EU) 2016/239). The present study aimed at the elaboration and validation of a method to determine PAs (echimidine, heliotrine, intermedine, lasiocarpine, lycopsamine, retrorsine, senecionine, seneciphylline, senkirkine) and TAs (atropine, scopolamine) in honey. QuEChERS sample treatment and HRMS, were used. The method results in good linearity ( $R^2 > 0.99$ ) and LOD and LOQ ranged from 0.04 to 0.2  $\mu\text{g}/\text{kg}$  and from 0.1 to 0.7  $\mu\text{g}/\text{kg}$  respectively. Recoveries ranged from 92.3 to 114.8% with repeatability lying between 0.9 and 15.1% and reproducibility between 1.1 and 15.6%. The characteristics of the method are in agreement with Commission recommendation (EU) 2015/976 which suggests the following LOQ for TAs: "preferably below 5  $\mu\text{g}/\text{kg}$  and not higher 10  $\mu\text{g}/\text{kg}$  for agricultural commodities, ingredients, food supplements and herbal teas and should preferably be lower than 2  $\mu\text{g}/\text{kg}$  for finished foods and 1  $\mu\text{g}/\text{kg}$  for cereal-based foods for infants and young children".

### NEW APPROACHES FOR DETERMINATION OF HONEY QUALITY IN RELATION TO ITS BIOLOGICAL ACTIVITY

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The identity and quality parameters of honey are considered useful for detecting possible adulterations, and also for confirming the hygiene conditions for the manipulation and storage of honey. One possible approach in honey quality control is to focus on the detection of specific honey constituents such as enzymes or peptides derived from bee salivary secretions. In this study we report the development and evaluation a polyclonal antibody based competitive ELISA for the quantification of bee-derived defensin-1 in honey. Bee defensin-1, an antibacterial peptide, is a regular but variable antibacterial component of natural honey. Its concentration in honey decreased after sterilisation process using gamma radiation as well as after microwave treatment.

This assay can be used for the purpose of the qualitative analysis of honeys, particularly in order to select honeys with high potential antibacterial activity. Furthermore, it can also be used for monitoring of honey antibacterial activity during its technological processing.

### ANTIOXIDANT CONTENT, FREE RADICAL SCAVENGING ACTIVITY AND IDENTIFICATION OF PHENOLIC/FLAVONOID

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Pollens is the male gametophyte of flowers found in the anthers of stamens in angiosperms, important for plants reproduction but also crucial to honeybees because it will provide them of all of the nutrients like proteins, fats, vitamins, and minerals. The aim of this study was to identify the HPLC-DAD phenolic/flavonoid profile and the antioxidant (in vitro) potential of pollen hand collected from fourteen plants : Anethum graveolens (Apiaceae); Capparis spinosa (Capparidaceae); Opuntia-ficus-indica (Cactaceae) ;Calendula officinalis (Asteraceae); Anacyclus clavatus (Asteraceae); Scolymus hispanicus (Asteraceae); Papaver rhoeas (Papaveraceae); Justicia adhatoda (Acanthaceae); Malva sylvestris (Malvaceae); Anacyclus radiatus (Asteraceae); Echium creticum (Boraginaceae); Mentha pulegium (Lamiaceae); Mentha spicata (Lamiaceae); Acacia pycnantha (Fabaceae).

The results show that all samples presented excellent antioxidant content and activities with different strength. The HPLC-DAD analysis of hydro-ethanolic pollen extracts showed the presence of a wide variety of flavonols and flavones, mainly glycosides of luteolin, apigenin...kaempferol and quercetin. The phenolic acids are represented by derivatives of caffeic acid, p-hydroxybenzoic acid and vanillic acid, in Malva sylvestris and Scolymus hispanicus some compounds found could be alkaloids, however in this work the confirmation of such chemical group was not carried out but it will be include in further studies.

### CRATAEGUS MONOGYNA VS. SALIX SP. BEE POLLEN: NUTRITIVE VALUE AND BIOLOGICALY ACTIVITY

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Knowledge about the botanical source of the bee pollen samples, as well as their chemical composition, it is important to classify and add value to the product. Crataegus monogyna and Salix sp., two types of spring bee pollen were analyzed regarding nutritional and biological value. The nutritional value was 352.64 Kcal/100g for C.monogyna and 328.83 Kcal/100g for Salix sp.

Fourteen fatty acids were identified in the pollen extracts, from which the most abundant was alpha-linolenic acid in Salix sp.(24.26%) and in C.monogyna (20.24%), followed by linoleic acid in the range of 30.65 and 33.21% from total fatty acids, in Salix sp. and C.monogyna respectively.

The variability regarding the phenolic content and antioxidant activity in examined pollen samples was not statistically different. The total polyphenols of C.monogyna was 8.80 mg GAE/g and to Salix sp. was 7.69 mg GAE/g. Total flavonoids were 5.93 mg QE/g for C.monogyna bee pollen and 5.07 mg QE/g for Salix sp.

The most important difference was determined in the antibacterial activity.

Inhibition zones diameters of pollen extracts for C.monogyna and Salix sp. on S.aureus bacterial strains was 5.33 and 12.33mm respectively, for B.cereus: 7.33 vs. 8.00mm, for B.laterosporus: 6.67 vs. 11.33mm and for P.alvei: 11.33 vs. 21.33mm.

### APPLICATION OF HS-SPME/GC-MS FOR CHARACTERISATION OF SATSUMA MANDARIN (CITRUS UNSHIU MARC.) HONEY: NECTAR / HONEY-SAC / HONEY HEADSPACE VOLATILES

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Citrus unshiu Marc. honey characterization by melissopalynological analysis is difficult with strongly underrepresented characteristic pollen due to the specific plant physiology of particular mandarin cultivars (aborted anthers, sterile pollen grains, partenocarpy). Therefore, there is a need for its detail chemical characterization and present research is focused on its headspace profile that in general can be considered characteristic of the unifloral honey.

Headspace solid-phase microextraction (HS-SPME) with the fiber polydimethylsiloxane / divinylbenzene (PDMS / DVB) followed by GC-MS was applied for monitoring the nectar (NE) / honey-sac (HS) / honey (HO) headspace volatiles. The major NE headspace compounds were linalool, terpineol, 1H-indole, methyl anthranilate and phenylacetoneitrile. The major HS headspace compounds were linalool, terpineol, 1,8-cineole, 1H-indole, methyl anthranilate and cis-jasmone. HO headspace composition was significantly different in comparison to NE and HS with respect to phenylacetaldehyde and linalool derivatives abundance that appeared as the consequence of the hive conditions and the bee enzymes activity. 1H-indole and phenylacetoneitrile were found as particular compounds of C. unshiu honey, not pointed out in previous researches of other Citrus honey types.

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### PALYNOLOGICAL ANALYSES OF HONEYS PRODUCED IN HONEY-FLOW SEASON IN OYO STATE, NIGERIA

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This study was conducted to evaluate the pollens source of honey harvested in the honey flow season in Oyo State, Nigeria. A total of 19 samples of honey were collected from 19 bee hives from two different villages in the January, 2015. The experiment was carried out at Tede Atisbo Local Government of Oyo State and Ladoja and Sons Plantation and Apiculture farm located at Saki-West Local Government of Oyo State. All the samples of honey collected were analysed palynologically in the Main Laboratory of the College of

Health Sciences, Ahmadu Bello University, Zaria, Nigeria. Result shows the presence of fifteen and twelve pollen samples in Atisbo and Saki West Local Government Areas respectively. The result revealed that all the honey tested were multifloral. The prominent pollens include: Ipomea batata (Convolvulaceae), Ocimum spp (Lamiaceae), Launea taraxacifolia (Compositae), Anarcadium occidentale (Anarcadiaceae), Gliricidia sepium (Fabaceae), Elaeis guinensis (Palmaceae), Glycine max (Fabaceae), Glycine max (Fabaceae), Helianthus annuus (Compositae), Brassica spp (Brassicaceae), Moringa oleifera (Moringaceae), Sophora japonica (Fabaceae), Cocos nucifera (Palmae), Psidium guajava (Myrtaceae), Gossypim spp (Malvaceae), Mimosa pudica (Fabaceae), Hymenocardia acida (Phyllanthaceae), Piliostigma thoningi (Fabaceae), Eucalyptus spp (Myrtaceae), Psidium Spp (Myrtaceae), Magnifera indica (Anarcadiaceae), Parkia biglobosa (Fabaceae).

### COMPARISON OF THE PROTECTIVE EFFECT OF MOROCCAN PROPOLIS AND THYMUS VULGARIS AGAINST OXIDATION OF COSMETIC EMULSIONS

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Emulsions encompass a vast number of materials and products including foodstuffs, personal care products, and pharmaceuticals. Such emulsions are thermodynamically unstable. The aim of this study was to compare the protective effect of natural products from propolis and thyme on the oxidative stability of oil-in-water (O/W) emulsions.

O/W emulsions were prepared by mixing almond oil (20%), wheat germ (20%), Tween 80 (1.1%), Span 80 (0.9%), Phenonip (0.8%), Xanthan gum (0.9%), and propolis extract, thyme extract, (0.01-0.04%) or BHT (0.01-0.04%). Distilled water was added to 100% and all ingredients were homogenized. Emulsion without extract or BHT were control samples. All emulsions physically stable were stored at 37°C for 9 weeks. Rheological characterization of different emulsions was performed throughout the storage period. The rheological behaviour was analysed using a fitted power law.

The results show that propolis extract at the different concentration used was the most effective as antioxidants in tested emulsions comparing with those of thyme, depending on the proportion of almond and wheat germ oil. The emulsions presented a clearly a pseudoplastic behaviour.

### QUALITY ANALYSIS OF EGYPTIAN POLLENS COLLECTED BY HONEY BEE, APIS MELLIFERA L., COLONIES

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Four dominant pollen species, i.e. Egyptian clover, Trifolium alexandrinum; maize, Zea mays; sunflower, Helianthus annuus and sesame, Sesamum indicum, collected by honey bees at Fayoum region, Egypt were chemically analyzed for their quality. Fresh pollen loads had ranges of 21.68% -24.91% moisture; 2.97- 3.27% ash (minerals were descendingly: K, P, Mg, Ca, S, Na, Fe, Zn, Mn and Cu); 18.46% -27.85% total carbohydrates; 8.06% -11.49% total lipids, and 22.25% -24.96% total proteins. Amino acids fractionation indicated that the ratio of each acid to total amino acids was nearly stable. Proline was the dominant amino acid in these pollens, whereas tryptophan was markedly high in clover pollen, with traces in maize, and sunflower pollens, but it was undetected in sesame pollen. Remarkable amounts of cystine were also detected in clover pollen, with traces in sunflower, while it was undetected in maize and sesame pollens. Honey bees collect pollen species with high nutritive value especially proteins and amino acids. The present findings would shed some light on the influence of the floral origin on the chemical composition of pollen species in order to monitor their nutritional and medical values and to contribute to the choice of criteria and quality standards of Egyptian bee-pollens.



### A PROJECT TO IMPROVE AND CHARACTERIZE SOUTH AFRICAN HONEYS: THE EDEN HONEY BEE PROJECT

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Beekeeping in South Africa is relatively small in terms of the overall agricultural sector: in 2006 the country produced 1,500 tons per year (the 64th largest honey producer), only the 0.1% of world production (SABIO, 2008). The estimated demand for honey in South Africa in 2008 was between 2700 and 3000 tons per annum, while production was at between 1700 and 2000 tons per annum; the shortfall of approximately 1000 tons was imported. South Africa is by far the largest importer of honey in the Southern African Region. In 2005 South Africa imported natural honey to the value of US\$ 1,2m which equals 73% of total imports; China is the main supplier of honey to the South African market (SADC, 2012).

In 2008 the bulk honey sold at a price in excess of R25 per kilogram, gave the annual honey crop a value of roughly R50 million.

South African honey is of high quality and could easily compete against other honeys on the world market. With available natural resources, the South African Bee Industry Organisation (SABIO) assume the industry could expand to twice or three times its present size.

The South African beekeeping sector shows a lack of accurate information, though honeybees are vitally important for agriculture and conservation of the floral reserves. It was estimated that less than 10% of beekeepers were registered in 2008, although the practice is mandatory.

The South African beekeepers on their own are not be capable of providing all the necessary funding and infrastructure to support and sustain the honeybee population. This is a matter of concern when considering the serious challenges currently facing the beekeeping field such as vandalism and theft, pests and diseases, competition from cheap imports, loss of forage and a lack of research capacity. In terms of institutional and financial capabilities, the Government is unable to cover all activities due to budgetary constraints and staff deficiency, hence the need to find other sources of funding to enhance Government intervention.

On the other hand, the local beekeeping associations are present but not involved in beekeeping projects, due to lack of knowledge and funding.

