Issue **5** • November **2024**

NO BEES LIFE

EBA MAGAZINE

27 COUNTRIES

FROM WHICH EBA HAS MEMBERS

(41 beekeeping organizations)

In order of confirmation of the Statute of EBA

366.368 beekeepers

Serbia
Slovenia
North Macedonia
Bulgaria
Greece
Romania
Malta
Germany
Hungary
Ukraine
Montenegro
Lithuania
Bosnia and Hercegovina
Sweden
Croatia
Czech Republic
Poland
United Kingdom
Netherlands
Italy
Ireland
Belgium
Cyprus
Türkiye
Switzerland
Prishtina
Portugal
Spain

THE ANALYZES ARE DISASTROUS, BUT FAKE HONEY IS STILL ON THE MARKET SHELVES

The results of the analyses are catastrophic, but without a change in legislation, fake honey (which should not even be called honey) will not be removed from the market!

Recently, beekeeping organisations across Europe have been trying to show, through various analyses, how much counterfeit honey is actually on the market. Using a variety of methods, the results are frightening, with fakes found ranging from 60% to almost 100%. These results should be a wake-up call for all European policy-makers and consumers alike.

The European Beekeeping Association advises consumers to be very careful when buying honey and, in particular, to buy honey directly from beekeepers, to buy honey from their local area, to choose honey of their own country's origin or at least of European origin.

The methods used to analyse honey vary widely, so it is now up to the experts. Experts in the field of honey analytics should study all methods, especially the newest ones, and then, through the Honey Platform set up by the European Commission, propose to the politicians which methods need to be legislated and harmonised. This must be followed by the immediate establishment of a reference laboratory for the detection of counterfeits and full control of honey already at its point of entry into Europe. Honey without clear traceability and quality control should not be on the shelves in Europe.

All of the above is already enshrined in EU documents, which are part of the European Commission's guidelines on the latest amendment to the EU Honey Directive, which stipulated that all honey must be labelled with an accurate indication of the country of origin. Unfortunately, the timeline that envisages a solution within three years is unacceptable for European beekeepers. What we want from politicians is clear – we need change now!

Our aim is that the analyses will be recognised by all inspection bodies in all countries in Europe and that counterfeit honey will be immediately removed from the market. Without this, we are completely powerless, despite the many analyses that point to, or even prove, counterfeits.

If all of the above is not immediately addressed by European policymakers, we propose that during this transitional period, they should IMMEDIATELY impose quotas or even an embargo on imports of honey from the countries where counterfeiting is most commonly found! Europe needs bees for pollination, which cannot be imported or bought, and if action is not taken now, beekeeping in Europe will be abandoned, thus ending the free pollination service that the bees provide, because unfortunately today bees cannot survive without beekeepers! We need to be aware that the disappearance of pollinators (due to changes in the environment and the use of pesticides) has made the honey bee an even more important pollinator (up to 90%, according to some data), and that the beekeepers are responsible for its existence, while other pollinators are largely left to fend for themselves.

The European Beekeeping Association would once again like to call on all European beekeeping organisations to join us (it is completely free of charge), and I am convinced that united we can be successful. At the moment, nearly 400,000 beekeepers in 27 countries trust the EBA. I am convinced that we will be even more successful if more join us. Beekeeping and bees know no borders, are neither left-wing nor right-wing, they do not come from the East or from the West... so there is no place for divisions in our EBA. We can only win against counterfeits together!

It really is now or never for the protection of bees, for the preservation of beekeepers and, above all, for the protection of consumers all over Europe!

Boštjan Noč
President of the
European Beekeeping Association

APPOINTMENT OF EBA AS A MEMBER OF THE HONEY PLATFORM EXPERT GROUP

On October 3, we were informed by the Directorate General for Agriculture and Rural Development from Brussels that EBA has been appointed as a member of the Honey Platform expert group.

The need for clearer rules on the origin and composition of honey led the European Commission to set up a platform for honey.

The Commission has published a call for the establishment of a honey platform to gather the best available expertise on the authenticity and traceability of honey. The call for applications was open until July 15.

The Head of the Scientific Committee of the EBA, dr Urška Ratajc, applied on behalf of the EBA. This group of experts will assist the Commission in harmonizing methods to detect adulteration in honey and tracking the product back to harvesting producer or importer.

The new common rules on the composition and labeling of honey will help consumers make informed choices by increasing transparency in the food chain and limiting fraudulent practices.

The upcoming Honey Platform, composed of up to 90 members, is expected, among other things, to gather data for methods to improve authenticity controls of honey, and provide recommendations for a Union traceability, composition criteria, and the possibility of establishing a Union reference laboratory.

SHORT-TERM ACTIVITIES OF THE EBA TO STOP COUNTERFEITS

The Executive Board of the EBA made a decision on the short-term activities of the EBA to stop counterfeits:

The European Beekeeping Association's (EBA's) main goal is to STOP counterfeits. The revised Honey Directive introduces requirements for uniform and clearer labelling of the origin of honey blends by country with the respective proportions, which is an excellent achievement. Additionally, Directive (EU) 2024/1438 states that harmonised methods for honey authentication will be selected, a European Reference Laboratory will be established, and recommendations will be issued for a traceability system to the importer and producer.

However, the projected timeframe for the full implementation of these measures—2028 or 2029—appears misaligned with the urgent nature of the issue.The EBA will direct all efforts to ensure that all commitments from the Directive are realized as soon as possible in the political field in the EU. Therefore, the first task of the EBA will be a conversation with all political decision-makers in this area. EBA believes that European policy will understand the problem and urgently protect consumers and beekeepers, because we believe that the EU Commission's data on 46% of fake honey is a clear enough warning and alarm!

The EBA also follows, cooperates and supports the work of all beekeeping organizations in Europe, which is aimed at the same goal – stop counterfeiting. In the short term, the EBA will direct all its forces to a dialogue with the decision-makers, after all, the new EU leadership must be given a chance.

We will decide on possible protests in case of unsuccessful negotiations.

CONSTRUCTIVE MEETING OF THE EBA WITH EPB

A constructive meeting of the EBA was held on October 2, which was represented by Mr. Boštjan Noč – President of the EBA, Mrs. Urška Ratajc – Head of the Scientific Committees of the EBA, Mr. Rodoljub Živadinović – Vice President of the EBA and, Mrs. Biljana Tomić – General Secretary of the EBA, with representatives of EPB (Estonian Proffesional Beekeepers) represented by Mr. Mario Kalvet, Vice President of EPBA and Mr. Peeter Matson, who was invited to agree on joint action in the future.

We discussed joint capacities, got acquainted with future plans and potential fields of cooperation.

EBA, as you know, is open to cooperation and the position of the EBA leadership is that cooperation should be achieved with all actors of beekeeping at the European level in order to achieve common goals for all bees, beekeepers and consumers in Europe.

Only united and together can we achieve our goals.

