STUDY OF GENETIC STRUCTURE OF HONEY BEE FROM CYPRUS (*Apis mellifera cypria*) WITH GEOMETRIC MORPHOMETRICS ANALYSIS

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In this research, the genetic structure of honey bee populations was studied, with geometric morphometrics analysis approach, using the coordinates of nineteen (19) landmarks located at vein intersections of wings on 16 samples from different areas of Cyprus. The aim of this research was to find out if there is still pure honey bee race, *Apis mellifera cypria*, in the island of Cyprus , according to Ruttner's (1988) morphometric analysis, to estimate the hybridization due to importation of foreign queens and colonies and to compare the results from this research with those of other studies on Cypriot honey bee. For data processing the program standarization of software package Excel as well as programs STAND, SAHN and TREEof software package NTSYS (Rohlf, 1990) were applied. Phylogenetic tree constructed by UPGMA (Sneath and Socal 1973) method, showed that the populations of our study are grouped in two main clades. In one clade populations from isolated areas are grouped and the other one is separated in two clades; in the first one populations from areas too far from the North part of island are grouped together and in the second one populations from areas near the North part of Cyprus island. Comparing the results from this study with those of similar studies with different approaches on Cypriot honey bee, the estimation that there are pure honey bee populations in Cyprus island is ensured as well as that there is hybridization due to the importation of queens and colonies.

MOLECULAR AND MORPHOMETRIC MARKERS FOR THE DETERMINATION OF GREEK HONEYBEE POPULATIONS HYBRIDIZATION

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Honey bee populations from different areas of Greece: Platamonas, Nisiros, Kos, Astypalaia, Larissa, Katerini, Trikala, Milos and Sifnos were studied, using molecular and morphometric markers, in order to detect the existence of hybridization in Greek honey bee populations.

Concerning the molecular approach, mtDNA was analyzed with PCR-RFLPs method. The gene segments studied were 16srDNA and COI. Total DNA was extracted, then the above gene segments were amplified using PCR method and finally the enzymes that recognized sites were for 16srDNA: *Sau3AI, SspI, DraI, HincII, EcoRI, PstI* and *AluI* as well for *COI: NcoI, Sau3AI, FokI, BclI, SspI, StyI, BstUI* and *XhoI* respectively. Polymorphism was detected for 16srDNA gene segment for the enzyme *SspI* in Milos population and for COI for the enzymes *StyI* and *NcoI* in Larissa, Astypalaia, Milos and Sifnos populations respectively.

According mtDNA analysis, there is probably genetic material transmission in Larissa, Astypalaia, Milos and Sifnos populations as more than one haplotypes were detected. Furthermore, in these areas the macedonian pattern was detected which supports the assumption that beekeepers prefer to buy queens that belong to *Apis mellifera macedonica* race.

The results of the mtDNA analysis as well as the phylogenetic tree which was constructed according to UPGMA method (Sneath and Socal, 1973), were statistically processed using the software packages REAP (McElroy et al., 1991) and PHYLIP (Felsenstein, 1993).

As far as the morphometric approach is concerned, the characteristics that have been measured were: length and width of fore wing, the cubital index, length and width of hind wing, number of hamuli, length of femur, length of tibia and finally length and width of basitarsus.

The conclusion from the morphometric approach is that there is no geographical discrimination and heterogeneity.

The results of the above method were statistically processed using STANDARDIZATION program of EXCEL package and STAND, SIMINT, SAHN, TREE programs of NTSYS software package (Rohlf, 1990). The phylogenetic tree was constructed according to UPGMA method (Sneath and Socal, 1973) using NTSYS software package too.

The two approaches were compared using MANTEL program (NTSYS package), and the conclusion was that there is no coincidence between them, possibly because mtDNA is maternally inherited while nuclear genes are responsible for the morphological characters.

The above results are probably due to migratory beekeeping and commercial breeding. It is well known that Greek beekeepers prefer *A. m. macedonica* because it is less aggressive, it has low swarming tendency, it is more productive and it is more resistant to pests.

RESEARCH ORIENTATIONS FOR THE GREEK HONEY AND THE OTHER PRODUCTS OF BEEHIVE.

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Research regarding the characteristics of Greek honey began in 1932 and is continued up to today. The research orientations are distinguished in the following categories.

a) Most research is focused on analyses of physiochemical and microscopical characteristic of honeys of different botanical origin to describe the identity of Greek monofloral honeys. So far, more than 30 parameters, have been determined. Results allow the description and differentiation of Greek monofloral honeys (pine honey, fir, thymus, castanea, Erica, cotton, helianthus, citrus).

V) Analyses concerning the quality of Greek and imported honeys. The Greek scientists have given particular concern on issues regarding the quality of both packed and beekeepers honeys.

Research includes methods of ascertainment of adulteration and localization of unfavorable repercussions of human intervention in the qualitative characteristics of honey (feeding, residues of acaricides and antibiotics, problems of overheating packing, etc).

c) Identification of characteristics of unifloral honeys from different regions that allows the geographic differentiation. Young scientists in apiculture use modern scientific instruments and techniques. In this research area the volatile characteristics, the aromatic substances, the flavonoids and the combination of pollen grains are examined.

d) Analyses of Greek honey in order to indicate the nutritional value of bee products. This kind of research is particularly important for the marketing of bee products. Research concerns the examination of the microbial and antioxidant action of various categories of honey of propolis and royal jelly.

From the volume of so far published work in international and Greek periodically it is apparent that the effort of Greek scientists in the identification of Greek honey, in keeping its excellent quality and in accents of its good characteristics is continuous and efficient.