The Eden Honey Bee Project ([www.edenhoneybeeproject.com](http://www.edenhoneybeeproject.com)) is an ethical project to help local beekeepers maintain healthy beehives. The Eden Honey Project also wants to conserve the Cape Honeybee (*Apis Mellifera Capensis*) and its natural environment, resulting in excellent bee health and smaller quantities of high quality ethically harvested honey. Named "Eden" because this project is developed in the Garden Route of Eden District, in South Africa, where no trace of the AFB (American foulbrood) illness has yet been found. Partners of this Project are: Apimondia, Conservation Global and the IZS Lazio e Toscana.

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### ORGANOLEPTIC AND MELISSOPALYNOLOGICAL ANALYSIS OF HONEYS FROM WESTERN CAPE PROVINCE IN SOUTH AFRICA

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The Apiculture laboratory of IZS Latium and Tuscany, in collaboration with Conservation Global and the Knysna Beekeeping Association realized in 2015 n. 39 samples to realize organoleptic, melissopalynological and humidity analyses on honeys coming from the Western Cape province (South Africa). This area is strongly characterized by a typical South African flora called "Fynbos". Organoleptic characteristics, humidity and pollen present in the honeys were studied. Ten honeys out of the 39 sampled (25.6%) were monofloral honeys, while 29 (74.4%) resulted wildflowers.

The microscopic examination of the sediment revealed a strong presence of the Eucalyptus genus, a pollen that seems to strongly characterize the South African honeys. The single-flower eucalyptus honeys were characterized by interesting organoleptic profiles, like a nice dark golden amber color and a strong flavor of toffee. Two samples out of the 29 monofloral honey, characterized by pollens with a botanical origin still unknown, showed optimum organoleptic characteristics: light amber color and very delicate vanilla floral flavor. In general, the humidity levels of the sampled South African honeys were higher than usual for the European honeys, with a mean value of 17.4%. Furthermore, fermented smell and taste (also smoke taste in some of them) were present in 21 (54%) samples. Regarding the hygienic aspects, only 11 (33%) of the samples had satisfactory traits. Many samples, in fact, presented dark particles of impurity, wax or other material. Frequently the consistence observed was elastic and stringy. This was probably due the processing of honey or to the wrong sampling of the honey delivered to the laboratory.

Further studies should be realized to characterize South African honeys (e.g. to quantify antioxidant compounds and content in methylglyoxal - MGO), to verify their potential application in apitherapy.

### BACTERIA ASSOCIATED TO DIFFERENT HONEY BEES' MATRICES

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The Apiculture Laboratory of the Istituto Zooprofilattico Sperimentale del Lazio e della Toscana (IZSLT), in collaboration with the Istituto Superiore di Sanità (ISS), performed from 2010 to 2016 the microbiological examination of different apicultural matrices (bee carcasses, hive matrices, honey and pollen) from both healthy and sick colonies, in order to improve the knowledge about the bacterial flora of bees and hives. Results from our work could help in identifying potential beneficial microorganisms to be used as antibiotic alternatives for the prevention and control of bee diseases in future studies, thus avoiding the environmental side-effects of antibiotics.

Samples of bees and bee products were collected for microbiology. Gut microbiota and microbiota of body surfaces of adult bees and larvae, as well as microbiota found in hives and honey bee products, were investigated. Both healthy and sick colonies were included in our study. Selective and non-selective media were used for cultures. Bacterial colonies were examined for biochemical characteristics and Gram staining. 16S rDNA PCR analysis was performed in the case of uncertain phenotypic identification.

A broad spectrum of germs was isolated especially from guts of larvae and adult bees. Members of the families Enterobacteriaceae, Gram-negative non-Enterobacteriaceae, Lactobacillaceae, Streptococcaceae, Rhizobiaceae, Alcaligenaceae, Corynebacteriaceae, Brucellaceae were identified. Less heterogeneity was found in

honey; members of the families Lactobacillaceae and Corynebacteriaceae were identified. Finally, members of the family Bacillaceae and some fungi (e.g. *Aspergillus*) were identified from feces and pollen.

### THE SCIENTIFIC VETERINARY MEDICAL ASSOCIATION FOR APICULTURE (SVETAP)

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On October 2016 it was founded in Pisa the first Veterinary Scientific Association for Apiculture. The acronym in Italian language sounds SVETAP and stands for: "Società Scientifica Veterinaria per l'Apicoltura".

SVETAP is a non-commercial and non-profit scientific association, which has technical-scientific, cultural and educational finality, based at the Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "Mariano Aleandri" (IZSLT) in Via Appia Nuova, 1411, Rome.

The establishment of the society's headquarter at the IZSLT is motivated by its wide experience (especially thanks to the presence of the Beekeeping Unit) in research, diagnostic, advice, assistance and training in beekeeping, and also by its continuous exchange and collaboration with other national and international institutions.

The founding partners are veterinarians from different institutions (Universities, AA.SS.LL., II.ZZ.SS.), and different aged that represent Italy from North to South, to ensure a diversified approach with an integrative view.

The president of the Association is Dr. Giovanni Formato, head of the Apiculture Laboratory of IZSLT and one of the main promoters of the scientific society, flanked by Prof. Carlo D'Ascenzi of the Veterinary Department of the University of Pisa as Vice President and Prof. Michele Mortarino of the Veterinary Department of the University of Milan as Secretary.

The main objectives of SVETAP are:

1. To promote and to enhance the veterinarians professional skills applied to the field of integrated protection of beekeeping patrimony, from the perspective of bee health, food safety and public health, helping beekeepers to produce respecting the Good Beekeeping Practices (GBPs), ensuring the quality of honeybee products;
2. To cooperate with the view "One Health" with:
  - The professional body, representative bodies and associations of Veterinary Surgeons;
  - Professional associations, representative bodies and associations of the other professional fields involved at various levels in beekeeping;
  - Universities, research institutions and scientific associations involved in beekeeping;
3. To promote the study and research within the veterinary disciplines applied in beekeeping;
4. To facilitate the technological upgrading and the development of innovation in beekeeping;
5. To establish working groups and committees for the study of specific topics in the field of beekeeping;
6. To set up awards and scholarships;
7. To promote research activities related to the field of beekeeping;

8. To ensure the continuing education of its members;
9. To promote and organize training activities (e.g. Conferences, workshops, seminars, courses of all types including undergraduate, master, round tables, etc.), both at national and international level;
10. To promote the activities of communication, dissemination and correct technical and scientific information of the beekeeping topics, also to public opinion.

Highly trained veterinarians in the apiculture sector will contribute to guarantee a proper support for beekeepers and a better quality and quantity of beehive products.

### MICROBIOLOGICAL QUALITY AND FOOD SAFETY EVALUATION OF BEE POLLEN: A PRELIMINARY STUDY IN TUSCANY

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Bee pollen is a natural product of the hive. Its high content of healthy nutrients make it appreciated in the human diet. It can be collected by beekeepers in pollen traps fitted to hives. The aim of this study was to evaluate hygienic quality and microbiological contaminations in a bee pollen production chain located in Tuscany (Italy). A total of 12 pollen samples were collected at different moments of the production cycle (collection, after dehydration, after 90 days of storage). There were also collected 10 environmental swabs and bee samples. The samples were submitted for physico-chemical (pH, water activity) and microbiological (*S. aureus*, Enterobacteriaceae, Salmonella spp., *E. coli* O157, *L. monocytogenes*, *Bacillus cereus*, colony count at 30°C, moulds, yeasts, *Pseudomonas* spp., total Coliform) analysis. According to the results obtained, Salmonella spp., *L. monocytogenes* and *E. coli* O157 were never found in bee pollen samples. Instead, a high microbial contamination was detected in fresh bee pollen, as expressed by colony count at 30°C values. The contamination was mainly relate to high levels of Enterobacteriaceae values; among these, environmental ones, such as *Pantoea* spp. and *Serratia* spp., were identified. The origin of enterobacteria is related to bees, whose contamination could come from the environment or from bee intestine itself. Regarding environmental swabs, it can be excluded human contamination during processing. High levels of moulds were detected in bee pollen after collection and dehydration and they were related to the genera *Penicillium*, *Alternaria*, *Aspergillus* and *Mucorales*. The stored pollen samples instead had lower microbial and fungal contamination values. The dehumidified pollen sample has a water-activity value < 0.6, that prevents the microorganisms multiplication, including moulds.

Preliminary results show a good quality of the bee pollen investigated as described by literature, despite the lack of legislation regarding standard microbiological criteria.

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### AFB AND EFB TREATMENT WITH OXYTETRACICLINE AND SHOCK SWARMING TO GUARANTEE HONEY BEE SURVIVAL AND NO DETECTABLE RESIDUES IN JAR HONEY - PRELIMINARY RESULTS

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American Foulbrood (AFB) and European Foulbrood (EFB) are the most widespread and damaging bacterial

honeybee (*Apis mellifera*) brood diseases.

Several methods have been adopted to control AFB and EFB as antibiotics (Thompson et al., 2006; Budge et al., 2010), destruction of infected hives (Roetschi et al., 2008), shook swarm method alone (Budge et al., 2010) or in association with oxytetracycline (Waite et al., 2003).

In this paper we tested the efficacy of shook swarm method in association with oxytetracycline (OTC) against AFB and EFB, and OTC residues in honey harvested, administering it in jar caps.

At the end of March 2016, 19 hives with AFB and 10 hives with EFB, 3-6 frames each were partially (leaving honey and pollen storage combs) shaken onto foundations. Partial shaken onto foundation was realized taking off all the combs provided with brood (both open and closed, egg included) and leaving combs with honey and pollen storages. All the combs taken off were replaced with combs with new foundations.

Authors were oriented towards partial shaken because of the low flowering being at the end of winter and subsequent impossibility of bees to build combs without honey and pollen storages. The shook swarm method was associated to a following treatment with a total of 2,4 g OTC/hive administration (0.48g of oxytetracycline/hive administered once a week, during 5 consecutive weeks to cover n.2 cycles of broods). One day after the shaken the colonies started the treatment with OTC administrations.

So far (i.e. after 7 months from the treatments start) only two hives presented an AFB relapse (7%) at 6th months from the treatment.

Only 5 (17.2%) of the 29 hives were able to produce honey for a total of 70 Kilos (average of 14 Kg/hive). Concerning the OTC residues in comb honey, that were verified by HPLC with fluorimetric detector (LOQ = 9 ppt), we found only 3 samples out of 29 (10.3%) that were positives after 7 days by the end of the treatments, respectively with 7 ppb, 6.5 ppb and 0.43 ppb. Only 1 sample (3.4%) was positive for OTC after 3 weeks by the end of the treatments with 0.05 ppb. And, finally, only 1 sample (3.4%) was positive for OTC after 5 weeks by the end of the treatments with 0.29 ppb.

### FEEDING EFFECT ON THE EVOLUTION OF HONEYBEES' SWARMS

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The products of bees feeding complement the usual classic food of the colonies, in conditions of dietary deficiencies which often occur naturally due to bad weather or a bad floral diversity.

The objective of this work was to assess the effect of different syrups of bee feeding on both the evolution of honeybees and on the state of honey and pollen stocks, by doing a test in an experimental apiary (12 homogeneous swarms divided into 4 groups) in the Higher Institute of Agronomy of Chott Mariem during the period which took place between the first of April and the end of June. Each week, each colony received 500ml of a 50/50 classic solution to the basis of white sugar, brown sugar, or a mixture of fructose and glucose. The consumption of various syrups by bees differs depending on the hours of the first day as well as the days of the distribution. The weight of the hives increased depending on time with a significant difference between the different batches. The evolution of the brood surface, the stock of pollen and honey during the period of feeding has been growing. The bees have drawn from the reserves of pollen to increase the brood. In the course of our trial, the mixture fructose-glucose was the most appreciated and the most favorable for the raising of the brood and making honey. The inversion of sucrose to glucose and fructose by the bees has a significant energetic power for the bee.

### HONEYBEE HEALTH STATUS IN THE WESTERN CAPE PROVINCE OF SOUTH AFRICA

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Beekeeping in the Western Cape Province of South Africa is a developed sector, thanks to the natural and productive areas of Fynbos and Forests of Rhenendal, but really few data are available on the health status of the colonies. A monitoring activity on the health status of the hives was realized at the end of February 2015 by Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri" (IZSLT), Conservation Global and Apimondia. 7 different apiaries were inspected in the area delimited by Wilderness (West side), the Craggs (East side) and the Outeniqua mountains (North side). A total of 31 beehives were visually inspected and sampled to evaluate their health status and the presence of the main honeybee diseases: *Nosema* spp., *Varroa destructor*, 7 honeybee viruses (ABPV, CBPV, DWV, SBV, KBV, BQCV, IAPV), American foulbrood (*Paenibacillus larvae*), European foulbrood (*Melissococcus plutonius*), *Aethina tumida*. Laboratory analysis were carried out by IZSLT adopting the following methodologies:

*Varroa destructor* infestation level after alcohol wash (OIE method) of the adult bees;

*Aethina tumida* by hive visual inspection.