MEDIA THAT REPORTED THE PRESS RELEASE OF THE EBA

Slovenia
https://si24.news/novice/2024-10-16-cebelarstvo-ne-more-preziveti-nelojale-konkurence
https://novicnik.si/novice/2024-10-16-cebelarstvo-ne-more-preziveti-nelojale-konkurence
https://pressnews.si/novice/2024-10-16-cebelarstvo-ne-more-preziveti-nelojale-konkurence
https://lokalnodogajanje.si/objava/2024-10-16-cebelarstvo-ne-more-preziveti-nelojale-konkurence

Serbia
https://agroportal.rs/na-svetski-dan-hrane-evropski-pcelari-porucuju-odmah-poostriti-mere-protiv-laznog-meda/
https://poljoprivrednik.net/newsdetails/670f83ca9b992f22fdbd9e2b/Evropsko-pcelarstvo-pred-kolapsom,-marketе-preplavio-lazni-med
https://domacinskakuca.rs/2024/10/16/eba-se-bori-za-skoro-400-000-pcelara/
https://biznis.kurir.rs/amp/9465637/svaka-tegla-meda-morace-da-ima-poseban-natpis
https://www.politika.rs/scc/clanak/638659/Ugrozeno-cak-400-000-pcelara
https://www.agrotv.net/pcelarstvo-tesko-moze-da-prezivi-nelojalnu-konkurenciju-falsifikovanog-meda/
https://www.youtube.com/watch?v=M6stn1T5SPQ
https://spos.info/pcelarstvo-ne-moze-da-opstane-od-nelojalne-konkurencije/

Croatia
https://www.dnevno.hr/gospodarstvo-i-turizam/u-trgovinama-zavladala-prevara-na-medu-kakva-se-ne-pamti-posljedice-mogu-biti-katastrofalne-2548192/?fbclid=IwY2xjawF_s9JleHRuA2FlbQIxMQABHQVf4Uc0SjUBf1artn1_WVU6m21E_EMUKG--2T0Y9kyBCyDcWaaKT3mArA_aem_mHhHGARdoP3rkxbXqkM0qw&sfnsn=mo
https://pcelinaskolica.wordpress.com/glavni-izbornik/novo-na-portalu-2/

Spain
https://www.agrodigital.com/2024/10/18/444861/

Portugal
https://vozdocampo.pt/2024/10/16/a-apicultura-europeia-nao-sobrevivera-a-concorrencia-desleal/

Bosnia and Herzegovina
https://radioassarajevo.com/eba-upozorava-pcelarstvo-ne-moze-opstati-od-nepostene-konkurencije/

CHALLENGES IN THE EUROPEAN BEEKEEPING AND HOW TO SOLVE THEM

TOPIC OF THE MEETINGS HELD IN SERBIA AND NORTH MACEDONIA ON SEPTEMBER 21 AND 22, 2024.

Challenges in the European beekeeping sector and how to solve them is the topic of the meetings held in Serbia and North Macedonia on September 21 and 22, 2024.

Since its establishment, EBA has been continuously dealing with challenges in the European beekeeping sector. The President of EBA visited Serbia and North Macedonia together with 50 beekeepers from Slovenia

On September 21, 2024. The 7th Beekeeping Fair of the Southeastern Balkans was held in Vranje, organized by the Serbian Federation of Beekeeping Organizations with the city of Vranje, in cooperation with the European Beekeeping Association (EBA), the Union of Beekeeping Associations of North Macedonia, the Union of Beekeepers Association of Republic of Srpska and the National Association of Beekeepers of Albania with the support of the Beekeeping Society "Matica "Vranje.

The topic of the round table, which brought together numerous beekeepers and visitors from

Serbia and the region, was: "How the European Beekeeping Association (EBA) will save bees, beekeepers and consumers".

EBA President Boštjan Noč, General Secretary Biljana Tomić and Head of EBA Scientific Committee dr. Urška Ratajc answered the questions of the attendees about EBA's activities and plans for further activities in a comprehensive report on EBA's work.

On September 22, 2024, the EBA President visited North Macedonia, Kumanovo, together with 50 beekeepers from Slovenia.

The host of the meeting at which the president of the EBA appeared with 50 beekeepers from Slovenia is Mende Trajkovski from North Macedonia, the president of the Union of Beekeeping Associations of North Macedonia and the president of the Supervisory Board of the EBA and the ambassador of Republic Slovenia also attended the meeting, Mr. Gregor Preskar.

This was also an opportunity for the Ambassador to learn about challenges in the European beekeeping sector, EBA activities and possible solutions. The meeting was attended by all presidents of beekeeping associations from North Macedonia.

The visit continued at the house of beekeeper Boban Stoševski in the village of Dragomance Kumanovsko and the beekeeping farm of Boban Stoševski near Kumanovo, which operates with 350 hives.

The conclusions of the meetings in Serbia and North Macedonia are the same: the problems in beekeeping are so great that without the continuous support of all actors in public life, the problem of beekeeping cannot be solved.

EBA will continue with similar activities in the coming period in order to resolve the difficult situation in the beekeeping sector in Europe with joint efforts.

EXPOSING JOINT RESPONSIBILITY FOR HONEY FRAUD

The Global Industry of Adulterated and Fake Honey Production

It is generally accepted that there is a global industry producing adulterated and artificial honey, which is being distributed as authentic in Europe. Many countries are involved in this fraud. According to the coordinated research of the Commission «From the Hive» (European Union, 2023), besides China—where 100% of suppliers were found to have at least one sample that did not comply with EU regulations, the same problem was identified in 100% of suppliers from the UK, India, Israel, and Zambia. The issue also extended to 80% of suppliers from Turkey, 63% from Brazil, 50% from Vietnam and Ethiopia, followed by other countries like Ukraine, Mexico, New Zealand, and Russia.

The honey market grows by about 100 million euros annually, with prices ranging from 0.80 to 2.54 euros. This situation is exacerbated by the U.S. imposing protective tariffs (antidumping duty) on cheap imported honey from China and planning to extend these measures to other countries exporting unjustifiably cheap honey. It

is further worsened by the rise of industries producing fake honey (honey without bees), which have already expanded to four countries: the U.S. (MeliBio and Bee-io), Germany (Veganz), Israel (Bee-io), and Australia (Bumble Bloom). This product is essentially a honey imitation syrup, advertised as having the same benefits for the human body as natural bee honey, despite no scientific evidence to support such claims. Furthermore, it is marketed as a solution to save bees from the alleged mistreatment they endure from beekeepers! They are essentially discrediting the real honey in order to market the fake one.

Europe has become a global dumping ground for adulterated and fraudulent honey. This product has already entered the EU market. A notable mention by the president of professional beekeepers, Bernhard Heuvel, stated that 80% of 30 honey samples tested from the German market were fake honey!!

The Free Entry of Adulterated and Fake Honey into Europe

This problematic honey is imported into European countries without proper controls. According to the EU's coordinated research, 100% of the honey imported by suppliers in Belgium, Denmark, Greece, Ireland, and the Netherlands did not meet EU quality standards. In Germany, the percentage reached 88%, in Spain and Poland, 85%, and in the Czech Republic, Lithuania, Norway, and Switzerland, it was 50%. With such high percentages of adulterated honey imports into Europe, it is clear that custom checks in European countries are not being carried out properly.

This means that the EU Directive 97/78/EC is not being implemented. This directive states that for a border post to be approved by the Community, it must have, among other things, an adequate number of veterinarians and specialized staff, who in addition to checking documents, should conduct sampling of imported products of animal origin, such as honey. This staff should have access to appropriate equipment and facilities for product inspections and collaborate with a specialized analysis laboratory for checks beyond their capability. The directive even specifies the methods for sampling and states that if a product does not meet EU quality criteria, it must be returned or destroyed.