THE QUALITY OF GREEK HONEY

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A great number of samples of Greek honey were analyzed during the last ten years and for these results the quality of honey is discussed. The criteria that were used were a) the physicochemical characteristics of product in relation to the current legislation b) adulteration c) the content of packing in relation to the label, and d) the present of dangerous compounds for the human health (pesticide and antibiotics residues).
Honey that is produced by the greek bees is excellent and corresponds in the current market provisions but the human` intervention can influence it negatively and downgrade its quality.

IDENTIFICATION OF THYMUS HONEY OF RHODES AND CYPRUS ISLANDS

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Thymus honey from the islands of Rhodes and Cyprus was analyzed to find their physicochemical and microscopical characteristics. Samples were collected from collaborated beekeepers to which certain directions were given.

The parameters that were analyzed were HMF, diastase activity, electrical conductivity, sugar content, pH, acidity, water content, volatile compounds, antioxidants and antibacterial activity.

Difference between the thymus honey that is produced in the two islands exist and mainly is due to the absence of mixture of honeydew in Cypriot honeys and are located in the physicochemical characteristics and in volatile compounds

THE USE OF PROPOLIS IN APITHERAPY

Koutsianas Nikos
APIVITA

"If we look deep into nature only then we shall understand everything better" Albert Einstein was quoted saying because:

Bees collect resins and balms from certain plants; they mix them with wax and pollen, enrich them with enzymes and trace elements to produce propolis in order to protect their city, the hive, from infections and unwanted intruders. Hence, the term propolis came about and it means "in front of the city". For millions of years now, the bee "knows" that in order to survive infections, predators, etc., it must produce propolis. In a few words, propolis is the raw material the bee uses to repair, disinfect, clean and spruce up the hive. The use of propolis is of fundamental importance in the bee colony, if one considers that the 50 litre space with a temperature of 35-38 ^{o}C, the 40.000-50.000 bees, the 70% humidity and the high sugar content which the hive offers constitute the ideal conditions for microbial growth. The absolute sterilization

achieved with the use of propolis is miraculous. The interior space of a beehive is the outmost sterilized natural environment in the world.

Propolis prevents the causation of unpleasant odors and microbial growth. It consists on average of 55% resins and balsam, 30% wax, 10% essential oils and 5% pollen. It also contains flavonoids, phenolic and aromatic substances and trace elements. Propolis is a true source of health, known since antiquity. The past few yeas scientific research has completely clarified the outstanding antibiotic, anti-inflammatory, antibacterial, antifungal, antioxidant and analgesic properties of propolis, which are responsible for a plethora of uses in contemporary therapeutics and cosmetology. It is complementary used in cases of respiratory infections, pharyngitis, otitis, dermatitis, arthritis, burns, circulatory dysfunctions, internal organ diseases, fungal infections, viral infections, gynecological problems, infections of the oral cavity, diseases and inflammations of the eyes.

The very high level of scientific knowledge and the know-how in the production, the purification, the extraction and commercialization of propolis is reflected by the high biological value, quality and effectiveness of the propoline products. The scientific foundation is a result of ongoing cooperation with the academic community that reached a milestone in 1995 with the Program Development Industry Research during which the pharmaceutical, chemistry and medicine departments of the University of Athens researched and rated the antioxidant, antimicrobial and antiseptic action of medicinal plant and propolis extractions and fractions for the production of new dermocosmetic products.

Comparative study onto the effectiveness of alternative thymol formulations against the mite *Varroa destructor*

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The Varroa mite (*Varroa destructor*) is an external parasite of honey bees. Despite the application of many synthetic chemical mitecides through the years, it still constitutes a major problem for the Greek and European apiculture. The present study was undertaken to examine and compare the effectiveness of thymol crystal formulations against the mite *V. destructor*.

The number of the examined thymol formulations were six and the experiment took place under real field conditions in Greece. Three of them were known by its commercial names which are Apilife var, Apiguard and Thymo var. The other tree formulations were thymol crystals (Thymol), thymol crystals mixed with clove essential oil inside a gel formulation (ThyGel) and thymol crystals mixed with baseline (ThyBaz). The ThyGel and ThyBaz formulations were made in the laboratory and they were examined for the first time under Greek real field conditions.

The results showed that the effectiveness of the tree commercial formulations (Apilife var, Apiguard, Thymo var) was high with no-significant deviations among them and an average value of 90.6%. The average value of the effectiveness of the other tree examined formulations (Thymol, ThyGel, ThyBaz) was 89.0%. Comparatively, thymol crystals (Thymol) was the formulation that reached the maximum effectiveness (91.8%±1.02) with the disadvantage of some adverse effects on bee behaviour such as erratic movements, medium aggressiveness and crawling of bees near the hire entrance. The effectiveness of the ThyBaz formulation was slightly lower (~5%) ($p<0.05$) from the others but taking into account the absence of any adverse effect on bee colonies during the application, the easy preparation procedure and the small cost, makes it a very important way in the control of varroa mite without harmful effects.

CERTIFICATION OF BIO-APICULTURE PRODUCTS

BIO-ELLAS

The requirements for biological Apiculture are described in the Regulation 2092/91 and **1804/99** of the E.U. *About the Biological way of production and the relevant indications on the agricultural and nutritional products.* The biological production label for the apiarian products has to do with the treatment of the beehives, the environment as well as the conditions involved in the collection, processing and storage of the apiarian products.

In order to obtain a certification of bio-products, the beekeeper submits an application towards the Organization and then a contract is signed among the beekeeper and the Organization which binds the beekeeper to follow all the clauses of Regulation 2092/91 and the Regulation of the Certification Institution. More specifically, the beekeeper must follow all the requirements concerning the origin of the bees, the conversion period, the places of the apiaries, the nutrition, veterinarian treatments, feeding and identification practices, use of allowed substances.