American foulbrood (AFB) and European foulbrood (EFB) by PCR from adult bees and by immunomigration from brood;

*Nosema* spp. (*apis* and *ceranae*) microscopy spore count and PCR by foragers bees;

viruses PCR on adult bees;

*Varroa destructor* was present in all the apiaries. Its mean infestation levels on adult bees was 1,39%±1,1%: amount able to give an economic damage, even though quite low considering the season (end of Summer). *Aethina tumida* was present in all the apiaries too.

Concerning AFB and EFB, no samples resulted positive to them and no clinical signs were observed in the inspected hives.

*Nosema ceranae* resulted always absent in all the samples, while *Nosema apis* was present in 60% of the samples with a mean amount of 500.000 spores/bee; only one apiary was pathogen free for *Nosema apis*.

The most spread viruses were ABPV and BQCV, found in 100% of the sample, and SBV, found in 86% of the samples. IAPV was found only in one apiary and the other viruses were absent in the samples.

The results obtained by the analysis of the samples are the first report of a wide spectrum health status monitoring of the apiaries in the Western Cape Province of South Africa.

### BOTANICAL ORIGIN OF HONEY FROM DIFFERENT AGRICULTURAL REGIONS OF THE REPUBLIC OF MACEDONIA

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The aim of the research was to determine the botanical origin of honeys produced in various agricultural regions in the Republic of Macedonia.

Materials for the work were 180 samples of honey that were taken directly from beekeepers who keep their bees in the six agricultural regions of the country: Mediterranean region, Pelagonija, region, Skopje- Kumanovo region, Western region, Eastern region and Golemozerski region.

Melissopalynologic analysis was performed based on the method of the International Commission for Bee Botany (ICBB), published by Loveaux et al. (1978).

Out of the 180 investigated samples, 83% were samples of honey with nectar origin, while 17% were honeydew samples.

Out of 151 samples of nectar honey, based on melissopalynologic analysis, 3 samples were classified in monofloral chestnut honey, while 148 were samples with polyfloral origin.

Out of 29 samples of honeydew, eight were pure honeydew honey, while 21 sample were mixed honeydew and nectar honey.

#### PRELIMINARY RESEARCH ON NECTAR SECRETION IN TWO CITRUS UNSHIU CULTIVARS: HONEY PRODUCTION POTENTIALS

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Honey production is a complex biological process that unites synergistic activity of plants and honey bees which starts with nectar foraging. Satsuma mandarin (*Citrus unshiu* Marc.) in Neretva Valley (Croatia) represents one of the northernmost Citrus honeybee forage in the world. A preliminary study on nectar secretion dynamics was carried out on two C.unshiu cultivars, Kawano Wase and Zorica, in the blooming period. The amounts of nectar and sugar concentrations per flower were recorded in the bud and fully open flowers three times a day (9:00/13:00/17:00). The results revealed statistically significant differences in the average amount of nectar/flower between studied cultivars ( $F=108.02$ ,  $p < 0.0001$ ). The average amount of nectar in Kawano Wase was 21.73  $\mu\text{L}/\text{flower}$  in the bud stage and 22.47  $\mu\text{L}/\text{flower}$  in open flowers, while Zorica cultivar showed lower values in both stages: an average of 14.31  $\mu\text{L}/\text{flower}$  and 18.50  $\mu\text{L}/\text{flower}$ . The results on the average concentration of sugar/flower also reflected significant variations ( $F=85.70$ ,  $p < 0.0001$ ); the average sugar concentration determined in Kawano Wase bud stage and open flowers was 4.50 mg/flower and 5.86 mg/flower, respectively, while results observed in Zorica revealed significantly lower sugar concentrations (2.64 mg/flower and 3.52 mg/flower). Monitoring of nectar amounts and sugar concentration over the course of the day revealed the highest average amount of nectar and sugar concentration in open Kawano Wase flowers at 9:00 and in Zorica at 13:00. The results indicate great potential of both cultivars for honey production. However, Kawano Wase cultivar exhibited better results in both qualitative and quantitative parameters.

#### A SEASONAL ANALYSIS OF HONEY BEE-COLLECTED POLLEN FROM MATELICA (MARCHE, ITALY): PALYNOLOGICAL ORIGIN, PHENOLIC CONTENT AND ANTIOXIDANT PROPERTIES.

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Honeybee pollen contains high level of proteins, lipids, carbohydrates and vitamins and possesses antioxidant properties which are known to be effective in fighting against free radicals; therefore, bee-collected pollen has high potential for nutritional and medical applications. However, the composition of bee pollen has not been yet fully characterized and it may changes according to the palynological, geographical origin and collection season. This study aims to assess the palynological origin, phenolic contents and antioxidant properties of multifloral bee pollen from a rural location in Matelica (Italy). The project involves the analysis

of pollen samples on a seasonal basis (April-September 2016) using the following assays: a) Total Phenolic Contents (TPC); b) 1, 1-diphenyl-2-picrylhydrazyl (DPPH) Scavenging and Hydroxyl Radical Scavenging (HRSA) assays. Preliminary results show that using gallic acid as standard, TPC values are 11.32 mg GAE/gr and 13.52 mg GAE/gr for April and May pollen samples. DPPH and HRSA scavenging activities for April samples are 63.46% and 55.47%. In May, DPPH and HRSA scavenging activities are 68.11% and 60.72%. Seventeen families were found in the pollen mixtures: with Fabaceae, Rosaceae, Salicaceae, Brassicaceae, being the most abundant. This study provides the first fingerprint of the seasonal pollen properties in the area.

#### THE QUALITY AND SAFETY EVALUATION OF EAST SERBIAN HONEY- DJERDAP HONEY

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The main objective of this study was to evaluate the quality and health safety of honey from east serbian Djerdap region, verifying their compliance with national and international regulations. Two honey types (Blossom honey and Acacia honey) were selected and analysed for several quality parameters such as content of reducing sugars and sucrose, moisture, content of HMF, diastase activity, free acidity and water-insoluble content. The obtained results completely agree with the European Commission, Codex Alimentarius and national Regulation indicating adequate processing, good maturity and freshness. Through the food chain, various contaminants can affect human health, therefore in honey were determined pesticides, metals, radioactive elements and residues of antibiotics. The content of pesticides and metals (As, Pb, Cd, Hg, Cu) were under the LOQ, and the concentration of Fe and Zn in all samples was in accordance with Regulations. The radionuclides content was significantly low for 137Cs and 40K, while for 238U was under LOQ. Antibiotic residues in all honey samples were negative. The results obtained in this study, indicate that honey produced in east serbian Djerdap region are characterized by excellent quality and health safety properties according to international standards.

#### AMINO ACID AND VITAMIN CONTENT OF PROPOLIS COLLECTED BY NATIVE CAUCASIAN HONEYBEES

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Polyphenol content of propolis draw huge attention due to their benign biological properties at the chemical composition studies. The present study, therefore, investigated the amino acid and vitamin composition of propolis collected by *Apis mellifera caucasia* from 60 colonies of nonmigratory beekeepers native to secluded Ardahan province, Turkey. *A.m. caucasia* is known for their distinctive propolis collecting capability. Vitamin combination of propolis determined by HPLC fluorescent detector, in the meantime amino acid analysis performed with UFLC (Ultra-Fast Liquid Chromatography) system consisted of binary pump and UV/VIS. Our findings record that vitamin and amino acid content of propolis samples collected from three areas of different altitudes in the same region differ from each other. Vitamin B1 content and Vitamin B2 content ranged between 0.025-0.16 mg/100g, and 0.304-0.777mg/100g respectively. Maximum amount of amino acid reported as leucine, while minimum amount of amino acid was seen as tryptophan in Ardahan propolis. Consequently, vitamin and amino acid content of propolis, which derived from secondary plant metabolites of resin, vary depend on their geographical altitudes. Those vitamin and amino acids found in

propolis composition is believed to contribute its beneficial therapeutical properties.

## THE APICULTURE LABORATORY OF THE ISTITUTO ZOOPROFILATTICO SPERIMENTALE DEL LAZIO E DELLA TOSCANA "M. ALEANDRI" TO GUARANTEE HIVE PRODUCTS QUALITY

M. PIETROPAOLI<sup>1</sup>, J. RIVERA-GOMIS<sup>1</sup>, M. MILITO<sup>1</sup>, V. BELARDO<sup>1</sup>, F. SCHOLL<sup>1</sup>, G. FORMATO<sup>1</sup>

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The Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri" (IZSLT) is a public institution of the National Health Service which, on behalf of the Ministry of Health, provides services and research related to animal health and food safety.

More in detail, its activities are related to: diagnostic service of animal diseases, microbiology, chemical and physical analysis, research, epidemiological surveillance, training and international cooperation. The laboratories are accredited according to the UNI CEI EN ISO / IEC 17025. IZSLT interacts with the Authorities at central level (Ministry of Health and other Ministries) and local level (Regions, Local Health Units - AASSLL, etc.) and other public and private organizations.

IZSLT is headquartered in Rome and has other eight provincial offices distributed both in Lazio and Tuscany regions, all closely linked together. Among other IZSLT in Italy (around 10) is a network of laboratories that represent a unique situation in Europe, with National Reference Centres. In IZSLT are the following National Reference Centres: Antimicrobial Resistance; GMO - Genetically Modified Organisms -; Equine Diseases; Equine Infectious Anemia; Quality of Milk of Sheep and Goat; Forensic Veterinary Medicine.

In 2008 the IZSLT established an Apiculture Unit (Unità Operativa di Apicoltura) (<http://www.izslt.it/apicoltura/>) in order to join and improve all the activities already carried out by the IZSLT in the beekeeping sector. In fact, in the frame of IZSLT activities, the Apiculture Unit's mission (<http://www.izslt.it/apicoltura/>) is to certify and guarantee the health and welfare of bees, and the hygiene and safety of the hive products, through: diagnosis of bee diseases and analyses on hive products, support to Governmental Institutions in drawing up legislation, research activity, collaboration with other laboratories or institutions, technical support and training for operators, pollution environmental monitoring using honey bees and protection of honey bee biodiversity.

## PROFICIENCY TESTING SCHEME FOR PESTICIDES IN HONEY

E. MESSINEO<sup>1</sup>, E. DIORE<sup>1</sup>, N. LE BARON<sup>1</sup>, A. BOUBETRA<sup>1</sup>

<sup>1</sup> BIPEA, Paris, FRANCE

BIPEA (<http://www.bipea.org>) organizes regular proficiency-testing schemes (PTS) in many analytical domains, including the analysis of pesticides in honey. As an example, in October 2015, one test was conducted using an organic honey spiked with 21 pesticides, at levels between 15 and 200 µg/kg, with 31 participating laboratories. This test was intended for the quantification of 20 molecules of pesticides residues as for the identification and the quantification of another one. The techniques used by the laboratories were GC-MS-MS, LC-MS-MS, GC-MS and GC-ECD, according to the molecules.

Participating laboratories were required to return their results on a dedicated website after a period of one month, and a statistical treatment of the data was as usual performed by BIPEA according to ISO 13528 [1]. Assigned (consensus) values were calculated from the participants' results and the performances of the laboratories could then be evaluated individually and collectively according to ISO 17043 [2].

These tests allow participating laboratories to draw up a general inventory of their analytical skills, and are a very useful tool to detect bias or non-compliant results; they act as a warning signal for the implementation of corrective and/or curative actions in the laboratory.