The problematic honeys imported into the EU did not meet the quality criteria of Directive 2001/110 EC. These quality criteria do not require specialized and expensive equipment. It is not a significant expense to equip a state inspection laboratory at border posts with a spectrophotometer, water bath, centrifuge, microscope, pH meter, heating plate, scale, conductivity meter, and refractometer. With these instruments, it is easy to check whether honey complies with the legislative provisions of Directive 2001/110 EC and now Directive 2024/1438 EU. There is even a provision in EU Directive 97/78/EC for border posts to collaborate with more specialized laboratories to detect exogenous sugars and identify adulteration through modern analytical methods.

Obviously, in violation of European law, several border inspection posts have been approved without meeting the legal requirements, lacking the necessary staff and equipment, or failing to enforce the law and conduct proper sampling and inspections.

At this point, the joint responsibility for honey fraud includes the European Union itself, which has approved border posts that do not meet the legal requirements. Additionally, each country bears responsibility for not adhering to the directive on proper checks, resulting in problematic honey being imported into Europe at alarming rates. Accomplices also include the staff at border posts, who, although aware of the issue and obliged to know the legal requirements of their profession, fail to act on the inadequate inspections of goods entering Europe.

The Distribution of Adulterated Honey in European Countries

The importer of adulterated honey, who is undoubtedly aware of the poor quality of the product they purchased, faces two challenges. First, how to pass consumer protection checks on the shelves, and second, how to overcome the distrust of consumers who have heard that imported honey is of lower quality than domestic honey.

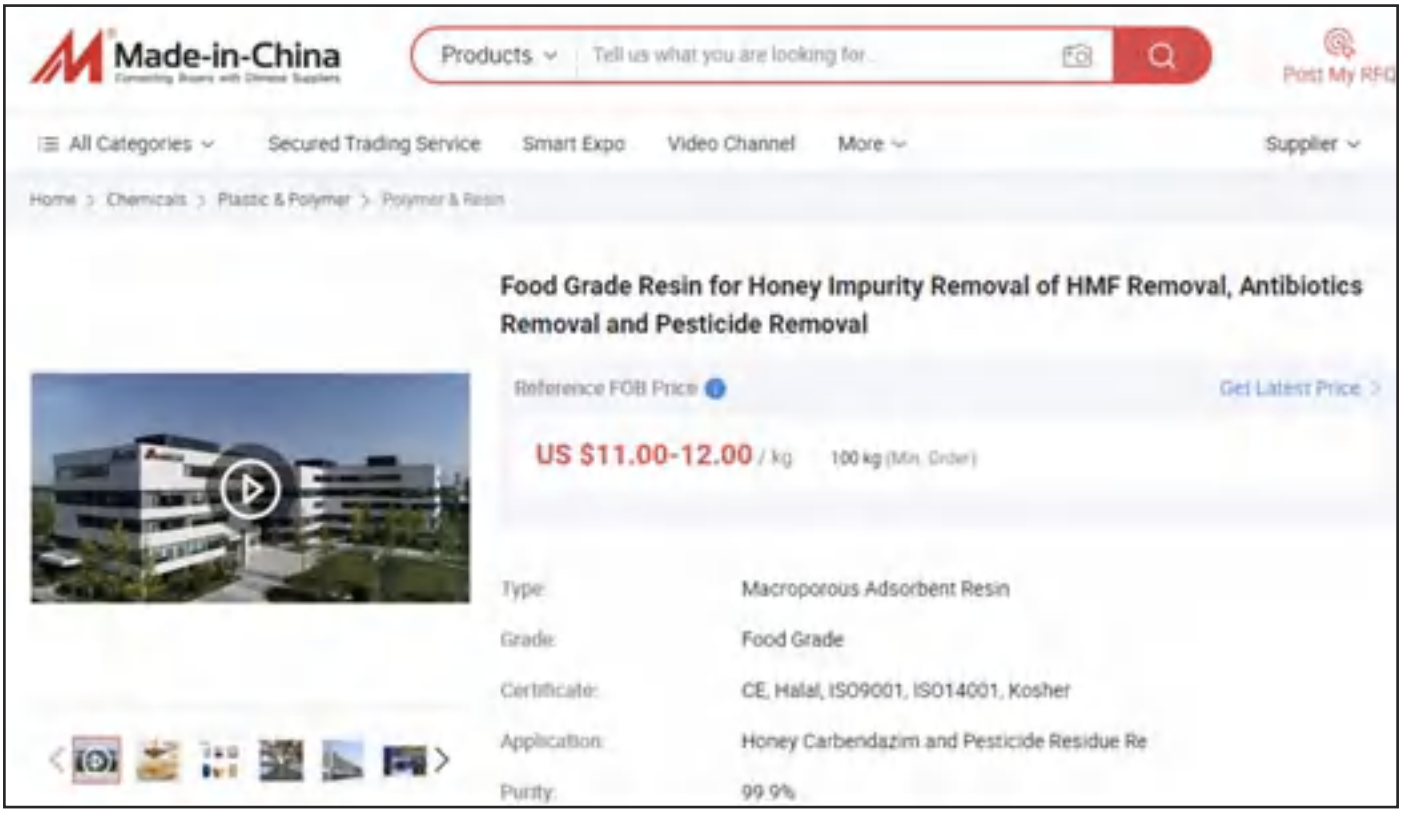

Figure 1. Advertisement for resin filters online, which remove HMF, antibiotics, chemical residues, and of course all pollen.

The first challenge is solved with the help of technology and science. Chinese manufacturers already advertise resin filters online, which help remove HMF, antibiotics, and chemical residues from honey (fig. 1). HMF is an indicator that honey has been overheated, possibly degrading its nutritional value. Removing HMF merely eliminates the indicator, but the degradation remains. However, this is of no consequence because the biological degradation of honey (antibacterial, antioxidant activity, aromatic profile, etc) is not legislated and won't be detected during consumer protection checks.

Of course, the resin filters will also remove the pollen that the product contains. This is not a problem for adulterators but an advantage be-

Quality problems in imported honey	Fix the problem
High HMF	Removal with resin filters
Pesticide residues	Removal with resin filters
Residues of antibiotics	Removal with resin filters
Diastase activity low	Addition
Absence of pollen grains	Mixing with domestic honey
Light color	Mixing with domestic dark honey
Sugars (HFCS)	Adjust according to Directive 2001/110
Antibacterial & antioxidant action	Cannot fixed but according to current legislation, their control is not foreseen

Table 1. How the degraded imported honey in the EU upgraded to normal

cause, by removing the pollen, the ability to identify the geographical origin is also removed. Mixing even a small amount of domestic honey makes the product “local”. It has been estimated that mixing even 20 kg of domestic honey into one ton of ultra-filtered honey results in over 1,000 pollen grains per 10 grams of honey. While it will be a honey low in pollen grains, this doesn't matter to the adulterator since EU law does not specify a minimum number of pollen grains for genuine, natural and unprocessed bee honey, even though this issue was identified 10 years ago in Directive 2014/63 EU.

In Table 1, some of the simple solutions available to an importer of adulterated or degraded honey are provided, allowing them to upgrade their product to pass consumer protection checks and fully comply with the parameters of the honey directive.

According to the coordinated research "From the Hive," the efforts of adulterated honey importers to "cook" degraded honey so that it meets EU quality criteria are aided by accredited honey inspection laboratories in the EU. This information is shocking, considering that these laboratories are likely developing methods to detect fraud or cooperating with state agencies to inspect honey. A true Trojan horse within the heart of Europe, always one step ahead of proposed measures to curb honey fraud.