The Certification Institution performs annual inspections in order to ascertain the continuous application of the Regulation requirements, as well as surprise checkouts. Samples are also taken for laboratorial analysis, in specific laboratories for pollenoscopic examination and examination for drug remains.

The observance of all requirements leads to the issue of a certification and the use of BIOHELLAS stamp on the bio-apiculture products.

ANTIMICROBIAL AND ANTIOXIDATIVE ACTIVITIES OF GREEK PROPOLIS

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Propolis is a resinous material produced by bees. The use of propolis in the traditional medicine is known since 3000 BC in Egypt. The chemical consistency of Greek propolis and its pharmacological activities have never been studied and published before.

The chemical constituents (volatiles and non volatiles) of propolis from 5 different regions of Greece have been studied (Preveza, Crete, Attiki, Andros, Chalkidiki). The n-butanolic extract of the samples from Preveza, was afforded 33 diterpenic and phenolic compounds like: 7-prenyl-stropopinin, pinostrobine, pinobanksine, chrysin, totarol, manool, isocupressic acid, agathodiol, isoagatholal, benzoic acid, caffeic acid and p-coumaric acid. Among those constituents, 7-prenyl-stropopinin is a new natural product while other ones like agathodiol and isoagatholal have never been reported as propolis constituents. The identification of the origin of our samples was based on their pollinic spectra microscopically.

The antimicrobial activities of all studied samples and of pure isolated compounds were determined, using the diffusion-dilution techniques, by measuring the MIC of them against the Gram-positive bacteria: *S.aureus* and *S.epidermidis*, and the Gram negative: *P. aeruginosa, E.coli, E.cloacae, K. pneumoniae,* the oral pathogens *S. mutans* and *S. viridans*, the pathogenic fungi: *Candida albicans, C. tropicalis* and *C. glabrata* . All strains were standard of American Type Culture Collection. Standard antibiotics were used in order to control the sensitivity of the tested bacteria and fungi. The results of the tests showed interesting and promising antimicrobial activity. Some of them exhibited antimicrobial activity similar to clinically used antibiotics

The samples have also been tested for their ability to protect cellular DNA from H_2O_2-induced single strand breaks in a T-lemphocytic cell-line (Jurkat cells). Formation of single strand breaks was estimated by using the highly sensitive methodology of comet assay (single cell gel electrophoresis) as well as DPPH method. The results obtained from these experiments shower that our samples exhibited the best protective activities.

COMPARATIVE STUDY OF SILKWORM (B*ombyx mori*) REARING ON MULBERRY LEAVES AND ARTIFICIAL DIET

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Silkworm is a monophagous insect that feeds only on mulberry leaves. Mulberry cultivation and leaf collection may be time consuming and often require high cost manpower, particularly when multiple and year-round rearings are realized. To overcome the limitations of rearing silkworms exclusively on mulberry leaves, artificial diets are used for silkworm rearing. The use of artificial feed was an important breakthrough in sericultural techniques for the following reasons. Firstly, it provides a new path to keep up with the sericultural development and favorable conditions for research on silkworm, which represents the model insects for many entomological and genetic researches. Secondly, it has shown the way for the mechanization and industrialization of silkworm production.

The objective of this paper is to make a comparative study of silkworm rearing on mulberry leaves and artificial diet in terms of cocoon production and post cocooning parameters.

Eggs of the Chinese hybrid (Feng1 X 54A) hatched under controlled laboratory conditions of temperature (25 ˚C), relative humidity (75%) and photoperiod (16L:8D). Newly hatched larvae were provided with mulberry leaves or with an artificial diet. The study focused on the nutritive value of both mulberry leaves and artificial diet. After completing the rearing, postcocooning parameters such as effective rate of rearing (ERR%), cocoon weight, shell cocoon ratio (SCR) and fibroin content (SC) were evaluated.

THE EFFECT OF P-DICHLOROBENZENE ON HONEY BEES ABILITY TO DETECT SUGAR CONCENTRATION IN NECTAR (GUSTATORY THRESHOLD)

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The gustatory threshold of honey bees is defined as their ability to detect the lowest sugar concentration in nectar. Given that p-Dichlorobenzene has been detected in honey, which is also used for bee consumption, the effect of p-Dichlorobenzene was examined on gustatory threshold of honey bees using two colonies of the Macedonian race. The two colonies used, were fed with sugar syrup (30%) for about a week. 400 mg of the substance in test was diluted in 1ml acetone and then in 1litre of syrup and given to the experimental colony. The control colony was given syrup containing only acetone in similar quantities. Two (2) series of sugar solutions were prepared with 0.1%, 0.3%, 0.5%, 1%, 3%, 5%, 10% and 30% sugar and p- Dichlorobenzene was added to one series of solutions. Each sugar solution was tested on the ability of honey bees to elicit the proboscis extension reflex (PER) after touching the antennae. The results are discussed.

MORPHOLOGICAL STUDY OF THE FEMALE INSECT *Marchalina hellenica* Gen. WITH SCANNING ELECTRON MICROSCOPE

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The most important honeydew producing insect for beekeeping in Greece is *Marchalina hellenica* Gen. (Hemiptera: Margarodidae). The female's immature stages feed on several species of *Pinus*, producing honeydew which is finally becomes honey known as "pine honeydew honey". In this study important

morphological aspects of the adult and immature stages of the female insect, are described with Scanning Electron Microscope. Specifically, is described by photographs the morphology of antennae, mouth parts, legs, spiracles, anus, haetotaxy and cuticle formations. Also significant differences in morphology between the stages are presented. Finally, the advantages and the contribution of Scanning Electron Microscope in studying the morphology of female *Marchalina hellenica* are discussed.

ALTERNATIVE METHODS OF CONFRONTATION OF *GALLERIA MELLONELLA* (WAX MOTH).