### KEYNOTE SPEAKERS

1. C. Mateescu - A new approach for honey and hive products - New medicines, supplements, cosmetics and new technologies
2. P. Perez-Gomez - Apipuncture: practical demonstration and clinical evidence
3. A. Couto - Bee Venom Teraphy: micro api-puncture application
4. A. Colonna - New perspectives for apitherapy among integrated therapies. Hive products and their application in human medicine
5. C. Aosan - Apitherapy in the daily practice - Clinical applications
6. M.A. Lopez-Pazos - Study of selected clinical cases by speciality: 15 years of Apitherapy in Veterinary Medicine practice from Southern Chile

### ORAL PRESENTATIONS

1. N. Iegaki - Royal jelly improves mental health
2. S. Silici - Essential and omega fatty acid content of beebread and its effect on heart diseases
3. P. Bhandari - Bee Bread: Various usage of bee bread and clinical experiences
4. A. Varadi - Practical api-nutrition - what happens when we introduce bee products in our daily diet
5. A. D'Angeli - "BeesAble Project": Beekeeping in Rehabilitation
6. D. Musa - Apiculture intervention for malaria-mitigation in Nigeria (AIM)
7. L. Crimaldi - Activity studies of characterized, standardized and highly purified propolis extract
8. K. Rihar - Influence of propolis and royal jelly on the antibacterial activity of honey against *Pseudomonas aeruginosa*
9. B. Lyoussi - Propolis: is there a potential for the development of new and efficient antimicrobial agents?
10. V. Zaccaria - Propolis: intracellular mechanism of action to clarify anti-inflammatory and antioxidant activities
11. E.H. Abd Al Rahman - Promising antiprotazoal activities of Bee Propolis
12. L.I. Daker - Bee venom acupuncture in multiple sclerosis
13. C. Salvatori - Evaluating the effectiveness of propolis in the non-surgical treatment of periodontal disease
14. M. Biagi - Bee propolis in the treatment of *Helicobacter pylori*: in the right way to clinical application
15. A. Miceli - Experiences and perspectives of infected ulcers and wounds treated with knitted viscose mesh impregnated with Manuka honey in the INAIL Physical Rehabilitation Center of Volterra (Pisa- Italy)
16. A. Zbucnea - Medicinal honey dressings for burns and other types of wounds: properties and clinical effects
17. M.V. Strant - Api-therapy in daily practice

### POSTERS

1. M. Ansari - Protective effect of Saudi Arabian sidr honey against lead-induced anemia and hepato-renal toxicity
2. R. Alleva - Organic honey supplementation reverses pesticide-induced genotoxicity by modulating DNA damage response in a population chronically exposed
3. B. Lyoussi - Bee Products: Chemical composition and therapeutic application
4. M. Mostafa - Bee Venom acupuncture in cognitive dysfunction in Parkinson's disease
5. B. Filipic - Water soluble propolis extracts and human interferon- $\alpha$  (HUIFN-ALFAN3) shows anti-influenza virus activity in vitro
6. A. Varadi - Api-feed & Veterinary apitherapy applications for livestock
7. B. Lyoussi - Antioxidant activity, hepatoprotective and antidiabetic effects of the hydroalcoholic extract of propolis in streptozotocin-induced diabetic rats
8. J. Majtan - Bee-derived antibacterial peptide, defensin-1, promotes wound healing in vitro and in vivo
9. N.J. Fernandez - A scientific note on the first report of honeybee venom inhibiting *Paenibacillus larvae* growth
10. A.G. Hegazi - Determination of oxidative stress in apiacupuncture of patients suffering from chronic low back pain
11. A.G. Hegazi - Evaluation of antibacterial activity of Saudi Arabia honeys
12. A.G. Hegazi - Nutritional Facts of Honey
13. A.G. Hegazi - Novel approach of bee venom as treatment of rheumatoid arthritis
14. B. Lyoussi - The protective effect of *Thymus vulgaris* honey, *Origanum vulgare* essential oil, and their aromiel against CCL4-induced liver injuries in rats
15. M. Soyulu - Glycemic index of sucrose, fructose and honey added highbush cranberry (*Viburnum opulus*) juice
16. C. Rossi - Application of two homeopathic remedies on honeybees against varroaosis: preliminary results
17. M. Pendleton - A Day in the Life of a Caribbean Bee Sting Clinic
18. B. Filipic - Effect of pH variation during propolis extraction with the use of PBS and consequently 70% ethanol as solvent on its antimicrobial/antiviral activity
19. B. Lyoussi - Moroccan propolis a natural anti-bacterial and anti-biofilm against *Staphylococcus aureus* with no induction of resistance after continuous exposure
20. C. Ene - An original method of bee pollen processing with encouraging therapeutic results

### A NEW APPROACH FOR HONEY AND HIVE PRODUCTS - NEW MEDICINES, SUPPLEMENTS, COSMETICS AND NEW TECHNOLOGIES

#### C. MATEESCU

The last developments in the world of bee products give a new perspective for their increasing use in various types of products: food supplements, functional food, medicines for both humans and animals and cosmetics. Honey is well known for its food quality but researches have shown that honey itself may be an excellent wound healing agent and a good antibacterial. New honey dressings which can be directly put on the wounds have been developed. A compound in honey called defensin-1 is a hope that one day it could be used to treat burns and skin infections and to develop new drugs that could combat antibiotic-resistant infections. Propolis with its composition diversity offers itself a large variety of biological actions (antimicrobial, antioxidant, anti-tumor, wound healing, to mention but a few) with direct effects on several human ailments and diseases. Bee collected pollen and bee bread are super-concentrated functional foods with direct effects on the good function of the liver, prostate, heart and circulatory system etc.). Royal jelly - known in many cultures as an elixirum shows new perspectives for human health. Bee venom in its natural form and apitoxin - the dry bee venom - represent a hope for many patients with degenerative diseases. New technologies meant to increase the bioavailability of their active ingredients (nanotech, preparative techniques, extraction methods) are developed. However, all these products have still to be clearly defined in respect to their health claims. The international regulations related to food, novel food and medicines require an attentive assessment of both their biological activities and side-effects. Clinical trials with well defined protocols are needed, but standardization, quality, the presence of contaminants are still many drawbacks in their credible use. It is with this approach that apitherapy could gain a respectful and credible position among the complementary and alternative therapies.

### APIPUNCTURE: PRACTICAL DEMONSTRATION AND CLINICAL EVIDENCE

#### P. PÉREZ-GÓMEZ<sup>1</sup>

<sup>1</sup> UAH - Alcalá de Henares University, Madrid, SPAIN

As apitherapist I practice a profession that combines art, science and spirituality. The confluence of everything that helps to heal. Integrative Medicine. Acupuncture and Apitherapy have been practiced for thousands of years. Beekeepers were apitherapists unknowingly since prehistoric times. Probably, observing the effect of bee stings on the body itself, in China they found the energy channels and the points to regulate them. Apitherapy is the use of bee products, to help the body heal. Acupuncture is the use of needles and other elements of Chinese medicine, to restore or maintain the body harmony. Modern medicine also has a very ancient origin: the Animus curandi. It is part of human nature. When both medicines come together is what has been called Integrative Medicine.

- What is the disease? The disease is not the problem, it is the solution of the problem
- Neither we, nor anything outside cure. It is our body that heals. What we do is help in this healing.
- The role of emotions
- What makes Apitherapy in all this? The first apitherapists. Acupuncture.
- How choose bees acupuncture points?
- Cases and practical applications. Special collaboration with M. D. Cristina Aosan in a case of Multiple Sclerosis

### BEE VENOM THERAPY: MICRO API-PUNCTURE APPLICATION

A. COUTO

Overview of the cases I treated since 2003. I practice Apitherapy since 2003.

My specialty is Micro Api puncture.

Micro Api puncture is a Japanese method of Bee Venom Therapy.

This method is less painful for the patient and is also more efficient because it allows to treat a wider area with the same stinger.

Quantity of bee venom with Micro-Apitherapy

In my practice office I receive any kind of patients.

From people who want to look younger or loose weight to people with serious problems like Cancer, Auto-immune diseases such as Rheumatoid Arthritis or Multiple Sclerosis

I started to receive patients for apitherapy treatments in 2003. I received more than 600 patients since 2003. My patients never felt any Anaphylactic Shock.

Only 3 patients felt a strong unpleasant reaction to Bee Venom.

### NEW PERSPECTIVES FOR APITHERAPY AMONG INTEGRATED THERAPIES. HIVE PRODUCTS AND THEIR APPLICATION IN HUMAN MEDICINE

A. COLONNA<sup>1</sup>

<sup>1</sup>Physician, President of the Italian Association of Apitherapy, Italy

The world of medicine is changing; therapies do not only address a single symptom, but rather medicine must consider the patient in its complex state as environmental and social disease factors must be addressed. Thus, a global approach to care is the only way to effectively prevent disease by addressing how food, air, water, and soil influences pathogens.

This holistic vision is increasingly popular as new clinical approaches and medical protocols emerge, including natural approaches that have synergies with synthetic drugs. Within this framework of innovation, beehive products could play an important role with several interesting components.

In my personal daily professional experience, I experiment with the benefits of this new approach by working with patients who request synthetic pharmaceutical therapy only after trying natural products first. This is what drove me to also create the Association of Apitherapy in Italy with the aim of spreading the knowledge of the benefits of bee products.

For complete functionality, it is paramount that we can verify that the bee product is completely free of chemical or environmental additives coming from human intervention. In my speech, I will provide several examples of bee products that could be instrumental in daily practice and disease prevention.

Some laboratory research is already being conducted studying the various fractions of bee product extracts that could be used against important diseases either as a main therapy or to support the effectiveness of a synthetic drug.

We can all do big and small things to preserve this important knowledge and maintain it over time. We cannot lose this ancient wisdom.

While studying this, it is important to remember to maintain attention and an open mind; we must understand that bees have lived on this earth for millions of years and their genetic code is complete. They have shared life with humans since the very beginning of our history, following their billions of years living on the earth. This history has real value and humans should respect and understand it. We have much to learn from such a small insect with such huge expertise.

The knowledge that we derive from the natural world requires continuously deeper studies, which go hand in hand with the new scientific and technological discoveries. More studies should be implemented and supported on the effects and the composition of the different substances produced by bees for the benefit of our health. These are the objectives of the Association: to disseminate the available knowledge about this

practice, promote research and raise public awareness on the importance that bees have for the humans lives, not only as pollinators.

### APITHERAPY IN THE DAILY PRACTICE - CLINICAL APPLICATIONS

C. AOSAN<sup>1</sup>

<sup>1</sup>Apitherapy Commission of Apimondia - member, Romania

Why about apitherapy in the daily practice? After a long experience working and living with apitherapy. Since being student in the Medicine University, I was using bees products for me and my relatives. After graduating in 1994, I applied apitherapy and phytotherapy in parallel with conventional therapy. From 2004 on, I'm practicing 100% natural therapy, meaning apitherapy associated with other complementary methods (e.g. phytotherapy, aromatherapy, diet, psychotherapy, massage, Ayurveda).

In fact, using the proper bees products for each patient, matching the doses and administering in specific manner according to the personal needs, apitherapy is applicable to the majority of sufferance and also for maintenance and prophylaxis.

In the presentation I will offer some clinical examples of apitherapy treatments:

Benign right broncho-pulmonary tumor, with extension in the left lung

- after three months of treatment it was disappeared

Ischemic vascular cerebral accident, with sequelae on visual field, paresis on superior and inferior left limbs, psycho-intellectual capacity

- started api-phyto-therapy after eight months from the cerebral stroke

- recovered progressively until complete, during one year of treatment

Clinical applications of Apilarnil - triturated drone larvae, for adults and children

Clinical study for a group of 33 patients with multiple sclerosis treated with api-phyto-therapy

- on MRI some demyelinated lesions have disappeared, some other diminished; positive clinical evolution

- the results give the hope that these patients can be helped much more than it is said conventionally.

### STUDY OF SELECTED CLINICAL CASES BY SPECIALITY: 15 YEARS OF APITHERAPY IN VETERINARY MEDICINE PRACTICE FROM SOUTHERN CHILE

M.A. LÓPEZ PAZOS

Veterinary Medical Doctor

Apitherapy & Chinese Medicine Specialist

Casilla 3103, Temuco, CHILE

Based on the traditional and biomedical history of the bee products - using the properties of each product of the beehive and the synergy of them - I developed different Treatment Protocols for different pathologies. Far and above Apitherapy, the protocols combine many different therapeutic tools (Apitherapy, Traditional Chinese Medicine, Phytotherapy, Nutrition Support, Allopathic Modern Medicine), to treat and support many animal species. However, Apitherapy has been determinant in therapeutic results of clinical cases that I present.

Fifteen years of work on Clinical Integrative Veterinary Medicine show that the beehive products are fundamental elements on the outstanding results CENTROVETERA - Apitherapy Veterinary Medical Center - offers.

For best results, we need to pay attention to quality, concentration, combination and dosis / time of treatment, specific for each patient's needs. To achieve the former, in the practical and private work of CENTROVETERA,

we included the sourcing of the “best” beehive products and the handmade, artisan production of the “api-phyto-medical formulations” for animals, to walk from the pathology to health. The presentation graphically shows examples of Integrative Treatment, including beehive products in different animal patients and applied by different ways. To simplify the presentation, the patients were ordered by Medical specialities, like Dermatology and Tegument Surgery (big wounds, skin and general dermatological diseases); Neurological / Neuromuscular diseases, Degenerative diseases, Autoimmune diseases, Reproduction, etc...

### ROYAL JELLY IMPROVES MENTAL HEALTH

N. IEGAKI<sup>1</sup>, Y. NARITA<sup>1</sup>, N. HATTORI<sup>1</sup>, K. ICHIHARA<sup>1</sup>

<sup>1</sup> API Co., Ltd., Gifu, JAPAN

Recently, a randomized, placebo-controlled, double-blind trial of royal jelly (RJ) was conducted in 61 healthy volunteers, in which subjects who received the RJ drink once daily for 6 months showed better mental health scores of the short-form 36 health survey questionnaires compared with the placebo group. In order to confirm the clinical effect of RJ, we performed experimental studies using the unpredictable chronic mild stress (UCMS) rodent model of depression. Mice were loaded with UCMS and given a diet containing RJ for 3 weeks. The RJ-fed group did not cause prolongation of immobile time, a depression-like behavior, in tail suspension test, suggesting that RJ prevents and/or relieves stress-induced depression. Thus, we have been investigating its mechanisms of action using analysis of gene expression of hormones involved in the hypothalamic-pituitary-adrenal (HPA) axis and microarray analysis of the adrenal gland. We have previously found that RJ facilitates the differentiation of cultured neural stem/progenitor cells into neurons and glial cells in vitro, and that RJ promotes recovery in trimethyltin-induced neuronal cell death in murine hippocampus in vivo. Taken together, our findings raise the possibility that RJ improves mental health in part through affecting the HPA axis response and the hippocampal neurogenesis.