This situation is further aided by EU legislation, which until now allowed for filtered honey that could remove a significant portion of pollen grains without defining a minimum number. While the filtered honey has been abolished in the new directive, it has paradoxically been replaced by baking honey, given a new definition—the same one that previously applied to filtered honey. It is

Figure 2. This honey is available in Greek supermarkets. It states its geographical origin from six different countries, among other information and images, in violation of Regulation 1169/2011. Most imported honey exhibits a similar problem on their labels and remains on the shelves despite complaints being made to the relevant authorities

honey that has been overheated or from which foreign organic or inorganic substances have been removed in such a way that a significant portion of the pollen is removed. This honey is imported without the mandatory indication of the country of origin, contains very few pollen grains that will disappear after mixing with the domestic honey, and will transform from baking honey of unknown origin into regular domestic honey.

The second problem facing the importer of adulterated honey, that of overcoming consumer distrust regarding the quality of imported honey, is solved in two ways. First, by purchasing invoices for domestic honey from local beekeepers, allowing them to market it as local, and second, by listing the countries of origin among other information and images on the back of the label, which the consumer is unlikely to see (fig. 2). This, of course, violates EU Regulation 1169/2011, which states that the harvest country should not be hidden, covered, or interrupted by any other text or images.

This is aided by the minimal allowable font size of just 1.2 mm and the absence of thorough inspections.

In Greece, for example, despite several complaints about misleading labels, no authority has acted to remove them from shelves. In this case, the accomplices include laboratories that assist the adulterators, local collaborators who provide invoices, EU legislation that favors the falsification of geographical origin and consumer deception, and the lack of substantial inspections.

Conclusions and proposal

Many are the joint responsible for the massive honey fraud currently taking place in Europe, and many are accomplices to the crime. First and foremost is the European Union itself, which either has legislation but fails to enforce it, resulting in adulterated and fake honey entering through its borders without proper inspections, or has gaps in its legislation that encourage honey fraud. At the same time, it delays proposing a unified traceability system for imported honey, leaving its movement in the domestic market entirely unchecked.

The same applies to many member states, which avoid conducting the thorough inspections required by law at customs stations. Some member states may have even enacted decisions that encourage adulteration. A prime example is Greece, which, with a national decision in 2004, set an upper limit for pollen grains in eight pure categories of honey, and successive gov-

ernments have completely ignored scientists' and beekeeping organizations' complaints, documents, and recommendations to amend the decision and set a minimum pollen grain limit instead of upper, to prevent the mixing of domestic honey with ultra-filtered products.

Import traders also bear responsibility for the fraud, as they import low-quality honey, often dangerous to consumer health, and resort to unethical practices to market it as regular or even domestic in the local market. Accomplices in this fraud are also some accredited honey inspection laboratories in the European Union that collaborate with adulterators and possibly act as consultants for the adulterated and fake honey production industry. The same applies to the local collaborators of adulterated honey importers, who provide cover with invoices and other conveniences.

In this corrupt system, the beekeeper-producer and the consumer are essentially alone, without any support, and completely powerless to face the situation that has been described. The interests are enormous, and the parties involved are many.

Beekeepers, of course, must continue to protest and demand solutions from the state for their survival despite their disappointment. However, the years have shown that the various governments that come and go either do not want or are unable to address the issue. During the beekeepers' protests, politicians either do not respond or resort to tactics such as a small subsidy per hive and numerous future promises. This situation has been repeating for years, and the problems not only persist but multiply.

Considering my country's handling of the beekeepers' demands for solutions to their problems, I personally believe that beekeepers, alongside their protests and demands, must also adopt solutions they can promote themselves. One of these is to continue producing quality honey while ensuring it is distinguished from imported honey. This can be achieved with the help of their associations, through a distinct label that should be advertised and made known to the

Figure 3. Distinct honey label that differentiates local from imported honey. The label is provided on the initiative of the beekeepers from the federation and the association of beekeepers without government involvement. The first label is from Germany and the second from Cyprus

consumer, as has been done in Germany, Cyprus, and possibly other countries (fig. 3).

At the same time, beekeepers must also work with scientists to inform consumers about the benefits of real bee honey. Consumers should also learn where to buy honey to be assured of its purity, quality, and safety.

They need to be informed that cheap honey is likely an adulterated product with high-fructose corn syrup (HFCS, HFIS). Consumption of such adulterated honey, according to clinical studies in humans and experimental animals, poses risks to consumer health (Abdulrhman et al., 2013, Samat, et al., 2018, Yaghoobi et al., 2008) Specifically, it raises blood sugar levels, causes hyperglycemia and type II diabetes, increases body weight, causes obesity, raises fat levels in the blood and liver, increases triglycerides, LDL cholesterol levels, and uric acid, causes high blood pressure, chronic kidney damage, early mortality and other adverse effects.

Consumers must also be informed that fake honey has nothing in common with bee honey, and no studies prove it has the same benefits as bee honey. On the contrary, there are numerous studies from clinical research that document the beneficial and therapeutic effects of natural honey on the human body (Agrawal et al., 2007, Al-Waili, 2004, Bahrami et al., 2009, Chepulis, 2007). The results of these clinical studies should be made known to consumers by industry scientists.

If the consumer believes, with well-documented arguments, that cheap imported honey might also be dangerous to their health, while natural bee honey is a valuable product for them and their family, they will turn to buy real honey directly from the beekeeper or from the producers' group that produces it. This is the only way to ensure the purity of the product for themselves and their family."

Andreas Thrasyvoulou
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Aristotle University of Thessaloniki (AUTH)
Hellenic Republic (Greece)

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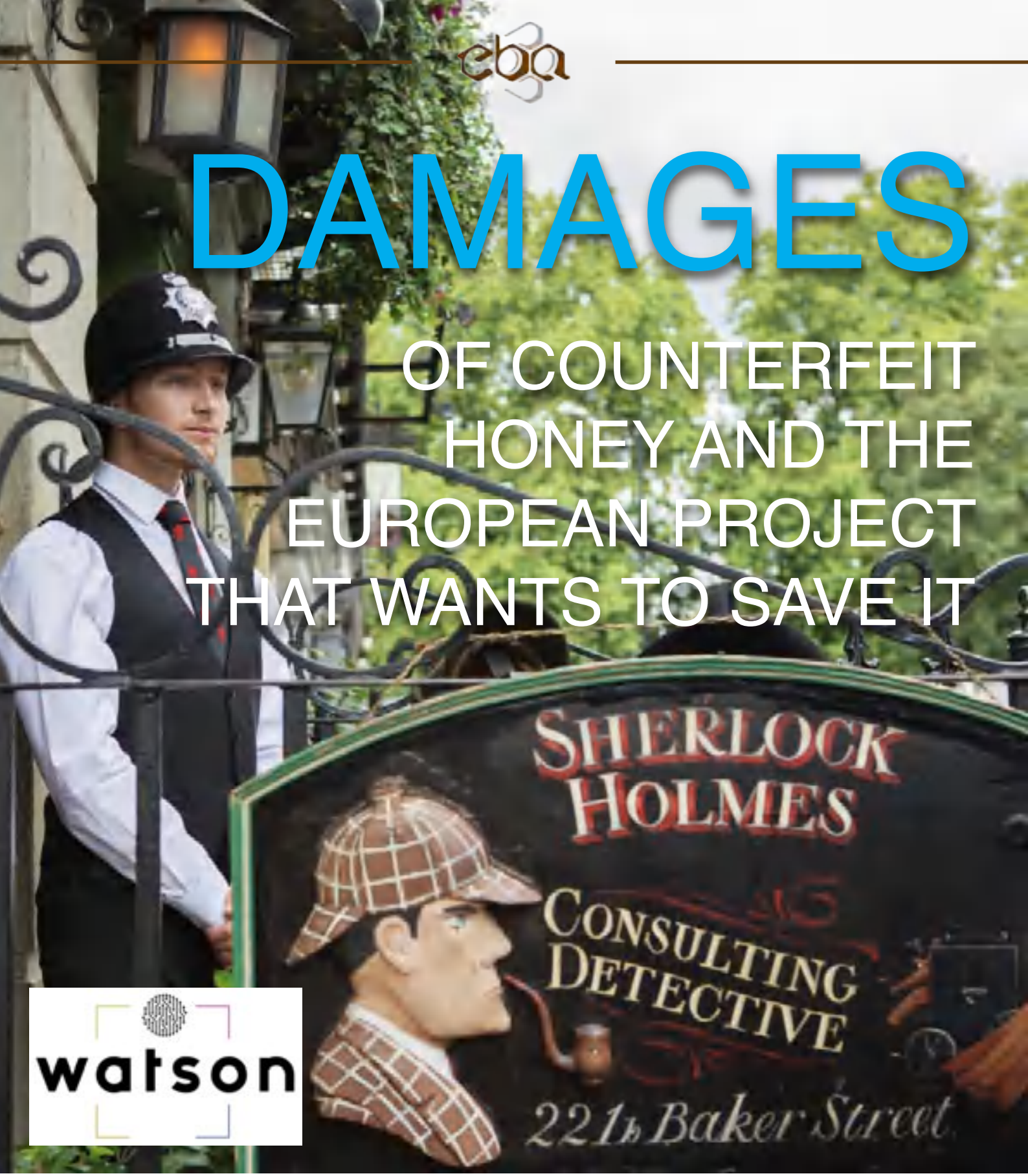
TO THE EBA WITHOUT MEMBERSHIP FEE