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Galleria mellonella (Lepidoptera: Pyralididae), wax moth, constitutes the major enemy of honeybees. The larvae destroy the honey combs, mainly in the deposit, but also in the beehive, when these are not covered by bees. The beekeepers use for the confrontation of problem chemical substances what suspend the biological development of insect or kill its biological stages, except the egg (larva, nymph and adult). The using substances are the sulphur, paradichlorobenzene (PDCB), the naphthalene, acetic acid etc. As all these substances are harmful for the beekeeper itself and because of the risk of high residues of 1,4 dichlorobenzene, the active substance of paradichlorobenzene, that was found in the Greek honeys, is investigated the possibility of use of alternative methods of confrontation wax moth, with friedly methods for the beekeeper and simultaneously sure for the honeybee products..
Are investigated the possibility of biological control by using the chalcid *Dibrachys cavus*, parasitoid of *Galleria mellonella*, but also the use of low temperatures.
The natural parasitism of wax moth from *Dibrachys cavus* can reach at the months of March, April and May up to 72%, while after the inoculation of honeycombs with adult parasitoids, the rate of parasitism can increase itself more reaching in March in 88,5%.
Respectively the evolutionary stages of *Galleria mellonella*, even the egg, are sensitive in low temperatures. Thus maintenance of combs in 0° C for 5 hours kills all the evolutionary stages of wax moth. Provided that the combs will protected after that from female ovipositing , they can maintain itself for as long as time it needs under any conditions.

EFFECT OF THE ENZYME SUBSTRATE ON THE MEASURED VALUE OF DIASTATIC ACTIVITY IN HONEY

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Abstract
A comparative study was performed on the two photometric methods that are used for the measurement of the diastatic activity in honey: the method after Schade that uses starch as substrate and the method that uses Phadebas tablets, with a cross-linked type of starch. In addition, a third different substrate was used for the method after Schade: starch prepared according to Zulkowski.
The measurements on 16 honey samples (3 replicates per sample for each of the three substrates) from producers of Crete, Greece, indicated that the starch acc.Zulkowski could replace the normal starch since it showed a very good correlation between the measured absorbance and the enzyme activity in Schade units and because the total time spent to the analysis was significantly decreased. Very good correlation has also been found between the enzyme activity in Schade units and the absorbance measured with Phadebas tablets. However, this correlation was found to differ from the one reported by Bogdanov et al.1997 & 1999 who have performed a big comparative study between the method of Schade and that with Phadebas tablets, using honey samples from the market. Additionally, in the present work, the

correlation observed with the Phadebas tablets was found to be significantly affected by the origin of honey samples (nectar, honeydew).

"MINERALS AND TRACE ELEMENTS IN GREEK HONEY-DISCRIMINATION OF GEOGRAPHICAL AND BOTANICAL ORIGIN"

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The variation in the concentration of various mineral elements has been studied in 48 honey samples from beekeepers in Greece, according to the botanical and geographical origin of the samples. The analysis was performed after honey digestion in a special microwave oven and subsequent examination with Inductively Coupled Plasma Atomic Emission Spectrophotometer (ICP-AES).
Quantitative differences have been observed among samples of different botanical origin (nectar, pine honeydew, fir honeydew) as well as among samples of the same botanical but different geographical origin. Differences and similarities with literature data are discussed.

CONTAMINATION OF HONEY WITH ANTIBIOTICS

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The contamination of honey with antibiotics is due to the use of them for healing the bacterial diseases of bees as well as from the spreads of fruit-bearing trees. According to EEC regulation 2377/90 on determination of maximum required limits in animal foods, there are no legalized MRLs for the antibiotics residues in honey. The analysis for the antibiotic detection in honey is performed by the use of chromatography and anther qualitative methods. In the present project, the results of antibiotic analysis in Greek honeys during the period from 1999 till 2003 are presented with the use of a simple column chromatography method.

TRADITIONAL APICULTURE. THE DEFINED PRODUCTION ACTIVITY WITHOUT THE USE OF PHARMACEUTICAL PROTECTION PRODUCTS, IN THE PREFECTURE OF LARISSA.

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The harmonic coexistence of man and nature is grandiosely confirmed in the philosophy and the practice of men, which worked on by tradition with apiculture. It is probably the simplest way to understand the term ‘’Aiforia’’ in the ecosystem, in society, in economy and so on. Most bees lived in

traditional hives (''kofinia'', ''kipseles'') or in unique brick or stone constructions (''pulitses'', ''melissoduvara'') and the beekeeper was forced to act with measure and self-limitation.

Purpose of this particular effort is the highlight of the traditional apiarian constructions and hives in the prefecture of Larissa and furthermore the philosophy and the practice of their proprietors.

The data was collected from thirty different apiarian families of the prefecture in two different ways. Prime material was collected via the questionary of E.DI.A.M. ''The Kirinthos'', which was properly adapted and contained seven groups of questions (hives, work, technique, products, infections, plants, folklore and other data). Furthermore information about apiaries and hives were collected by phone or written communication or using bibliographical resources.

The ''harvest'' has been rich in every occasion. Apiarian monuments (''melissomantri Anatolis''), museums of folklore regarding this issues (at Gonnoi), narrations, testimonies and memorials not only from elder people, but also from young beekeepers who continue the tradition (apiaries in hard to reach areas, ''kouveli'' in the house yard, accidental confrontation of a hive attack, practical healers and hunters of wild bees, eloquent wasp nests near a bee hive, queen-breeding in small baskets, bee-cultivation in ''pulitses'', the testimony of the oldest apiarian family of the east, the former apiarian folklore museum and others).