### ESSENTIAL AND OMEGA FATTY ACID CONTENT OF BEEBREAD AND ITS EFFECT ON HEART DISEASES

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<sup>2</sup> TUBITAK, Istanbul, TURKEY

Beebread is processed pollen stored and packed in the honeycomb cells following the addition of various enzymes and nectar or honey it undergoes lactic acid fermentation. Proximate and fatty acids (FAs) composition of beebread samples from different botanical origins were examined and significant variations were observed. A total of thirty-seven FAs were determined with most abundance of (9Z, 12Z, 15Z)-octadeca-9, 12, 15-trienoic acid, (9Z, 12Z)-octadeca-9, 12-dienoic acid, hexadecanoic acid, (Z)-octadec-9-enoic acid, (Z)-icos-11-enoic acid and octadecanoic acid. Among all, cotton beebread contained the highest level of omega-3 FAs, 41.3 %. Unsaturated/saturated FAs ratios ranged between 1.38 and 2.39 indicating that the beebread can be a good source of unsaturated FAs. The essential fatty acids such as omega-3 and omega-6 cannot be synthesized by human body. Essential fatty acids are main structural constituents of membrane phospholipids, influencing fluidity, and ion transport through the cell membranes. The omega-3 FAs may also cause increase in bleeding time due to decreased blood viscosity, platelet aggregation and fibrinogen content. They have a favorable effect on decreasing low-density-lipoprotein (LDL cholesterol) levels in blood. Therefore they prevent coronary heart diseases.

### BEE BREAD – VARIOUS USAGE OF BEE BREAD AND CLINICAL EXPERIENCES

P. BHANDAARI<sup>1,2</sup>

<sup>1</sup> Wonder Organics, Jaipur, INDIA

<sup>2</sup> Member of National Bee Board of India, INDIA

Raw bee bread, less known and used compared with other bee products deserve to come closer in our attention. Bee bread is the pollen which the bees process in their hives. There it suffers a process of fermentation in a stable condition of temperature and humidity. Bee bread contains more reducing sugars than



pollen from the same plant species (CASTEEL, 1912). It has higher acidity due to the presence of lactic acid and larger amount of vitamin K. The higher activity of bee bread causes a good preservation of bee bread due to the inhibition of the growing of moulds as well as of microorganisms. Another important thing to be mentioned is that the outer shell of pollen (exine) is broken during this process, releasing nutrients. Among its medical properties, some laboratory tests also showed an antitumoral activity of bee bread extracts. Bee bread is a food containing naturally occurring compounds. In the future, bee bread will apply more and more as health food and medicine due to its functional properties such as antioxidative and scavenging activities of free radicals.

In the final of our presentation we present some clinical cases and experiences using bee bread as remedy and superfood as well.

### PRACTICAL API-NUTRITION - WHAT HAPPENS WHEN WE INTRODUCE BEE PRODUCTS IN OUR DAILY DIET

A. VARADI<sup>1</sup>, M. STRANT<sup>2</sup>, R. GROSU<sup>3</sup>

<sup>1</sup> SC CasaBIO cu sanatate RL, Cluj-Napoca, ROMANIA

Seeing American Food Pyramid we noticed that animal products are consumed too often and bee products as food was not found. We also know that American lifestyle and diet has led to the almost most obese population in the world.

Raw pollen, bee bread, honey, royal jelly, queen larva by its composition and properties is a great food for human being, even a superfood.

It has been shown in scientific literature that: "Bee products are definitively high source of natural nutrients and also a rich source of biologically active compounds".

For 7 years we have experienced a diet containing bee products and we used this alternative way to eat for a very wide range of patients and the results were very good. We have introduced new food groups (pollen, bee bread, royal jelly, queen larva) and we took out some food from the patients' diet where we observed that are not helpful for them.

We experienced a 40 days cure with raw pollen or bee bread or raw pollen and bee bread, fresh juices and raw vegetables with blood tests before and after the cure. Results are very encouraging and can pave the way for new researches.

### "BEESABLED PROJECT": BEEKEEPING IN REHABILITATION

A. D'ANGELI<sup>1</sup>, C. PILATI

<sup>1</sup> Apis intacta onlus

The "Beesabled Project" in the promotion of the concept diversity as a tool for social integration and acquisition of a better physical and mental well-being state, has offered an interesting training course in beekeeping dedicated to people with different abilities.

Since 2016, at the Center "Apis intacta", placed in Rignano Flaminio (RM) for the start-up of the beekeeper job, a training course in beekeeping is active for people with disabilities. At this Center the voluntary associations Etica&Solidarietà onlus and Azimut onlus realized a learning apiary accessible with the wheelchairs, where disabled could practice and learn proper beekeeping techniques to breed honey bees. To achieve the project's goals a continuing cooperation among the therapists, the beginner beekeeper and the expert beekeeper resulted crucial to a successful hive inspection and to discover together the secrets of the beehive.

According to the doctor this kind of activity could: "surely are for the patience, an interesting gym for the acquisition/re-acquisition of different skills, like: communication, emotions, cognitive processes, sensory-motorial competences, etc., as well as guaranteeing a moment of absolute feeling of freedom and well-being in close contact with nature".

The strong emotions felt by disabled working with the beehives, merged the wonder and the astonishment. To better adapt the beehive structure to the novice beekeeper wheelchair in the apiary, a new beehive was created an adapted (so-called "drawer beehive"). In fact, the "drawer beehive" shows the nest's frames placed in a "cold way" (that is perpendicular to the hive opening) that have a half reduced length compared to the Dadant nest combs; while the honey super frames run "warm way" (this means that they are parallel to the hive opening). In addition, the drawer beehive has a nest formed by twelve combs with "Mussi" space among them (2.2 - 2.8 mm inter frame space) and shows a 5 honeycombs super, so that the colony can go in a short time to the honeysupers since the beginning of April. The extraction and replacing movements of the honeycombs by disabled were made with the use of the lever normally used in apiculture. Thanks to this system, a total overcoming of physical barriers was allowed, with a feeling of absolute freedom in contact with nature. With the help of the technical and medical staff together the possibility to acquire the basis of an art like the beekeeper profession was reached for disabled in the "Beesabled Project".

### APICULTURE INTERVENTION FOR MALARIA-MITIGATION IN NIGERIA (AIM)

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Apiculture Science /Beekeeping Bio-Tech can serve as a tool for reducing the prevalence of Malaria through the use of Beeswax Mosquito Repellent Candles.

The Economic Impact of Malaria on Nigeria according to the National Malaria Control Programme: Malaria is responsible for 60% outpatient visits, 30% childhood death, 25% death in children below 1 year and 11% maternal death. Malaria annually costs 132 billion Naira.

Our proposed intervention:

Industrial production and circulation of Citronella Beeswax Candles that can mitigate malaria through repelling mosquitoes utilizing Artemisinin-based combination therapies.

Citronella Beeswax Candles are, effective mosquito repellents, organic & Environmentally safe (Bio degradable/ Aroma therapeutic, slower combustion, brighter illumination, negligible toxin emission, environmentally safe)

Research Summary:

55 volunteer households were given free samples of Citronella/beeswax candles that delivered up to 86 hours of emission. Safety measures were strongly emphasized.

51% observed 80% reduction of mosquito presence during burning periods.

24% observed 95% reduction.

8% experienced less than 10% reduction.

78% experienced no malaria infections during the period of using the candles.

Citronella Beeswax candles possess potential to mitigate malaria infection rates, and reduce rural poverty in Nigeria.

Our Action Plan

Carry out a more comprehensive research project in partnership with key development stakeholders in Nigeria & Africa to mobilize/encourage Beekeepers to trade beeswax for candles at fair rates. This will ensure sustainability.

### Conclusion

This initiative is aimed at exploiting the broad spectrum benefits of bee products and utility of beekeeping in Nigeria & Africa towards achieving at least 4 SDG's within the foreseeable future.

### ACTIVITY STUDIES OF CHARACTERIZED, STANDARDIZED AND HIGHLY PURIFIED PROPOLIS EXTRACT

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Propolis is a resinous substance collected and transformed by honeybees from various plant sources. It is rich in polyphenols compounds that vary depending on geographical and botanical origin. The new patented productive process, called M.E.D.® (Dynamic Multi Extraction) is able to extract the completeness of polyphenolic fractions present that are: phenolic acids, bioflavonoids, (aglycons and glycosilated forms) in a specific blend of brown propolis, coming from selected areas. Thanks to M.E.D.® process it is possible to obtain the first standardized and highly purified polyphenols extract from propolis. The standardization is based on the content of the functional compounds such as total polyphenols in which six of them (Apigenin, Pinocembrin, Chrysin, Pinobanksin, Galangin) represent more than 25%, signifying a quality indicator of extraction method. The characterization is performed with HPLC-UV-ESI-MS and tandem mass, to give us a qualitative and quantitative profile of purified polyphenols extract and its derivatives. Based on this standardized and purified polyphenols complex, we have been performed in vitro studies to test its antimicrobial activity against a large panel of microorganisms, in particular against *Staphylococcus aureus* spp. and *Staphylococcus aureus* Methicillin-Resistant spp; *Streptococci* spp.

### INFLUENCE OF PROPOLIS AND ROYAL JELLY ON THE ANTIBACTERIAL ACTIVITY OF HONEY AGAINST PSEUDOMONAS AERUGINOSA

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The Gram negative bacteria *Pseudomonas aeruginosa* is the main cause of burn wounds sepsis. Additionally important is the increasing resistance of *Pseudomonas aeruginosa* to many different antibiotics. Propolis is a mixture of balsamic substances, flavonoids, minerals and proteins with antibacterial activity against Gram positive bacteria and Gram negative bacteria. The antibacterial activity of Royal Jelly against Gram positive bacteria is connected to 10-HDA and different proteins. Honeys show antibacterial activity against Gram positive and negative bacteria. The aim of the experiments was to explore the enhancing effect of Propolis on RJ on the antibacterial activity of Honey against *Pseudomonas aeruginosa*. All ingredients (RJ, WSPGreit120, Honey) were dissolved in saline at 1:10/1:100. In the combination experiments RJ diluted 1:10 and 1:100 and Propolis were mixed in 1:1 and added as 10% to the 90% Honey. The antibacterial activity was

expressed as MIC (Minimal inhibitory concentration) (mg/ml). The highest MIC (0.003) was obtained when the RJ was 1:10 and Propolis was 1:100. Enhancing activity of Propolis or RJ on antibacterial activity of Honey is concentration dependent in a the mixture of 1:10 of RJ and 1:100 of Propolis as 10% to the Honey show the best MIC (0.003) against bacteria *Pseudomonas aeruginosa*.

### PROPOLIS: IS THERE A POTENTIAL FOR THE DEVELOPMENT OF NEW AND EFFICIENT ANTIMICROBIAL AGENTS?

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Propolis plays a key role in the prevention and control of bacterial invasions. In addition to its bactericidal activity widely documented, propolis also has an inhibitory effect on the expression of virulence genes of some pathogenic bacteria without affecting bacterial growth. The bacteria are thereby disarmed, and can not trigger their virulence mechanisms in presence of the host organisms.

In *Pseudomonas aeruginosa* PAO1, propolis inhibit the expression of LasI/R and RhlI/R, two main Quorum Sensing (QS) regulatory mechanisms in this opportunistic bacterium.

In addition to this inhibitory effect of QS, propolis also affect the production of biofilm phenotype is altered as a result of modification in its architecture, and therefore of its molecular composition. This alteration of the biofilm architecture allowed a better penetration of tobramycin into the biofilm and increases the accessibility of the antibiotic to encapsulated bacteria in the extracellular matrix.

Overall, propolis properties are particularly interesting in the perspective of preventive use of propolis in the antibacterial fight. Moreover, the development of such preventive approach should significantly reduce the emergence of resistant strains.