At the meeting of the EBA Executive Board, on the proposal of the EBA President Mr. Boštjan Noč, an important decision was made regarding membership in the EBA in the upcoming period: "**Membership in the EBA is free for the duration of the mandate of the EBA President Mr. Boštjan Noč**."

Decision of the EBA Executive Board is another confirmation that the EBA continues to work only in the interest of bees, beekeepers and consumers in Europe.

DAMAGES

OF COUNTERFEIT HONEY AND THE EUROPEAN PROJECT THAT WANTS TO SAVE IT



According to the European Anti-Fraud Office, 46% of honey imported into the EU doesn't comply with European regulations.

In addition to the benefits, globalization of food supply chains has brought also the exponential increase in fraud, bringing to our tables food produced or grown in countries with less stringent regulations and fewer safety controls.

In Italy alone have arrived more than 25 million kilos of foreign honey of poor quality and at bargain prices. This is wrecking Italian honey producers.

The honey that arrives on our tables is increasingly poor.

Adulterated honey is as much as 74% in the case of imports from China and 93% from Turkey, but the most surprising figure concerns the United Kingdom, from which we import honey with a rate of possible adulteration of 100%, due, probably, to the fact that honey is produced in other countries and then blended in the UK before entering Europe.

The Watson project

Combat of the food fraud along the supply chain is one of the key lines defined in The Farm-to-Fork Strategy, the heart of the European Green Deal, aiming at making food systems fair, healthy and environmentally friendly.

Unione Nazionale Consumatori, the oldest consumers association in Italy, collaborates with 45 partners across 20 EU and non-EU countries, part of Watson's consortium, that will develop a traceability framework that will integrate data-driven services and intelligence-based toolsets, enabling food safety authorities to identify and prevent food frauds.

Watson (A holistic frameWork with Anti-counterfeit and intelligence-based technologieS that will assist food chain stakehOlders in rapidly identifying and preventing the spread of fraudulent practices), is a 3-year project that has been funded by the European Union's Horizon Europe research and innovation programme (HORIZON-CL6-2022-FARM2FORK-01-11).

Why Watson?

The project will investigate the characteristics of six pilot food chains and the chains stakeholders' attitudes and behaviour towards adulterated food, to identify the frauds motivations and the quantification of the economic dimension of the problem.

Exactly as a detective would do, the project investigate to fight counterfeit and find the truth. Traceability and authenticity: these are the two keywords of Watson project.

Watson will provide a wide range of innovative solutions that will assist food control and safety Authorities to rapidly identify fraudulent activities throughout the whole chain, ensuring food product traceability and authenticity, and will evaluate different food authenticity-related databases existing in Member States and the EU institutions, creating a central database to improve fraud detection and enforcement actions by the competent Authorities.

These solutions will be data-driven, fast, energy efficient and low-cost and will leverage the power of AI coupled with spectroscopy and chemometrics methods to prevent food fraud.

The Watson toolsets will be evaluated in real-life settings, in six different European countries, one food chain for each one: wine in Portugal, honey in Spain, extra virgin olive oil in Italy, meat in Germany, dairy in Finland and fish in Norway.

How is honey adulterated and what's the dangers?

According to previous studies main unfair practices in the honey sector include in that order:

• adulteration of honey with addition of sugars such as high fructose, glucose, and saccharose syrups, and low-quality honey added to high-priced honey;

• mislabelling respect geographical and botanical origin

Counterfeit honey is achieved by mixing authentic honey with beet and cane syrups, by adding flavourings and other food additives or low-quality honey to high-priced honey. The result is a product very similar to honey, but of much lower quality.

Beekeepers can feed sugar to bees as well, placing sugar solutions near the hives.

And even if the sugar consumed undergoes the same transformation processes as nectar, the honey obtained is lacking in the substances naturally present in the flowers and the nutritional value of counterfeit honey is very low.

Counterfeiting honey rarely poses health hazards. The consequences are above all economic for citizens who pay high prices for poor quality honey. One of the most unfair practices includes mislabelling with respect to the geographical and botanical origin.

Imported fake honey or adulterated honey distorts the market and makes unfair competition to producers of high-quality honey, which must have much higher prices to maintain standards. This makes the activity for EU beekeepers not economically sustainable.

The Watson solutions

Most of technologies to assess the adulteration are time-consuming, destructive, expensive, the results are obtained after several days (not real-time) and require experts for the interpretation of the produced results. Watson wants to develop fast, non-destructive, easy to use and low-cost analytical methods to detect and quantify adulterations in honey.

Near Infrared Spectroscopy (NIR) obtained relevant results in terms of adulteration and mislabelling control efficacy, but they still rely on spectrometers that are mainly conceived for laboratories, heavy and large, still expensive and managed by expert technicians.

Watson will use advanced data analysis tools based on Artificial Intelligence techniques: low cost, portable/miniaturized technologies: a digital NIR based sensor, and a digital HSI camera, that will be fast, non-destructive, easy to use, with real-time results and low-cost analysis.

The multi-sensor device is being tested for honey authenticity verification and results will be displayed in a mobile application. Consumers will

have insightful, easy-to-perceive data from the digital product passport coupled with advice on well-being based on honey products.

Scientific articles (recently published) have shown relevant results related to the use of NIR technology for the two applications that are targeted in Watson's honey case study: adulteration and mislabelling control. Regarding HSI, the research works utilising this technology for honey analyses are still limited.

However, these works still rely on spectrometers that are mainly conceived for laboratories and bench use, expensive, normally managed by expert technicians as well as heavy and large. In addition, these technologies (NIR or HSI) are integrated with chemometrics for the modelling of acquired data and the extraction of the chemical fingerprint of the analysed compound. Most of chemometrics used until now employ traditional linear modelling techniques, that are not able to model the performance of non-linear variables commonly involved in the determination of food quality.