The above mentioned comprise tokens of the cultural heritage of Larissa prefecture and of Thessaly in general. Many apiaries were constructed using the method of ''xerolithia'' (dry-stone). Together with the rest of the constructions of the vicinity they consist the so called ''xerolithika topia'', which are stately protected areas (in Marktsas, Anatolis and Kodritsi Gonnon). The only way to fight the ''cachexia'' of the bees, is to provide them with syrup and especially their movement.

Surely the knowledge of former beekeepers must be saved and this could be done effectively with the foundation of the Center of Environmental Education in the municipalities of Lakereias end Gonnon of Larissa, that will have a beekeeping character and dimension.

The folkloric museums and the regions with interesting apiarian elements (monuments, apiarian flora etc) in Thessaly it is needed to be shown by printed and electronic material as well as to be protected.

NUTRITIVE-FUNCTIONAL, THERAPEUTIC ACTIONS AND MEDICAL APPLICATIONS OF THE BEE PRODUCTS

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ABSTRACT

Whether pure secretion products (royal jelly, bees wax, bee venom) or collected products (pollen, propolis, honey), bee products offer to human organism the richest spectrum of biochemical compounds with nutritive, functional and therapeutic actions. If only vitamins, proteins, enzymes, minerals, pigments (carotenoids and flavonoids), nucleic acids, complex lipids (phospholipids), hormone-like substances or hormone precursors, etc., are to be mentioned, it is enough to support the choice of these precious resources in preparing complex products meant to ensure the normal function of human organism.

Moreover, many of the above mentioned compounds are known for their important anti-oxidative potentiality, acting effectively to prevent the excessive production of free radicals - incriminated for the occurence of several functional disturbances and even of pathological processes.

These products are especially designated to regulate the metabolic processes in both healthy organisms and those affected by several deficiencies generated by pathological processes or suffering the effects of special environmental and working conditions.

Medicines developped with bee products are on their way to become an important support for the classic medicine – apitherapy , and can also be a natural, harmless alternative to the chemical synthetic preparations. The only required condition: standardization for both their chemical composition and therapeutic action (s).

Keywords: royal jelly, pollen, propolis, honey, pollen extract, soft propolis extract, food supplement, antioxidants, apitherapy.

STUDY OF MORPHOLOGY AND PHENOLOGY OF THE MALE INSECT *Marchalina hellenica* Gen.

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The insect *Marchalina hellenica* Gen. (Hemiptera: Margarodidae) is the most important honeydew producing insect in Greece since 60 % of the produced honey comes from its secretions. In this study the morphology of the adult male is presented for first time with Scanning Electron Microscope. Very important morphological aspects as head and antennae, thorax, wings and legs, abdomen and genital organs are described by various photographs. Also for the first time, the flight period of the adult male in various pine forests is recorded by two types of traps. Finally, the male's significance in insect's biology is discussed.

IMPLEMENTATION OF THE LEGISLATION CONCERNING HONEY AND TRADING OF HONEY IN THE GREEK MARKET

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In the framework of the implementation of the legislation, which was current until the 1st of August of 2003, there were several cases in which specific characteristics of standardized products were not in accordance with the legislation, due to physical conciseness of honey. The consequense of the fact above was the committal, unjustly most of the times, of the ones who standardize and beekeepers until the present day.

The new legislation is in harmony with the Direction 2001/110 EC on honey. However, several points need to be elucidated, in order to interpret sufficiently and implement normally the new legislation.

In addition, there are unclarified matters, concerning the determination of honey quality standards and product marking, which are not included in the legal framework, causing problems to the trading of honey in the greek market.

The disposition of great quantities of imported honey, which is being sold as greek product, continues to be an issue of great importance concerning the greek apiculture. The state audits are hardly operated, causing the continuation of this situation and destructive consequences on the greek product

DISSIPATION OF COUMAPHOS IN THE HIVE AFTER THE APPLICATION OF CHECK MITE STRIPS 10%

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Coumaphos is the active ingredient of 3 different drugs, which are used by beekeepers to fight the acari *Varroa destructor* Anderson. These drugs are Perizin, Asuntol and the new one CheckMite Strips 10%. Perizin is approved in Greece and Asuntol is used without approval. Coumafos Bee Strips is partly approved in U.S.A. The way of the application of the drug is entirely different from those of Asuntol and Perizin. To be more specific, 2 plastic strips are placed between 3rd-4th and 7th-8th frame. We study the percent of residues in honey after the use of check mite strips 10%.

The level of residues in every frame was studied, in order to conclude from which combs the honey have more residues. By this way, we could propose the producers to avoid harvest of the frames, that would maximize the pollution of the honey and we could achieve the reduction of the final concentration of coumaphos in the product.

Apart from the concentration of residues and the dissipation of the drug in the hive, a third factor, which was examined, was was the level of honey contamination in relation to the time between treatment and collection. Concerning the level of concentration of residues it was found that the frames which were in contact with the strips had residues that exceeded the MRL, while the others had lower concentrations. Furthermore, it was found that concentration of coumaphos in the frame which were in contact with the drug, were at the MRL 102 days after the removal of the strips. The previous is an indication that the time of two months between the application and the harvest is not enough. In addition to that, the exclusion of the harvest of the frames contacting with the strips, reduces the concentration of coumaphos in the final product.

It should be noted, that the experiments took place in 4 hives, from which all the combs were removed. Before the start of the experiment, the stored honey was examined for coumaphos residues. The method which used included an extraction with a 50:50 solution of ethanol and water, a clean-up step with Solid Phase Extraction and analysis with GC-μECD.