### PROPOLIS: INTRACELLULAR MECHANISM OF ACTION TO CLARIFY ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITIES

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Propolis is a natural substance collected by honeybees from different plants. A large body of evidence shows that propolis exerts many physiological and pharmacological properties such as antimicrobial (antibacterial, antiviral and antifungal activities), antioxidant and anti-inflammatory. The mechanism of action at the basis of propolis capacity to counteract oxidative stress and inflammation has not been elucidated yet. Thus, the aim of this study is to show that a standardized, characterized and high quality propolis extract (prepared according to a patented technology) is able to modulate the expression levels of miRNAs and mRNAs connected with oxidative stress and inflammation. The effect of propolis was studied by evaluating the expression levels of four miRNAs, miR-27a-3p, miR-17-3p, miR-203a and miR-19a-3p, in cells HaCat (human keratinocytes), treated with growing concentrations of propolis tested at sub-toxic concentrations. Moreover, the study of the expression levels of mRNAs coding for NFE2L2 (Nuclear Factor, Erythroid 2 Like 2), and TNF-α, validated target of miR-27a-3p, miR-203a and miR-19a-3p respectively, revealed a decreased in agreement with the increase of the corresponding miRNA, while the mRNA coding for GPX2 (glutathione peroxidase2) increases according to the decrease of miR-17-3p. In conclusion, we showed that propolis exerts its anti-oxidant and anti-inflammatory effects acting on transcriptional level.

### PROMISING ANTI PROTOZOAL ACTIVITIES OF BEE PROPOLIS

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Propolis is one of honey bee products used in hive protection against invaders and in hive repair. Its composition varies according to plant floral sources, but it consists mainly of 50% resins, 30% waxes, 10% essential oils, 5% pollen, and 5% of various organic compounds.

In other hand, chemotherapy of some serious parasitic infections as toxoplasmosis, trypanosomiasis, leishmaniasis and malaria is not effective with relapsing parasitemia and clinical signs. In the search for new alternative therapies, attention is being directed to the antiparasitic activity of propolis. The effect was dose dependent and the activity of the active principals was more potent than crude extract. The antiprotozoal effect of propolis was verified by reducing parasites growth after its incubation with different concentrations of propolis. Increasing scientific interests are directed to correlate anti microbial effect of propolis with its chemical composition as an essential step for its effective utilization in apitherapy

### BEE VENOM ACUPUNCTURE IN MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is a chronic inflammatory, demyelinating and neurodegenerative disorder of the central nervous system (CNS) that begins in young adulthood and may be the result of the interaction between genetic and environmental factors, together with certain pathological hallmarks of an autoimmune disease.

The therapeutic benefits of honeybee venom have been known for a long time to relieve pain and to treat inflammatory diseases particularly for treatment of arthritic and rheumatic conditions in humans and in animals. Specific immunotherapy with bee venom can result in an almost complete protection against adverse (or allergic) reactions from stings in the great majority of cases.

The pathogenesis of MS is complex and only partially understood, hampering diagnosis and thus the choice of appropriate treatment. Nevertheless, a group of experts revised the MS phenotypic classification that includes the five MS subtypes: Relapsing-remitting MS (RRMS), clinically isolated syndrome (CIS), radiologically isolated syndrome (RIS), primary-progressive MS (PPMS) and secondary-progressive MS (SPMS).

It was postulated that Bee Venom administration could reduce the proinflammatory activity such as INF-gamma producing CD4(+) T cells, IL-17A producing CD4(+) T cells and inflammatory cytokine production including INF-gamma, IL-17A, TNF and IL-6. Moreover, it was suggested that Bee Venom could be a potential therapeutic agent for anti-inflammatory effects in an animal model of experimental autoimmune encephalomyelitis (EAE). Although Apitherapy is not a curable therapy in MS, but it can be used to minimize the clinical symptoms of MS such as fatigue and spasticity, and can be included among programs of MS therapy

### EVALUATING THE EFFECTIVENESS OF PROPOLIS IN THE NON-SURGICAL TREATMENT OF PERIODONTAL DISEASE

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Periodontitis is a multifactorial etiology's disease, that recognizes the primary causative agent, in the bacterial biofilm. The combined use of mechanical instrumental therapy (SRP) and antimicrobial agents, has been proposed for the non-surgical treatment. The aim of this study is to compare the efficacy of propolis associated with the SRP treatment, the chlorhexidine associated with SRP and the singular treatment of SRP. 24 subjects with chronic periodontitis were divided randomly into three groups: only SRP, SRP and propolis, SRP and chlorhexidine.

Clinical evaluation was performed by: Intraoral Systematic and Radiographic Exam (ERSE), photos, Plaque Index (PI), Pocket Probing Depth (PPD), Gingival Index (GI), Bleeding On Probing (BOP), impairment of furcations, Mobility (MOB ), Gingival Recession (REC) and Clinical Attack Level (CAL). The data were recorded 6 weeks before and after the treatment, except the Rx Status.

Results: the statistic analysis shows that treatments with propolis and chlorhexidine have a sort of effectiveness, but the first treatment has resulted be more effective than the second. Comparing treatments, through comparison tests for the average of the independent samples, the significance has not been proven due to the low number of subjects enrolled. This pilot project has to be interpreted as a baseline to consent the structuring of a more enlarged project, based on a greater sample, that allows an inferential statistic comparison, between both aforementioned methods.

### BEE PROPOLIS IN THE TREATMENT OF HELICOBACTER PYLORI: IN THE RIGHT WAY TO CLINICAL APPLICATION

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Helicobacter pylori (HP) is the infectious agent that cause the most common bacterial infection overall. Common and serious gastric diseases are strictly linked to HP infection. The pathogenetic factors of HP determine a massive inflammatory response in the stomach. Currently, HP infection treatment consists of antibiotics and proton pump inhibitors, even if eradication rate is estimated not to be over 80%. We focused our work on bee propolis. We first compared anti-HP activity of many different propolis dry extracts and we focused our attention on a standardized propolis extract (50% total polyphenols) (SPE). SPE showed a very good antimicrobial activity both against metronidazole and clarithromycin resistant HP strains and clinical isolates and against CagA+ and VacA+ strains with identical minimal inhibitory and bactericidal concentrations. Further investigations highlighted that bactericidal effect is recorded after 2 hours.. We furthermore tested SPE with all the antibiotics of I and II line therapy and we recorded synergistic effect in most cases against different HP strains. Recently we found also that SPE exhibited a strong in vitro anti-inflammatory effectiveness in two hours on human PBMC stimulated with bacterial LPS. These findings outlined a consistent profile of effectiveness of propolis against HP and as gastro-protective agent.

### EXPERIENCES AND PERSPECTIVES OF INFECTED ULCERS AND WOUNDS TREATED WITH KNITTED VISCOSE MESH IMPREGNATED WITH MANUKA HONEY IN THE INAIL PHYSICAL REHABILITATION CENTER OF VOLTERRA (PISA- ITALY)

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Manuka honey is characterized by antimicrobial activity. It contains high amounts (from 100 to 900 mg/kg) of methylglyoxal (MGO), aside of other as yet unknown compounds.

MGO is an organic compound, stable at light and heat, deriving by the degradation of the th pyruvic acid of the carbohydrates. It is highly cytotoxic

Manuka honey has the property to keep the wounds humid, concurring their healing. Moreover, Manuka honey has an anti-inflammatory action.

It can be used by topical use application, giving good results in case of: psoriasis, dermatosis, acne, eczema. From 1st of March 2016, in the INAIL Physical Rehabilitation Center of Volterra (Pisa), Central Italy, post-surgical wounds and infected ulcers have been treated with knitted viscose mesh impregnated with 100% Manuka honey (Activon tulle®, Advancis Medical, Lowmoor Business Park, Kirkby-in-Ashfield, Nottingham, NG17 7JZ, England – www.advancis.co.uk).

So far, in the application on wounds it seems a very promising tool with positive effects, especially in the quick tissue reparation and resolution of the infection through the iatrogenic debridement and, not of secondary importance, for a decisive action in the malodorous injury.

Preliminary results are documented and will be shown with pictures of the skin lesions during the different stages of healing and the medical history of the patients.

### MEDICINAL HONEY DRESSINGS FOR BURNS AND OTHER TYPES OF WOUNDS: PROPERTIES AND CLINICAL EFFECTS

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Honey has been used since ancient times for treatment of wounds and has been the subject of numerous studies and research that reveal its multiple properties and effects, which are favorable for wound healing. Resistance to honey application in wound therapy, due to lack of standardization and to its sticky and fluid nature, can be overcome now by the production and marketing of medicinal products based on honey, licensed and approved for the topical treatment of wounds. This work presents the composition, properties and therapeutic indications of the product range of dressings L-Mesitran, based on medicinal honey, and also a preliminary clinical trial that was conducted in the Department of Plastic Surgery of the County Emergency Hospital of Ploiesti, Romania, in patients with burns and other types of wounds. L-Mesitran range of products exerted a favorable action on healing of burns and other types of wounds, sometimes in combination with surgical treatment, being applied in these cases before or after surgery. Further clinical studies are necessary to establish the best indications, methods and forms of administration for each type of wounds, as well as the selection over other treatment modalities, of course depending on preference and experience of people involved.

### API-THERAPY IN DAILY PRACTICE

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The use of bee products in human and veterinary medicine proved its efficacy, extraordinary sometimes, over time. Apitherapy has a long tradition in our country and around the world also. Each bee product has specific therapeutic actions, scientifically documented, that we have used over the years in treating various diseases. Because of their complex and rich chemical composition, bee products can be used as remedies but also as food in many situations; they can be used separately or in combination. In this presentation we will show some clinical cases for each bee product, as topical use of honey in burns and cheratitis, raw pollen in a case of male infertility or in losing weight cure, royal jelly in nervous disorder or flu and topical use of bee venom in cases of warts. We will also approach a less known and used bee product as queen larva and some clinical experiences with it.

### PROTECTIVE EFFECT OF SAUDI ARABIAN SIDR HONEY AGAINST LEAD-INDUCED ANEMIA AND HEPATO-RENAL TOXICITY

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Natural honey has many biological activities including protective effect against toxic materials. The aim of this study was to evaluate the protective effect of Saudi Arabian Sidr Honey against lead-induced hepato-renal toxicity and lead-induced anemia in Albino Mice.

Methods: Thirty two albino mice were allocated into four groups eight mice each; group 1: control group, received distilled water (0.1 ml / kg.b.wt /daily); group 2: received oral lead acetate (2 g/kg.b.wt/daily); group 3: treated with oral honey (1g /kg.b.wt/daily) and oral lead (2 g/kg.b.wt/daily), and group 4: received oral honey (1 g/kg.b.wt/daily). Honey and lead were given daily during 24 days of experimentation. Laboratory tests and histopathological evaluations of kidneys were done. Results: Oral administration of lead induced hepatic and kidney injury and caused anemia during two weeks of the exposure. Treatment with honey prevented hepato-renal lead toxicity and ameliorated lead-induced anemia when honey was given to animals during lead exposure. Conclusion: It might be concluded that Sidr honey has a protective effect against lead-induced blood, hepatic and renal toxic effects.

### ORGANIC HONEY SUPPLEMENTATION REVERSES PESTICIDE-INDUCED GENOTOXICITY BY MODULATING DNA DAMAGE RESPONSE IN A POPULATION CHRONICALLY EXPOSED

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Genotoxicity is the primary risk factor for certain chronic and long-term health effects, and a number of studies reported formation of DNA adducts from exposure to pesticides. Association between pesticide exposure and incidence of certain types of cancers, in particular hematopoietic, lung and prostate cancers has been reported. Honey is a polyphenol-rich food, which has been shown to exhibit several biological activities, including antimicrobial, anti-inflammatory, antioxidant, cardio-protective properties and anticancer. In this study, the protective effect of honey was investigated in a population chronically exposed to mixtures of pesticides with genotoxic potential. DNA damage response (DDR) including DNA damage and repair was evaluated at three different period of pesticide exposure before and after honey supplementation. DNA lesion accumulation occurs as consequence of a reduced DNA repair activity induced by pesticide exposure. The honey containing polyphenols affects in vivo the DNA damage response, thus limiting the toxicity induced by pesticides. These results provide new insight regarding the effect of honey containing polyphenols on pesticide-induced DNA damage response.

### BEE PRODUCTS: CHEMICAL COMPOSITION AND THERAPEUTIC APPLICATION

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There is an increasing interest of the consumers, pharmaceutical and food industries in products originating from the honeybees (honey,royal jelly,bee pollen,propolis,beeswax,bee venom (apitoxin), and pheromones). All of these substances have been reported to possess medicinal properties, including, antiarrhythmic,an-

tiatherogenic,antibacterial,anticancer,antidiabetic,antifungal,anti-ischemic,anti inflammatory,antimicrobi-al,antioxidant,antiparasitic,antiplatelet,antiproliferative,antithrombotic,antitumor,antiviral, and immuno-stimulant, vasorelaxant, cardio-, gastro- and hepato-protective activities. Honeybee products have been successfully used in treating human pathologies, including allergy, asthma,bronchitis,upper respiratory infections,anxiety,stress-relate migraine,burns,wounds,pressure ulcers,inflammation,stomach ulcers,digestive disorders,anemia, hypertension,diabetes,immune disorders,cardiovascular disease,hepatitis,tumors, and cancer.We have investigated the antioxidant properties of Moroccan propolis collected from various localities (using standard methods, such as DPPH, ABTS, ORAC and chelating activities).The capacity for pre-venting lipid peroxidation and scavenging free radicals was generally correlated with the phenol and flavone content.Some of the propolis samples were also able to inhibit lipoxygenase and acetylcholinesterase.In vi-vo,propolis and honey preparations were able to attenuate diabetic hepato-renal damage,probably through antioxidant and detoxification properties.The protective role of some honeybee products against reactive oxygen species-induced damage and nephrotoxicity in diabetic rats,gives hope that some of these products will have similar protective action in humans. In the rat diabetic nephropathy model,honey,propolis and pol-len also showed significant effect on glucose homeostasis and improving kidney function.A review on thera-peutic properties of honeybee products in experimental animal models and human health will be presented

### BEE VENOM ACUPUNCTURE IN COGNITIVE DYSFUNCTION IN PARKINSON'S DISEASE

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Parkinson's disease (PD) is a neurodegenerative and neuro-inflammatory disorder of the central nervous system, it has been known as movement disorder, also it is becoming recognized that for its non-motor characteristics that includes cognitive difficulties.