The specific objectives of the Watson Project honey pilot and the state of the art:

The Watson project's goals are ambitious:

1. The development of an Early Warning System (EWS) to be used by the food control authorities

2. The deployment of advanced optical sensors based on Near Infrared (NIR) and hyperspectral imaging (HSI) technologies to detect specific frauds adulterations, mislabeling of botanical variety and external sugars or syrups in honey in multiple stages of the value chain: producers, packers and points of sale.

3. Data from the honey value chain will be collected, as well as data from various databases related to food fraud providing alerts and recommendations on fraudulent activities that may occur in a national food chain. As result, a risk and vulnerability assessment framework will be elaborated, identifying sources of uncertainty and introducing them into the probabilistic modelling process to be propagated to the end result. Finally, the risk-based decision support engine will be coded in a software and its trigger thresholds will be calibrated against historical data, to be iteratively updated.

4. At a second level, the project aims to inform consumers of the nutritional benefits that honey can offer and other honey facts, through a specifically designed mobile app, increasing this way their trust in the honey value chain.

On September, Watson kicked off the first stage of validation for honey fraud detection tool, a portable and user-friendly device equipped with an optical sensor based on Near Infrared technology and powered by an advanced algorithm capable of detecting fraudulent practices in honey, such as the presence of added sugars and syrups.

NIR technology works by using light wavelengths just beyond the visible spectrum, to analyse the chemical composition of substances and allows rapid identification of food fraud, like adulteration in honey.

The testing phases along the honey supply chain will be carried out to support the authenticity of the Northwest Spanish honey.

The validation round's objective was to present the device to control agents of 'PGI Honey from Asturias' and the Consejería de Medio Rural del Principado de Asturias, alongside providing targeted training on its use and handling.

The second stage of this validation process will follow in the coming months, where the device's performance will be assessed by control agents during routine inspections.

For more information on the results and tools of the Watson project, to contact the partners in charge of the case studies www.watsonproject.eu

To be updated on the activities and the information campaign aimed at Italian consumers and stakeholders:
https://www.consumatori.it/news/watson-frodi-alimentari/

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DO YOU KNOW WHAT YOUR CHILDREN EAT?

Slovenia is a good pilot example of how locally produced food can be distributed to consumers while ensuring that they are aware of the benefits. It all started in 2006, when beekeepers and beekeeping associations came together to launch the nationwide campaign "one day for breakfast – honey from Slovenian beekeepers in our kindergartens". Every year, honey is collected and donated for children in kindergartens and primary schools, and on the third Friday in November, after breakfast, workshops are held in kindergartens and schools to teach children about beekeeping and the importance of bees for the environment.

At the same time, it is also a good example of how beekeepers, professional institutions in the field of agriculture, local communities and education have joined forces in a united approach to implement Honey Breakfast in kindergartens and primary schools. As such honey breakfast campaigns have proved to be well-received and beneficial, the Slovenian Beekeepers' Association proposed to the relevant Slovenian ministries (Ministry of Agriculture, Forestry and Food; Ministry of Education and Sport; Ministry of Health) to make the third Friday in November the "traditional Slovenian breakfast" day, which was adopted in 2011. Later, they proposed to the Government of the Republic of Slovenia the "Slovenian Food Day", which was adopted at the national level in 2012. Honey was joined by other foodstuffs traditionally produced in Slovenia, such as bread, milk, butter and apples. Initially, the food was donated by beekeepers and farmers, but for the last couple of years, it has been co-financed by the state. With the implementation of the project and the support of the many educational and learning contents available, activities on beekeeping and agriculture are taking place in kindergartens and schools. Every year, the Slovenian Beekeepers' Association, together with the Public Advisory Service in Beekeeping, publishes a leaflet for children with educational topics related to beekeeping.

However, in order not to focus on the importance of agriculture in general, the consumption of locally produced food and the promotion of a healthy lifestyle on just one day of the year, the Slovenian Beekeepers' Association and other stakeholders proposed to the Government of the Republic of Slovenia to declare a "Slovenian Food Week", which it did in December 2023. We will celebrate it in the third week of November this year for the first time. Awareness raising will be extended to other public institutions, catering establishments, households and the general public.

Benefits brought by a series of activities since 2006:

- young children are educated about the importance of beekeeping and farming, and the food, breakfast and environment we have an impact on,
- networking on common activities and strengthening relations between different stakeholders (beekeepers, farmers, different ministries, educational institutions, local communities),
- short supply chains, so the food delivered is fresher and tastier,
- increased visibility of locally produced food, raising the profile and reputation of the farmer as a key food producer.

The public awareness-raising on beekeeping carried out by the Slovenian Beekeepers' Federation has certainly contributed to the positive trends in honey consumption. Based on the average of the last ten years of data on the production and purchase of honey of Slovenian origin kept by the Statistical Office of the Republic of Slovenia, it can be concluded that on average 9% of the honey produced was sold to purchasers, while 91% of the honey was used by the beekeepers themselves or sold through other sales channels such as home sales, markets, schools, kindergartens and other end consumers.

Slovenia wanted to take good examples beyond its borders, so every year since 2014, the Slovenian Beekeepers' Association encourages beekeeping associations across Europe and beyond to join the "European Honey Breakfast" initiative. Slovenian embassies are also helping to spread this good practice by organising breakfasts in their respective countries.

Join us! Follow Slovenia's example by taking part in the "European Honey Breakfast" on the third Friday in November, namely on 15 November 2024. Spread awareness among children and the general public about the importance of locally produced honey and about having breakfast.

Lidija Senič
Head of Services at the
Slovenian Beekeepers' Association

More information:

-A video about what the world without bees would look like:

https://www.youtube.com/watch?v=Z6BhwaLcxK4

-The European Honey Breakfast and World Bee Day:

http://www.ohranimo-cebele.si/Upload/Initiative%20for%20The%20European%20Honey%20Breakfast%20-%20brochure.pdf

-European Honey Breakfast Initiative:

http://www.czs.si/Upload/European_Honey_Breakfast.pdf

CHILDREN AS CONSUMERS

THE RIGHT OPPORTUNITY

The basic marketing principle is the awareness of the customers' purchasing behaviour. A beekeeper, the immediate producer of honey and other bee products, in the conditions of unorganized distribution upon updated principles of sale, remains the first and only marketing manager.

Having this role, he/she needs to know his customers, individual consumers, well, in order to identify mutual and specific needs of buyers. Getting to know the buyers is the initial position in the creation of a personal, individual marketing strategy.

The customer purchasing behaviour is the studying of marketing environment of the purchaser and generally comprises of: political-legal, economic, technological, socio-cultural and environmental surrounding. From analyzing the environment, we come to the findings about needs and wishes, attitudes and beliefs of the consumers and their purchasing motifs.

The examining of the consumer behaviour provides beekeepers with in-

formation in the function of keeping the current and obtaining new purchasers, i.e. in the function of the increasing of honey consumption.

A deeper understanding of customer purchasing behaviour can be obtained through analyzing the answers to the questions: how, when and where a customer buys, his/her selection criterions and who is important in the purchasing decision making.

Answers to the question asked can be provided through immediate, personal contacts with customers or through a market research. In our country, there has not been a thorough and systematic honey market research. Truly, a significant attempt in this field has been performed by the Beekeeping Society from Trstenik, but upon a pretty modest sample. In this article, I would like to draw attention to a very important category of purchasers, children.