Keywords: GC-μECD, residues, coumaphos, check mite strips, dissipation

CONTAMINATION OF HONEY WITH SUBSTANCES USED AGAINST *VARROA DESTRUCTOR* ANDERSON

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The need of beekeepers to protect their property from the ectoparasite *Varroa destructor* Anderson, leads to the use of chemical substances. These substances are effective against acari, but they contaminate the final product with undesirable residues. The purpose of this assay was to check the usual concentrations of residues in honey, which appear in Greek market. Seventy two samples, which were coming from the markets of Athens and Thessaloniki, were analyzed. Furthermore, the concentrations of residues in honey collected in Greece were analyzed. It was considered useful to analyze samples from different areas in

Greece, in order to be able to conclude, the kind of veterinary drugs, which were used and also the trend that exist, about the way of application. The agricultural area can frequently confront with the exchange of opinions and recipes concerning the use of drugs. Samples of honey (n=148) from7 different areas of Greece were collected and were checked for the presense of residues of the veterinary drugs Perizin, Asuntol, Apistan, Mavrik, Folbex-VA and Malathion. The most frequently found active ingredient, was coumaphos, followed by fluvalinate and finally malathion. Bromopropylate was found in none of the samples. The kind of the substances used was found to depend on the area, from which it was selected. A multi-residue method was applied for the detection of acaricides. This method includes an extraction with a 50:50 solution of ethanol and water, a clean-up step with Solid Phase Extraction and analysis with GC-μECD.

Keywords: GC-μECD, residues, coumaphos, fluvalinate, bromopropylate, malathion, Greek honey

BOTANICAL DIFFERENTIATION OF GREEK UNIFLORAL HONEYS USING SOLID-PHASE MICROEXTRACTION

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The aroma profile of 10 unifloral types of honey was studied by means of Solid-Phase MicroExtraction (SPME), followed by Gas-Chromatography/Mass Spectrometry (GC/MS). The SPME fiber employed was a Carboxen/PDMS/DVB and the conditions for the isolation of the aroma compounds were: 6 mL sample volume, 60 ^{o}C waterbath temperature, 30 min equilibration time and 60 min sampling time.

Most of the categories are characterised by different components. Orange honey contains the three isomeric lilac aldehydes, methyl anthranilate, lavender lactone, dill ether, the two isomers of dehydroxy-linaloxides and three isomers of alpha-4-dimethyl-3-cyclohexen-1-acetaldehyde. Thyme honey is characterized by the hydroxycetones 3-hydroxy-4-phenyl-2-butanone and 3-hydroxy-1-phenyl-2-butanone, 2-hydroxy-1-phenyl-ethanone, phenylacetonitrile, carvacrol, alpha-ethylidene-phenylacetaldehyde, 3,4,5-tromethoxybenzaldehyde and two unknown isomers, while it also contains increased amounts of benzaldehyde and phenylacetaldehyde. Cotton honey is characterized bu benzenepropanol and (E)-cinnamaldehyde, while heather honey by p-anisaldehyde. In chestnut we find 1-phenylethanol, acetophenone and 4′-aminoacetophenone. In eucalyptus, increased proportions of pelargonic acid and the corresponding methyl ester are contained, while in jerusalem sage we find greater concentrations of linalool. Finally, strawberry-tree honey is characterized by an array of norisoprenoids, such as isophorone, 3,5,5-trimethyl-3-cyclohexenone, 2,2,6-trimethyl-1,4-cyclohexanedione, 4-hydroxy-2,6,6-trimethylcyclohex-1-en-1-one and the oxidized derivative at the -4- position and it also contains 3,4,5-trimethylphenol. Concerning pine and fir honey, no characteristic compounds were found.

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POLLEN GRAIN CHARACTERISTICS OF PLANTS FROM PELOPONNESE

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During the course of the project ‘Study of bee flora of Peloponnese’, pollen was collected from colonies located in 4 areas of Peloponnese: Achaia, Tripolis, Lakonia and Argolida. The pollen collected was originated from about 100 plants. The color spectrum of all pollen loads was recorded, all different pollen grains were photographed, and identification of pollen species was performed based on pollen grain characteristics. We present the microphotographs and the color spectrum of 60 plants identified at list to the level of genus and of 10 plants identified to the level of family

INTRODUCTION SUMMARY

“ ORGANIC BEEKEEPING”

Beekeeping is an important activity that contributes to the protection of the environment and agricultural and forestry production. The rules of organic production have been legislated within the European Union in the framework of the Council Regulation 2092/91 which was amplified with the requirements of the livestock production by Council Regulation 1804/99.
These Regulations include specifications regarding plant production , the origin and conversion of the animals, feeding , stable locations ,veterinary treatments , management of the manure, processing as well as obligations of the producers. The specifications of beekeeping are described separately by REG (EU) 1804/99. In these Regulations is also determined the Control and Certification System of organic products as well as the labeling.
Beekeeping products can be sold as organic only when the provisions laid down in the above Regulation have been complied with for at least one year. In the choice of breeds , account must be taken of the capacity of animals to adapt to local conditions , their vitality and their resistance to disease.
The siting of the apiaries must ensure enough natural nectar ,honeydew and pollen sources for bees and access to water. The artificial feeding of colonies is authorized where the survival of the hives is endangered due to extreme climatic conditions and can be carried out only between the last honey harvest and 15 days before the start of the next nectar or honeydew flow period. The disease prevention shall be based on the selection of appropriate hardy breeds and the application of certain practices. The use of allopathic chemically synthesized medicinal products for preventive treatments is prohibited.
The use of combs , which contains broods , is prohibited for honey extraction as well as the use of chemical synthetic repellents , the destruction of bees in the combs and mutilations such as clipping the wings of queen bees.
Final the beeswax for new foundations must come from organic production units or from the caps.