Cognitive function is broadly defined as an intellectual pro-cess by which one becomes aware, perceives, or compre-hends ideas. It involves all aspects of perception, thinking, rea-soning, and remembering. Cognitive impairment means a deterioration of the mental processes such as memory, decision, understanding, and reasoning. Cognitive impairment may be associated with behavioral disorders. There are different cognitive domains that accompany Parkinson's disease, the most common affected domains include: executive func-tions, attention, learning & memory.

Many studies reported that Bee venom therapy has anti-inflammatory and anti-neurodegenerative effects that improve PD symptoms. Also it attenuates the activation of the microglial response, reduces expression of the inflammation markers and reducing glutamatergic cell toxicity. Also it was reported that Bee venom therapy plays an important role in improving cognitive functions through blocking small conductance calci-um-activated potassium channels (SK channels), also can increase brain-derived neurotrophic factor (BDNF).

### WATER SOLUBLE PROPOLIS EXTRACTS AND HUMAN INTERFERON-ALFA (HUIFN-ALFAN3) SHOWS ANTI-INFLUENZA VIRUS ACTIVITY IN VITRO

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Influenza virus infects the respiratory tract in humans causing a variety of different symptoms which could

become severe. Propolis has also anti-influenza. HuIFN-AlphaN3 is a multi-subtype protein showing antiviral activity. The aim of experiments was to elucidate the Anti-influenza activity of the combinations of Propolis and HuIFN-AlphaN3. Influenza A and B viruses were obtained from the the Institute for Microbiology and Immunology in Ljubljana. 10 % Propolis water extract was prepared from 30 % water-soluble propolis (BNatural, Italy) and HuIFN-AlphaN3(Institute for Immunology, Zagreb, Croatia) were used in different ratios (1:1, 1:2 and 2:1). Influenza A and separately Influenza B viruses were added, and plates were incubated, when in the control 100 % CPE with small plaques was developed. In the experiments the HPLC column Purospher®STAR RP-18; 5microm 150x4,6 mm. was used: 25oC, 90-100 Bar, 20microl, 290 nm Absorbance. The best results (ID50) were obtained when the combination between 10 % Water extract of propolis and HuIFN-AlphaN3 in ratio 1:2 were used. (ID50 12±2 microg/ml for Influenza A and 19±6 microg/ml for Influenza B). In case of 10 % Ethanolic extract and HuIFN-AlphaN3, the best ratio was 2:1, where 22±7 microg/ml for Influenza A and 15±4 microg/ml for Influenza B.

#### API-FEED & VETERINARY APITHERAPY APPLICATIONS FOR FARM ANIMALS

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Today, demand on healthy life increases concern of bee products. Bee products use to protect health and treatment. Last years, they also used in veterinary apitherapy of farm animals, intensely. Using honeydew honey in the wound management enhances healing and decreases inflammatory oedema in farm animals. It treats mastitis in cows with inhibiton of bacteria species of highly antibacterial characteristics. Adding pollen in ration of race horses increase the performance. Application of royal jelly improved the success rate of closure and effective in increasing healing of tympanic membrane perforations in guinea pigs. Propolis supplementation to the starter diet improved the digestibility of feeds in broilers. Daily weight gain of female calves increased by using propolis. Neonatal diarrhea did not observe in calves. Apilarnil administration stimulated the development of secondary sex characteristics of male broilers and reduced blood glucose and cholesterol concentrations, stimulated sexual maturation and reduced fearfulness in male broiler chickens. Sperm quantity, motility, live weight gain, pruriency and growth parameters increased in male goats with apilarnil application. In our practice we concluded that these findings are observed in humans, when they consume bee products.

#### ANTIOXIDANT ACTIVITY, HEPATOPROTECTIVE AND ANTIDIABETIC EFFECTS OF THE HYDROALCOHOLIC EXTRACT OF PROPOLIS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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The objective of this study was to determine the antioxidant activity and the protective effect of Hydroalcoholic extract of propolis in streptozotocin-induced diabetic rats. Hydroalcoholic extract of propolis was prepared and evaluated for their antioxidant activities by DPPH, Reducing power, ABTS and Total antioxidant capacity, as well as the total phenolics, total flavonoids and flavones and flavonols. Experimental diabetes was induced by a single dose of STZ (60mg/kg) administered by intraperitoneal. The animals were treated orally once daily for 2 weeks by the oral administration of the hydro-alcoholic extracts of propolis at the dose of 50 and 100mg/kg b.w. Glibenclamide (2.5mg/kg) served as a standard. At the end of experimen-

tal period, blood samples were collected for the measurement of the blood glucose; triglycerides (TG), total cholesterol (TC), HDL-C, LDL-C and VLDL-C were measured. Biochemical parameters including renal function test and liver function test were also performed. The results show that propolis sample has relatively strong antioxidant activity accompanied by high total polyphenol contents and produced a significant decrease in blood glucose levels in normal rats, and even more in diabetic rats. This hypoglycemic effect might be due to an extra-pancreatic action of the hydro-alcoholic extract of propolis. In the other hand, the effect of the hydro-alcoholic extract on the plasma cholesterol, TG, TC, HDL-C, LDL-C and VLDL-C were also significant in both normal and diabetic rats. The effect was more pronounced with higher dose of the propolis extract. The results of this study indicate that Morocco propolis is an important source of total phenols showing antioxidant properties and a strong hypoglycaemic effect in diabetic rats.

#### BEE-DERIVED ANTIBACTERIAL PEPTIDE, DEFENSIN-1, PROMOTES WOUND HEALING IN VITRO AND IN VIVO

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Historically, honey bee products such as honey and royal jelly (RJ) have been used in a treatment of broad spectrum of injuries. RJ is part of the diet of honeybee larvae, and is secreted from the hypopharyngeal and mandibular glands of worker honey bees. The findings following topical application of RJ to treat human diabetic foot ulcers provided compelling evidence that RJ can accelerate wound healing. However, the mechanisms of action associated with the effects of RJ in wound healing remain enigmatic. In this study we report the identification and characterisation of a RJ component, bee-derived defensin-1, responsible for RJ-induced matrix metalloproteinase 9 (MMP-9) secretion from human keratinocytes and keratinocyte migration in vitro. This bee peptide in its recombinant form also improves wound closure and re-epithelialization in rat, and thus promotes wound healing in vivo.

Bee defensin-1 is a regular but variable component of royal jelly as well as honey. Defensin-1 is an antibacterial peptide belonging to insect defensin group with a molecular weight of 5.52 kDa. For the first time, we showed here, that bee defensin-1 possesses immunomodulatory properties and positively affects healing of non-infected wounds.

#### A SCIENTIFIC NOTE ON THE FIRST REPORT OF HONEYBEE VENOM INHIBITING PAENIBACILLUS LARVAE GROWTH

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In the highly eusocial honeybee *Apis mellifera*, worker bees use a stinging apparatus for defense. The sting is supplied with venom from the abdomen glands. Honeybee venom (BV) extraction has become a standard-

ized practice, characterized for being safe, simple, and causing no harm to bees. BV is composed of at least 18 bioactive molecules, ranging from biogenic amines to proteins. Melittin and PLA2 are the most predominant proteins in bee venom, representing 50 and 10-12% of its dry weight, respectively.

Furthermore, venom gland has been recently reported as an important source of antimicrobial substances with proven antibacterial and antifungal action. Despite this, antimicrobial broad spectrum data about the effects of BV on honeybees infectious pathogens is previously absent from the literature. This paper is the first report on honeybee venom antimicrobial activity against *Paenibacillus* larvae and on its effect on the fed bees survival. Results hold particular promise, with a minimal inhibitory concentration (MIC) from 3.12 to 6.25 µg/ml and minimal bactericidal concentration (MBC) ranging from 4.16 a 10.42 µg/ml against this microorganism. This research also constitutes the first record of oral administration of BV to an invertebrate biological model. No lethal or behavioral effects on bees after a week of BV ingesting were revealed. High doses of BV resulted much less toxic for bees than some botanical extracts. Further studies should be conducted to address the effects of higher concentrations of BV and determine the toxicological risks and the optimum dosage in field assays.

#### DETERMINATION OF OXIDATIVE STRESS IN APIACUPUNCTURE OF PATIENTS SUFFERING FROM CHRONIC LOW BACK PAIN

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Chronic non-specific low back pain (CLBP) is a common medical problem considered as a multifactorial disorder in which musculoskeletal pain and psychosocial factors interact with each other. Aims: The aim of this investigation was to evaluate the role of oxidative stress by measuring levels of plasma antioxidant enzymes malondialdehyde (MDA) concentration, Glutathione S-transferases (GST), Catalase, Alanine transaminase (ALT) and aspartate transaminase (AST) activities and Creatinine of patients with chronic low back pain (CLBP) and normal controls. Methods: We determined the activity of plasma antioxidant enzymes malondialdehyde (MDA) concentration, Glutathione S-transferases (GST), Catalase, Alanine transaminase (ALT) and aspartate transaminase (AST) activities and Creatinine of 25 chronic low back pain (CLBP). patients and 25 controls by using the spectrophotometric assay method. Results: There was statistically significant increase in the levels of these parameters of patient group compared to the control group ( P < 0.001). Conclusions: There is increased oxidative stress in chronic low back pain (CLBP) as is indicated by high levels of malondialdehyde (MDA) concentration, Glutathione S-transferases (GST), and low levels of Catalase in the serum of chronic low back pain (CLBP) patients.

#### EVALUATION OF ANTIBACTERIAL ACTIVITY OF SAUDI ARABIA HONEYS

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The objective of this investigation was to evaluate the antibacterial activity of twelve Saudi Arabia honeys against five pathogenic bacterial strains which has antibiotic-resistant including *Staphylococcus aureus*, *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa*. The antimicrobial activity of twelve Saudi Arabia honeys against five pathogenic bacterial strains All honeys tested exhibited antibacterial activity against all five bacterial strains with different levels according to the type. The antimicrobial activity was differing according the pathogen and honey type. The results of the current study revealed that Saudi honeys inhibits the growth of bacterial strains.

#### NUTRITIONAL FACTS OF HONEY

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Honey is healthy natural old sweetener; raw honey offers many nutritional benefits without the man-made chemicals found in many other forms of sweeteners.

Objective: The goal of this review is to evaluate honey facts and nutrition.

Results: The nutritional facts vary slightly which depending on the floral variety, Honey is a source of carbohydrates 76 % containing mainly fructose and glucose. Also honey contain 16-20 % water, 2% minerals, the minerals found in honey include calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc. Vitamins, pollen and protein are present in different level. The vitamins present in honey are B6, thiamin, niacin, riboflavin, pantothenic acid and certain amino acids.

Conclusion: one of the most encouraging honey nutrition facts, it has antioxidants and is free of fat and cholesterol

#### NOVEL APPROACH OF BEE VENOM AS TREATMENT OF RHEUMATOID ARTHRITIS

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Bee venom acupuncture (BVA) was potentially used as complementary modality for treatment of many diseases. The aim of this study is to evidence of bee venom acupuncture as novel trend for the long term treatment of rheumatoid arthritis (RA). This study is a randomized, controlled clinical trial. The study done to compare the effects of BVA by bee sting and pharmacotherapy in patients with RA. Patients with Rheumatoid arthritis disease treated with BVA (bee sting) therapy at apiacupoints twice a week. While other group treated with drug therapy. Tender joint count, swollen joint count, morning stiffness, visual analog scale (VAS), health assessment Q, ESR, CRP, Tumor Necrosis Factor (TNF), Interleukin 1(IL1) Interleukin 6(IL6), Nuclear Factor and Kappa B (NF-KB). All these parameters will be assessed before and after treatment. The results revealed that the bee venom acupuncture showed significant improvement in patients received bee venom group compare to patients received drug therapy. It is concluded that both modes of treatment for RA gave improvement regarding pain intensity, disability and quality of life being more evident in bee venom group supported with improved serum TNF, IL1, IL6 and NF-KB.