Children – great purchasers

The purchase of honey is individual. An impulse for making the decision about the purchase can come from an authoritative individual, friend's recommendation, or a group such as family, household, institution or similar. Economic propaganda, having a significant impact on the purchasing decision making in the world, hardly exists in our country. The first move in this area has already been done in Serbia, by printing the brochure entitled "Honey, miraculous food and medicine" in the record volume of over 650,000 copies.

The brochure aims to illuminate a little bit yellowed picture abut honey. Honey, periodically in the shade of seasonal raspberries, sour cherries and water melons, needs to provide its presence on the table throughout the year. Therefore, we need always new actions and ideas.

The initiators of a purchase can be various family members. In our community, children are the rarest initiators of honey purchases, although there are the most important population; they are the present and future purchasers. In the purchase of many food products (crisps, chocolate hazelnut cream, ice-cream, chocolate) children put a strong pressure on their parents who make decisions about the purchase. Therefore, children are our insufficiently used opportunity. It can be used by attracting the attention of children by a free toy, special packages in the shape of a toy or an award when buying.

The most common honey package, the 1 or half-a-kilo jar, is completely uninteresting to children. The imagination of package producers seems to be ended by the designing of the bear-shaped jar. The only purchasing motif is the motif for food, the decision of which is made by parents. The emotional impact of children has been completely neglected. Children know how to start crying, be angry or silent if they do not get the toy or the product they want.

For a bigger consumption of honey in children population, it is not sufficient to have the traditional way of consumption, by spreading it over a slice of bread. Children would love to accept honey if we offer it in many other ways (with hazelnuts, sesame, peanut), which are at the same time great creams. Pastry, pancakes or yoghurt with honey, as well. For our habits, it may be unusual, but fried eggs or hot dog covered with honey have been well accepted (in the USA, there has been an extremely popular sauce made of a very tasty mixture of garlic and honey for meat specialties). Furthermore, honey is used for pouring over fruit salad and ice-creams. Sour cherries covered with chilled black-locust honey are extremely delicious as a summer desert. Beekeepers should know bee products very well, as well as possible ways of consumption, and recommend them to children and parents in a popular and amiable way. If we want a higher consumption, the honey market needs to be expanded to a variety of food products.

Researches in developed countries have shown that in family purchases, men make more than a half of decisions related to the purchase of food products such as fruit, vegetables, jams, non-alcoholic drinks and similar (D. Jobder: Principles and Practice of Marketing, Megraw-Hill Book Company, London 1995).

Children belong to the category of emotional purchasers, because, generally, they are not very or are rarely interested in their parents' purchasing power, and decide to buy a product upon the influence of the environment (sellers, friends, ambient, trend, TV commercials and similar).

Children as consumers make various markets: primary, when they individually make decisions and do the purchasing; influential, when they directly or indirectly influence their family members; future, when by growing up their influence becomes stronger and stronger and leads toward their independent and more and more active behaviour as consumers (Jams U. McNeal, Kids as Customers, A. Handbook of marketing to children, Lexington Books, New York, 1992).

As consumers, children attract more and more attention of marketing agents, considering the fact that they make a complex, dynamic and challenging market. Children have always had a strong influence on product purchasing, where they often independently make decisions when buying sweets, toys, fast food and similar. Furthermore, children have more and more influence on mutual decisions within the household related to the acquiring of certain types and brands of food products.

According to researches in the USA, (36 million boys and girls between the age of 4 and 12) more than ever before, children make the main market with 24.4 billion dollars of immediate purchasing power and 187.7 billion dollars of direct and indirect influence on purchase. The yearly in-

crease rate in the children expenses in the purchasing of goods is as high as between 10 and 20%. The influence of children on family purchases is estimated to 110 billion dollars for food products, 26 billion dollars for purchasing toys and 22 billion dollars for the purchase of fast food. The estimate is that, by the beginning of the third millennium, children consumers in the USA will yearly spend about 35 billion dollars of "their own" money and 300 billion dollars of their parents' money, for buying food products.

According to the census from 2022, the Serbian market includes 6,623,183 citizens. Regarding the population's sex structure, the participation of male (48.6%) and female (51.4%) population is nearly equal. Children younger than 15 make 953,750, i.e. 14.4% of the population in total (Republic Institute of Statistics, Statistical Yearbook of Serbia, Belgrade, October 2024). If the children of this age would consume approximately only 1 kg of honey per year, it would make the consumption of 953.75 tons of honey, which is 11.9% of the total yearly production in Serbia.

Conclusion

Marketing activities begin and end with consumers. Whether we are ready to create adequate personal marketing strategy depends on how well we know the consumers.

Without information about the needs and motifs of consumers, their behaviour in various purchasing situations and various factors influencing the purchasing process, we can not imagine a success in the distribution of products, in our case honey, on our and foreign market.

The text you have just read draws your attention to children as one of important categories of consumers.

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MYSTERY REVEALED

HOW BEES INFORM ABOUT THE LOCATION OF THE SOURCE POLLEN, PROPOLIS AND WATER

A bat has no eyes but can "see" to fly, a bee has a tongue but does not use it to speak, hears but has no "ears", smells very well but has no "nose". The bat lives and hunts in the dark. If you remove him from his habitat during the day, when you release him he will return to his habitat, regardless of whether it is day or night, because he has memorized where he should return.

Unlike the bat, the bee uses its eyes to fly, but it needs partial light to do so. Beekeepers in cities know how the bees return even in the dark using a streetlight (since there is no Sun at night, I personally think that the bee has a kind of compass in it, we can talk about this in the future). If we take it away from its habitat, it will return like the bat, but for the bees some light is needed. That's why I think that both the bee and the bat have a common way of memorizing and using the memorized data.

Apart from flying, the bee, like the bat, also does everything in the dark in its habitat, including communication among bees. If her habitat is on a branch (picture 1) she againcommunicates and works in the dark, because the outer shell of the bees that regulates the microclimate in the nest makes the inside of the nest dark, as in any other habitat.

How bees communicate was unknown until Carl Von Frisch in the year 1967proved to the whole world that they do it by means of vibrations (he spoke about it for the first time in 1924). From then until today, it has been discovered and explained how the bee, from the source of nectar, pollen, propolis and water, measures the distance traveled to the habitat and "converts" it into vibrations. Through vibrations and sounds, it transmits information to the sisters, and they convert it into a code known to them. As I said, bees don't have ears, but they can hear, which tells us they communicate on a higher level.

We know that a bee has multiple eyes (three smaller eyes and two larger compound eyes), but we also know that bee doesn't recognize some colors or doesn't see them. Given her accuracy, I dare to think that, like the blind mouse, uses ultrasound or infrared spectrum. It has been proven thatthey see polar and ultraviolet light, and based on the coded mode they go exactly there where they were shown by other bees by the way of vibration. The color and size of the flowers were not transferred to her by vibration, but the smell and the place where the source is located.

All this about the communication of bees by means of vibrations and sound is written in detail in Professor Terzin's book "The Honey Bee, the Gospel of Nature" on page 23. In the mentioned book, it is also written that the bee makes both vertical and horizontal vibrations. In the attached video (scan the QR code on the next page), you will see that the same bee vibrates horizontally on the saddle, and then enters between the frames and vibrates vertically.