DETERMINATION OF P-DICHLOROBENZENE AND NAPHTHALENE RESIDUES IN HONEY USING ULTRASOUND ASSISTED EXTRACTION AND SOLID-PHASE MICROEXTRACTION

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The potential of determining p-dichlorobenzene and naphthalene residues in honey was studied. Two methods of isolation were evaluated, that is ultrasound assisted extraction (USE) and solid-phase microextraction (SPME), while the analysis was performed with a GC/MS equipment. More than 80 samples were analysed from various regions of Greece.

Concerning the two isolation techniques, SPME seems more promising as it provides a more clear GC profile that allows a better quantification. Moreover, it requires very small sample, while both techniques are easy to perform and have very low cost. It must be mentioned that the linearity is good for only up to 0.2 mg/Kg of honey and thus the SPME procedure is not recommended for higher residue levels than that.

Of the samples analysed, 14.5% contained p-dichlorobenzene and 9.6% naphthalene at levels greater than the MRL's. We must mention that there are no specific MRL's for these two residues. According to the European Code for honey, MRL's are arbitrarily set at 0.01mg/Kg of honey for those compounds that no particular limit has been established.

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PARADICHLOROBENZOL. A CHALLENGE FOR IMPROVEMENT

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It is a creepy problem which appeared suddenly and like a snowdrift. If there was not a coordinated and precise reaction from all, this problem could cause a very big crisis in the greek honey due to the interference of media.

What would happened if such a problem appeared in the decade of 1970, when the organizing, scientific, communicational substructure of apiculture was from insignificant to inexistent? The answer is: total disaster of greek apiculture.

Today with my 35 years of experience and occupation in the greek apiculture and promotion of greek honey, I am optimist. Today there are the proper circumstances and the means for the support of a greek traditional product which recently acquired its "national" character and meaning.

Today there is not only the experience but also the knowledge, not only the will but also the organization, not only the thought but also the science, not only the proposition but also the organized and systematic communication, not only the dream but also the target.

The paradichlorobenzol as a problem proved that we have the ability to rationalize the apicultural process in order to look at the future with optimism and security.

PROTEIN CONTENT OF MULBERRY LEAVES AS RELATED TO THE DEVELOPMENT OF SILKWORM (B*ombyx mori*) AND COCOON PRODUCTION

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The development and growth of silkworms as well as the cocoon and raw silk quality entirely depends upon the quality of mulberry leaves. The quality of mulberry leaves is closely related to the mulberry tree varieties, environmental conditions and cultivation practices. Most of the time, mulberry leaf quality is determined by its chemical contents (protein, carbohydrate, minerals, water content etc.).

This paper describes a study on the development of silkworms and cocoon production as related to protein content of mulberry leaves. For the experiment, eggs of the Chinese hybrid (Feng1 X 54 A) were hatched under controlled conditions of temperature (25˚C), relative humidity (75%) and photoperiod (16L:8D). Eight hundred newly hatched larvae (200 X 4) were reared on the leaves of two mulberry cultivars (Heyebai and Tang 10 x Lang 109). Leaf analysis was performed by Kjeldahl method to determine leaf composition in terms of nitrogen and protein contents.

After completion of the rearing, postcocooning parameters such as effective rate of rearing (ERR%), cocoon weight, shell cocoon ratio (SCR) and fibroin content (FC) were evaluated.

PRODUCTION OF ROYAL JELLY IN QUEENRIGHT COLONIES

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We developed a method of production royal jelly in queenright colony without restriction of the queen. We compared this method with others that produced royal jelly a) without the queen (queenless colonies) and b) have the queen restricted in triple – horizontal colonies.

The production of royal jelly from three (3) queenright colonies was 830 gr (0,205 gr / cell) after 48 graftings. From three (3) queenless colonies we produced 1580 gr (0,220 gr / cell) after 41 - 50 graftings. The two (2) triple – horizontal colonies produced 1297 gr (0,181 gr / cell) after 48 graftings.

The success of this method is based on the use of young queens (less than 1 year old) so that can accept the artificial queen cells that are grafted. Also it is important to use at the first (and only the first) grafting a queenless colony which will keep the grafted queen cells for one day so that increase the acceptance.

Key words : production of royal jelly, queenright colony

USEFULNESS OF HONEYBEE PRODUCTS IN MAN.

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The man from the dawn of his history developed all the elements, in which he could have access, as foods or as medicines. Among them important place they possess the products of honeybee, honey, pollen, royal jelly, wax, venom and propolis. Reports that prove the important place that these products possessed in the daily life, as foods or as therapeutic means, exist regularly in the archaeological discoveries of last at least 8.000 years of man history.

From the beginning of 16th cent. products that are produced by the man replaced in the daily life the products of honeybee, beginning from the wax for the lighting and the honey as sweetening substance. In the beginnings 19th cent. this replacement is complete, not only in the alimentary habits but also mainly in the use of products of bee in medicine, as the "modern" medicine puts the bases of utilization artificial therapeutic substances.

Today the world production of honey reaches the 1 million tones by year and its trade has been developed in the entire world**.** Overtaking the "dark" 19th and 20th century, when the honey had been ostracized by the basic foodstuffs and much more by the substances with therapeutic action, the western world discovers again the honey as natural, ecological food, but also as therapeutic factor, in the antipode of polypharmacy and its consequences.

EFFECT OF HIGH TEMPERATURES DURING SILKWORM REARING

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Many factors contribute to the production of cocoons of good quality and high production. Among them, temperature in the rearing room is one of the most important factors.

The present paper investigates the effect of high temperatures on silkworm growth and quality of the produced silk.