#### THE PROTECTIVE EFFECT OF THYMUS VULGARIS HONEY, ORIGANUM VULGARE ESSENTIAL OIL, AND THEIR AROMIEL AGAINST CCL4-INDUCED LIVER INJURIES IN RATS

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CCL4 is a xenobiotic used in laboratory animals to cause chemical hepatic injuries; it is highly toxic and can rapidly bring liver necrosis following a single dose. The current investigation aimed to evaluate the protective potential of thymus vulgaris Honey, Origanum vulgare essential oil, and their aromiel by analyzing the biochemical parameters (enzymatic liver profile, serum lipids, mineral and bilirubin values), physicochimique analysis of honey and chemical composition of essential oil are studied. Hepatic lesions were induced by intraperitoneal injection of carbon tetrachloride (dissolved in olive oil, 10% solution). One mL per 100 g was

administered, 2 times per week for 2 weeks. Hepatoprotection was achieved with 1g/kg of thymus vulgaris Honey, 200mg/kg of Origanum vulgare essential oil dissolved in 2% of tween 80 and 1ml/100g by the combination of 1g/kg of thymus vulgaris Honey and 200mg/kg of Origanum vulgare essential oil. The results reveal that the studied biochemical parameters were significantly protected with thymus vulgaris Honey, and Origanum vulgare essential oil against the toxicity of CCl<sub>4</sub>, this protection is more important by the combination of honey and essential oil as an aromiel, revealing the presence of a synergistic effect.

### GLYCEMIC INDEX OF SUCROSE, FRUCTOSE AND HONEY ADDED Highbush CRANBERRY(VIBURNUM OPULUS) JUICE

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Honey is considered as a healthy sweetener. Highbush Cranberry (*Viburnum opulus*) juice is used in indigenous medicine for the maintenance of general health. Glycemic Index (GI) is the effect of nutrition on blood glucose and epidemiologic studies have provided suggestive evidence that a diet with low GI may contribute to prevention and treatment of chronic diseases.

In the current study, it was aimed to determine the glycemic index values of highbush cranberry juice in unsweetened, fructose-sweetened, sucrose-sweetened and honey-sweetened forms, on total 24 healthy volunteers, with the mean age of 20.57±1.39 for men, and mean age of 19.37±1.02 for women. Anthropometric measurements of each participants were made and their diet histories were taken.

As the reference food, 50 grams of pure glucose was given to volunteers, while test portions of highbush cranberry juice samples in sweetened and unsweetened forms equivalent to 50 grams of carbohydrate were consumed by the volunteers. Blood samples were taken at the 15th, 30th, 45th, 60th, 90th, 120th minutes, and the "incremental area" method was used to determine glycemic index values.

Glycemic index values of highbush cranberry juice in unsweetened, fructose-sweetened, sucrose-sweetened and honey-sweetened forms were observed as 39.95, 63.76, 53.42 and 46.74; respectively. Therefore, highbush cranberry juice sweetened with honey has a low GI. In conclusion, referring to the beneficial effects of low-GI in the context of preventing chronic diseases, highbush cranberry and honey combination can be considered in a healthy diet for the maintenance of health.

### APPLICATION OF TWO HOMEOPATHIC REMEDIES ON HONEYBEES AGAINST VARROATOSIS: PRELIMINARY RESULTS

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*Varroa destructor* is the most damaging honeybee pathogen worldwide. The homeopathic remedies are ease of use, low cost, no impact on environment, safe for beekeepers and honeybee products. Unfortunately, limited data are available on the application of homeopathic treatments to honeybees.

A one-year-long field trial on 30 colonies started in Modena (Italy) in July 2016 and here are presented the preliminary results.

The apiary was divided in 3 groups of 10 colonies each, homogeneous in terms of strength:

group 1) treated with *Lachesis*, a homeopathic remedy obtained from the snake *Lachesis mutus*;

group 2) treated with *Iridium metallicum*, a homeopathic remedy obtained from the metal iridium;

group 3) a control group, untreated.

The treatments were applied with syrup in the feeder every 20 days for a total of 4 times.

Aim of the trial was to verify if the application of the homeopathic remedies could reduce the *varroa* infestation level of the treated colonies.

The preliminary results of the trial showed that the infestation of the treated and untreated beehives in Summer 2016 was so high at the clinical inspection that the beekeeper had to apply a conventional treatment in order to avoid the collapse of the colonies. The remedies' administration will be continued monthly until August 2017 to verify if better results will be obtained in the long-term period.

### A DAY IN THE LIFE OF A CARIBBEAN BEE STING CLINIC

M. PENDLETON

My submission: A day in the life of a Caribbean Bee Sting Clinic! -I spent 4 months consulting a St.Lucian clinic assisting to establish a professional clinical intake of clients treated with thorough record keeping and waiver of responsibility. During this time I was administering BVT to up to 40 patients a day and to myself. Some clients had a great fear these were particularly laughed at, i helped instill a sense of calm by using breathwork. -honey eye drops were administered to every patient and has a great implication for disease of the eye as well as general overall health. -Corresponding herbal protocol using local plants -every patient drank a cup of bitter bush tea in coming to the clinic, often to their dismay. -contraindications: what should be avoided during BVT? There are mixed ideas in what should be abstained from prior to BVT, here no processed foods, no alcohol, and if on nsaid's u cannot do the therapy. -what was the protocol of the bee sting clinic, for how long are patients treated in duration of subsequent visits and what symptoms treated or denied? -massage/BVT combination for increased effectiveness - i performed massage on my patients prior to treatment and found the best locale for the sting with greater ease this way. -following TCM guidelines for ailments without formal training. In studying the energy pathways of the body for years prior and receiving acupuncture personally up to 100 times, i was able to research acupuncture protocol for specific ailment i was treating ie. Diabetes, seizure, anxiety and tumor along with the help of LAC friends and share this info and literature with other BVT practitioners at the clinic. -product development with the medicines of the hive including honey vinegar, wine, soap and healing balm with local plants. - the concept of Laughter out of place is exonerated here & the presentation includes short videos, pictures and funny stories! Manda Pendleton is a clinical herbalist, nutritionist and apitherapist beekeeper from Denver, CO. She has 20 years of herbal formulation and health/business consultation. Her work for the last 9 years in West Africa, Costa Rica, St Croix USVI and St Lucia has prompted her commitment to Assisting others with the medicines of the hives with a focus on sustainable and organic methodology for assisting the bees in their current plight. Looking at the current schedule, I am thinking this is a 15 minute presentation. My powerpoint for the below past workshops is normally one hour so i would be thrilled to present the first submission in a succinct and thorough short talk with the benefit of added humor. Herbal Apitherapy - Let's talk about the nutritional aspects of the healing medicines from the hive & bee venom therapy (BVT): their modern applications and history. Also investigating the plants that Bees love and what we can do Now to positively assist pollinators and the Earth in these crucial times. Powerpoint 1 hour- can be shortened. Bee venom therapy: Bvt is thought to be the origin of modern acupuncture, taught by ancient Egyptians to the ancient greeks including hippocrates, the "father" of "modern" medicine who called bee venom "arcanum" in greek, meaning miracle cure. Learn the fascinating history and practical applications of this healing art based on Manda's extensive research and work in a BVT clinic in the Caribbean island of St.Lucia. Talk with videos- 1 hour that can be shortened. Thank u kindly for your consideration!



### EFFECT OF PH VARIATION DURING PROPOLIS EXTRACTION WITH THE USE OF PBS AND CONSEQUENTLY 70% ETHANOL AS SOLVENT ON ITS ANTIMICROBIAL/ANTIVIRAL ACTIVITY

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Propolis is a substance showing a variable chemical composition with antimicrobial and antiviral properties, associated with concentration of flavonoids and phenolic compounds. The objective of this work was evaluating the effect of pH variation on propolis extraction, prepared with PBS (Phosphate buffer saline) and 70% EtOH as solvent. Propolis extracts were prepared using 3g crude crushed propolis for each 15 mL of solvents: PBS and consequently 70% Ethanol. Seven different raw propolis samples (Medex d.o.o., Ljubljana, Slovenia) were prepared at pH 6 (6.4), 7 (7.2) and 8 (8.0). The flavonoids were measured by colorimetric method, phenolic contents were calculated by the Folin-Ciocalteu method. All samples were analysed by RP-HPLC. The antimicrobial activity against G+, G- and *Candida albicans* was measured by MIC method. The antiviral activity was measured against Influenza A, B and NDV. In PBS extracts the results were better in basic pHs (8.0), with increase of 160% in flavonoids and 25% in phenolics. The antimicrobial activity was higher at pH 8.0 so in PBS extracts, as well as 70% EtOH extracts compared with the sample without pH variation. At pH 6.4 there was the highest antiviral activity against Influenza A and B viruses as well as NDV virus. The use of PBS as propolis solvent in basic pHs produced an extract with higher concentration of flavonoids and polyphenols. In case of acidic pH (6.4) Caffeic acid was found. Depending on its content the level of antiviral activity was found. The final microwave extraction increase the flavonoid content.

### MOROCCAN PROPOLIS A NATURAL ANTI-BACTERIAL AND ANTI-BIOFILM AGAINST STAPHYLOCOCCUS AUREUS WITH NO INDUCTION OF RESISTANCE AFTER CONTINUOUS EXPOSURE

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The increase of bacterial resistance to antibiotics is a threat to public health at a global scale as it reduces the effectiveness of therapeutic and increases morbidity, mortality and health care cost. The aim of this study was to evaluate the adaptation response of *Staphylococcus aureus* ATCC 6538, and Methicillin-resistant *Staphylococcus aureus* (MRSA2, MRSA15 and MRSA16) to hydro-alcoholic extract of Moroccan propolis. The minimum inhibitory and minimum bactericide concentration (MIC, MBC) were determined. Adaptation to propolis was done by sequential exposure of the pathogens to increasing concentrations of the extract. The impact on the adherence ability was evaluated by the crystal violet assay, and the effect of adaptation to the extract on virulence was tested by the *Galleria mellonella* model, as well the assessment of the anti-quorum sensing capacity of propolis were investigated. Gas chromatography coupled to mass spectra (GC-MS) analysis of the propolis sample was also done.

Propolis extract has as main group of secondary metabolites, the flavonoids (31.9%), diterpenes (21.5%) and phenolic acid esters (16.5%). Propolis extract at the MIC value (0.36 mg/mL) reduced the virulence potential of *S. aureus* and MRSA strains (MRSA2, MRSA15 and MRSA16) with no development of resistance. It showed a high efficacy as antistaphylococcal biofilm agent. Adaptation at subinhibitory concentration of propolis extract protected the larvae against staphylococcal infection, ( $p < 0.001$ ). The results showed the ability of propolis extract to reduce violacein production in a concentration dependent manner, evidencing its anti-quorum sensing properties.

### AN ORIGINAL METHOD OF BEE POLLEN PROCESSING WITH ENCOURAGING THERAPEUTICAL RESULTS

C. ENE

Bee pollen is naturally transformed inside the cells of the combs into the so called bee bread. In our laboratory studies, together with a multidisciplinary team of medical doctors we developed an original method of processing bee collected pollen using the Kombucha culture. Kombucha is produced through the aerobic and anaerobic fermentation of black/green tea with symbiotic colonies of bacteria and yeasts. Kombucha contains microorganisms (bacteria and yeasts) and nutrients (organic acids, enzymes and minerals).

The aerobic and anaerobic fermentations of the polyfloral pollen in Kombucha culture, break the exine outer layer of the pollen grains releasing proteins, oligo-peptides and all the essential amino acids, a multitude of enzymes fatty acids, polyphenols, flavonoids and nucleic acids, lipo- and hydrosoluble vitamins and various minerals (Na, K, Ni, Ti, etc.). With a complete range of nutrients and a high number of active therapeutic principles the new product may be considered a "natural pharmacy", a complex of prebiotics, probiotics (selective bacteria) and post-biotics (nutriments), but also of antioxidants.

The final product of fermentation is an adaptogen and tonic with cholesterol-lowering and hypoglycemic effects as well as the ability to decrease platelet aggregation, an important antibiotic action against Gram+ bacterium (*Staphylococcus aureus*), Gram- bacteria (*Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*) and of yeast (*Candida albicans*). Due to its anti-inflammatory, antioxidant effects and the improvement offered in cases of anxiety and mental depression it may be a valuable dietary supplement.

To prove the beneficial effects of this product, the following surveys were performed: a) electrographic recordings in electroluminescence of modified Kirlian type, as well as spectrometric recordings in electroluminescence. The electrographic recordings of the thumb were performed before and after 24 hours from the administration of the supplement; b) testing of the energies of the body organs with Metatron in terms of chakras, and on the emotional and energetic level, on the left/right symmetry and the balance of organs; c) real-time survey with NLS METATRON -4027- System with a 47 years old patient with respiratory failure and sleep apnea in which changes at tissular, cellular and mitochondrial level were observed following the administration of the new products. Details on the clinical studies are discussed.

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