The rearings were realised in two rearing rooms under two different environmental conditions. In the first (experiment) room, the temperature was maintained between 30-35 ^{0}C and the relative humidity at 60%, while in the second (control), a constant temperature of 25 ^{0}C and relative humidity of 75% were maintained. Both for the experiment and the control, 4 replications were used, of 200 larvae in each replication. In this experiment, rearing duration, mortality rate, cocoon weight, shell weight and ratio of sericin to fibroin were estimated. The results showed that in high temperatures the rearing duration was shorter, the cocoon weight higher, but the mortality rate higher.

BEE FLORA OF PELOPONNESE

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We report the results of the two year project ‘ Study of bee flora of Poeloponnese’. Several approaches were used during the above study such as a) experience of beekeepers, b) visits, observations and photographs of the flowering plants, c) identification of plant species based on pollen collected by honey bee colonies located in 5 selected areas and d) bibliographic resources. Some information related to technical methods of utilization of the main bee plants of Peloponnese as fir, pine, thyme, heather, orange and eucalyptus are also given. Finally, a list of the main bee plants of Peloponnese is provided in respect of administrative boundaries (e.g. prefectures) as well as in respect of geographical morphology (mountain, valley, coastline).

A STUDY OF HONEY'S ABILITY TO ABSORB VOLATILE COMPOUNDS FROM ITS ENVIRONMENT

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One of the honey's characteristics that are associated with the costumers' preferences is its aroma. This characteristic is greatly depended on the volatile and semi-volatile organic compounds. Most of these are derived from the origin source, the nectar or honeydew, and few of them arise during processing or storage. Surprisingly enough, never before was a study made on off-flavours of honey, although is widely known as a product that can easily absorb smells from the surrounding environment.

In this work, the ability of honey to absorb volatile compounds from the environment was studied. In the first phase the parts of the glass vessels with metal lips was studied to find which of them can absorb volatile substances. In the second phase different spices were put in new glass vessels and stored at room temperature. Subsequently, the spices were removed, the vessels were thoroughly washed and pine honeys were stored in each one, at room temperature for 3 days. The absorption at volatile compounds from these previously stored spices was analysed in a Purge & Trap – gas chromatograph – mass spectrometer system.

The result of the first experiment suggests that the volatile compounds of the previously stored spices were absorbed by the plastic wrap under the lip and not by the glass of the jar. From the second experiment it had been found out that honey has the ability to absorb volatile compounds from its environment.

A COMPARISON OF THE VOLATILE CHARACTERISTICS OF GREEK AND TURKISH PINE HONEY.

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Pine honey is produced from the honeydew secreted by the insect *Marchalina hellenica*. This kind of honey is produced only in Greece and Turkey. The annual Greek honey production is estimated at 12.000 tones and the Turkish at 71.000.

In this work 46 samples of pine honey were analysed, 24 were from Halkidiki and 22 two from Turkey. A Purge & Trap – gas chromatograph – mass spectrometer was used for the extraction, separation and identification of volatile. From all samples, 84 volatile and semi-volatile compounds were extracted and intentified. In particular, higher percentages of benzaldehyde, phenylacetaldehyde and decanal were found in Greek samples compared to the Turkish ones. Turkish honey samples were found to have a higher percentage of toluene, octane, a- and b-pinene, limonene, a-terpinolene, cis-linaloloxide, nonanal, nonanol, 1,2,3-thimethyl-indene, p-menth-1-en-9-ol and juninen. Apart from common substances, some characteristics compounds were detected in samples from each country. In particular 1,4-dichloro-benzene was found in Greek pine honey, while 3-carene, trans-linaloloxide, santene and an unidentified compound which marked as unknown (m/z 70, 93, 123, 185) were found in Turkish samples. The 1,4-dichloro-benzene is exogenous origin.

This work is the first part of a research in a large number of pine honey samples from different areas with an aim to detected volatile compounds as markers. These markers could be used to distinguish pine honey from different areas.

COLOR SPECTRUM RECORDINGS OF POLLEN LOADS

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The color spectrum of pollen loads collected by bees is different for each plant and it can be used for the identification of the plant although this is not a very accurate method. This is the first attempt to record the color of pollen loads in Greece and create a reference color chart starting from plants of Peloponnese. The color chart was created based on the 4 color system (CMYK). There are 6.000 colors included in the chart with percentages of black (K) from 0-50%. The chart was printed as posters and as a book and it can be extended to other 5.000 colors. The pollen loads were separated according to their color, and the code number was recorded for each color representing the percentage of each of the 4 separate colors of the chart. Species identification for each pollen load followed with the use of a microscope. One to four pollen colors (color spectrum) were recorded for each plant species.

QUALITATIVE PROPERTIES OF GREEK COTTON HONEY

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In this survey several qualitative properties of cotton honey, taken from different Greek areas, were studied. Moreover, its antioxidant and antibacterial (bacteriostatic or/and bactericidal) capacity and the total phenolic content were determined.

The moisture, the Hydroxymethylfurfural (HMF), and the diastase (enzyme) fluctuated between 15,0-18,1%, 0,68-12,07 mg/kg, and 19,5-33,23 DN (Diastase Number) respectively, while the electrical conductivity was between 0,445mS/cm and 1,149mS/cm.

In order to calculate the antioxidant capacity we adapted the FRAP assay (Ferric Reducing Antioxidant Power) and the results were between 16.573,86 – 33.278,74 μm/g of honey.

The total phenolic content of diluted honey samples varied from 13,17-,32,61 μg Gallic acid equivalent (GAE) / g of honey.

The antibacterial activity of honey samples against tow phytopathogenic bacteria *Bacillus subtilis* and *Pseudomonas syringae* was determined using the broth dilution method. Specifically the minimum inhibition (MICs) and bactericidal concentration (MBCs) of honey have been estimated. The suspending of bacteria development (B. subtilis and P. syringae) was found in 10% (w/v) and more of honey dilution and the bactericidal action was also found when the concentration was 15% (w/v) and more of honey.