In addition to the mentioned book, in other literature that was available to me, I found answers to many questions that have been bothering me

for a long time. All this refers to communication, i.e. information transfer. We know that the bee has more eyes, but I have not found information about what and how the beesees in the dark, because we know that it makes these vibrations in the dark. The bees are only a few millimeters from the bee that transmits information to them, i.e., vibrations. She vibrates very fast, and her companions should see (feel) it in complete darkness and at such a short distance. I believe the information they receive is through the antennas, because the dancer hits the partner's antennas, which you can see in slow motion.

Unknown vibration

There is a vibration - an information that they do not recognize neither bees nor beekeepers. I'll call it the unknown vibration. My conclusion about the unknown vibrations is that they arise when the bee is attacked by a parasite, for example Braul Coek's louse, and for the last fifty years it has also been varroa. Bees also make vibration when they are sick. These vibrations lack the figure-eight movement of the bees, so they are unknown to beekeepers but also to bees. It also lacks the sound that is normally produced by the butt hitting the honeycomb, they just vibrate uncontrollably. I noticed the unknown vibrations for the first time more than five years ago in my apiary. Then I thought about the intelligence of bees, the framework to recognize those previously unknown vibrations. Then I realized that if they did not recognize it themselves from the time of their creation until today, then they cannot learn (picture 2).Also, the American foul brood-AFB is accompanied by a specific smell, those bees that recognize AFBdon’trobthe hives infected with AFB because that's how they will destroy themselves, just as they are being destroyed still today. From picture no. 2 you will not be able to see and understand it, because you can watch the video at the link https://youtu.be/ckd0XhxuH8 and this video supports the writing.

From the attached picture and video, we can see that a bee attacked by parasites vibrates but does not make the well-known figures of eight and other well-known vibrations, and the bees around it do not recognize it, so they simply move away. I realized that they do not understand this because it is simply missing in their "program",

even though they have been evolving for millions of years.

I want to emphasize that only healthy and vital bees have the intelligence to deal with parasites on their own. Varroa bothers them, because of it they tremble intensely until they excite the parasite, and when it moves, it is much easier for them to deal with varroa and destroyit with their legs, but this has nothing to do with unknown vibrations caused by parasites.

Transmission of information about water, pollen, propolis

In the already mentioned book by Professor Terzin on page 114. the other side states that the bee transmits information about water, pollen, propolis and nectar in the same way. This is because it concerns the length of the path to the source and the orientation of the bees in space. This has been bothering me ever since i started researching in more detail and getting to know the life of bees.Since then, until five years ago, I was bothered by the fact that the bee, when it goes for pollen, there it can find both pollen and nectar, as for example on a dandelion flower (picture 3). For that action, she needs honey for theflight to the location of the dandelion, where pollen and nectar await her for return flight. If bee must go for pollen on a hazelnut (picture 4) where there is no nectar waiting for her at the door, she must take more honey (fuel) for the work she

needs to do, as well as for the flight back. Flying to the source in relation to returning from the source (flower) is much harder than when it carries nectar, because for return with full baskets of pollen consumes more "fuel" or honey due to bigger wind resistance. When bee needs to go to collect propolis (picture 5), there is certainly no nectar to collect, so fuel for the trip needs to be planned for both directions, as well as for pollen. For water, the information is the most complicated and must be very precise, compared to all other information. Are you wondering why? Because the distance must be very precise, since it must have honey for both directions. It has no flight resistance like with pollen and propolis, but if it takes more honey it will have less room for water. It is not the clearest and it is not known to this day, it would be necessary to make appropriate measurements, whether the bee mixes the water with the honey taken in the honeycomb, or whether the honey passes into the intestines, before taking the water, so that there is enough food in the intestines for returning to the hive. This second variant is of course a much more logical one. It is even worse if bee takes less honey and risks not having enough fuel to return.

Vibration when swarming

There is another vibration - information that occurs during swarming, when the swarm leaves the hive and temporarily settles on a branch, and then looks for a new habitat if it has not already found it, sometimes because the bees find it months before. When the Bee Scouts finally find new habitat, it's different, specific but more recognizable vibrations where is that new habitat. Bees do not inform their sisters about discovered habitat before they swarm. That's exactly why swarm first temporarily catches on a branch, then the scout bees that have already found a new home start to dance and are informing the rest of the bees where the swarm should go. They perform the dance on the surface of the swarm, and it can be easily seen. Meanwhile, since the bees could find more new locations for rehoming, then on the branch the swarm decides by consensus which coordinates to choose, and when it decides, it moves towards it. If the bees do not find a new habitat, the swarm stays on the branch and builds its nest there.

There is another vibration - information that occurs during swarming, when the swarm leaves

the habitat and temporarily settles on a branch, and then looks for a new habitat if it has not already found it, which is a more common occurrence, because the bees find it. not when and months before. When the Girl Scouts finally find new habitat, it's different, specific but more recognizable vibrations where is that new habitat. Bees do not inform their sisters about found habitat before they spawn. That's exactly why they swarm first he temporarily catches on a branch, then the bees that have already found a place to dance, and both know where the swarm should go. They perform the dance on the surface of the swarm, and it can be easily seen. Meanwhile, since the bees could find more possible stands, then on the branch the swarm decides by consensus which stand to choose, and when it decides, it moves towards it. If the bees do not find a new habitat, the swarm stays on the branch and builds its nest there.

No information about this in the literature

All the above information about pollen, propolis and water are different from nectar information. There was no information about this in the literature. Five years ago, here in New Zealand, while inspecting the hive, I saw a bee with pollen, making well-known vibrations like a bee, but together with pollen on its legs. From the excitement, I almost missed the moment to record it all. At that moment, the bees finally gave me the answer I had been looking for so long. When returning to the habitat with pollen or propolis on its legs, the bee dances the same dance as with nectar, but with one addition,in order for the bees to know that it is pollen, it vibrates with the pollen on the legs, but before the vibrations start bee takes the starting position, flutters his wings for a moment, as if shaking something of wings. When bee completes the vibration cycle, it pauses for a moment and makes a short vibration of its wings again. It does not make such vibrations with its wings when it vibrates and shows where the nectar is (picture 6). The vibrations with pollen "left to right" are shorter than the vibrations for nectar, because pollen and propolis, due to their weight and size, make it difficult for the bees to dance. Bees memorize, in addition to the distance and the orientation to source, and the smell of that pollen or propolis. The flowers of the plants above the bushes and callas have no nectar, so the bee that performed the vibrations did not reward her sisters with nectar at the end of the dance, because there is no nectar at the source. This is the slow-motion part of the video Irecommended with this text. If there was nectar, as in the case of the mentioned dandelion at the end of the dance, the dancing bee would reward the companions with nectar waiting for them there, as it always does when it finishes vibrating with nectar (picture 7 on the next page). So they know that they don't need honey to return, as in the case of kale and gorse bush. The same happens, for example, in the hazelnut pasture.

When it comes to collecting propolis, the dance of bees is the same as with pollen, only with propolis on their legs (picture no. 5). By the

propolis, so there is no reward for companions, so they know that they need honey to return.

In the case of collecting water (to cool the hive, because bees do not drink water), I have already described how important it is that the information, i.e. the dance, be very precise. The bee dances as it does for nectar, but at the end it dances with its companions to give water, which is a sign that the information is about water and not about nectar. After five years of observing about the transmission of vibrational information about pollen, propolis and water, as well as information at birth, I thought that was all and that there was nothing more to do.

However, along the way, new questions arose about new mysteries about the transfer of information in the bee community I.e., how the departure for mating...We can leave this subject for a future text.

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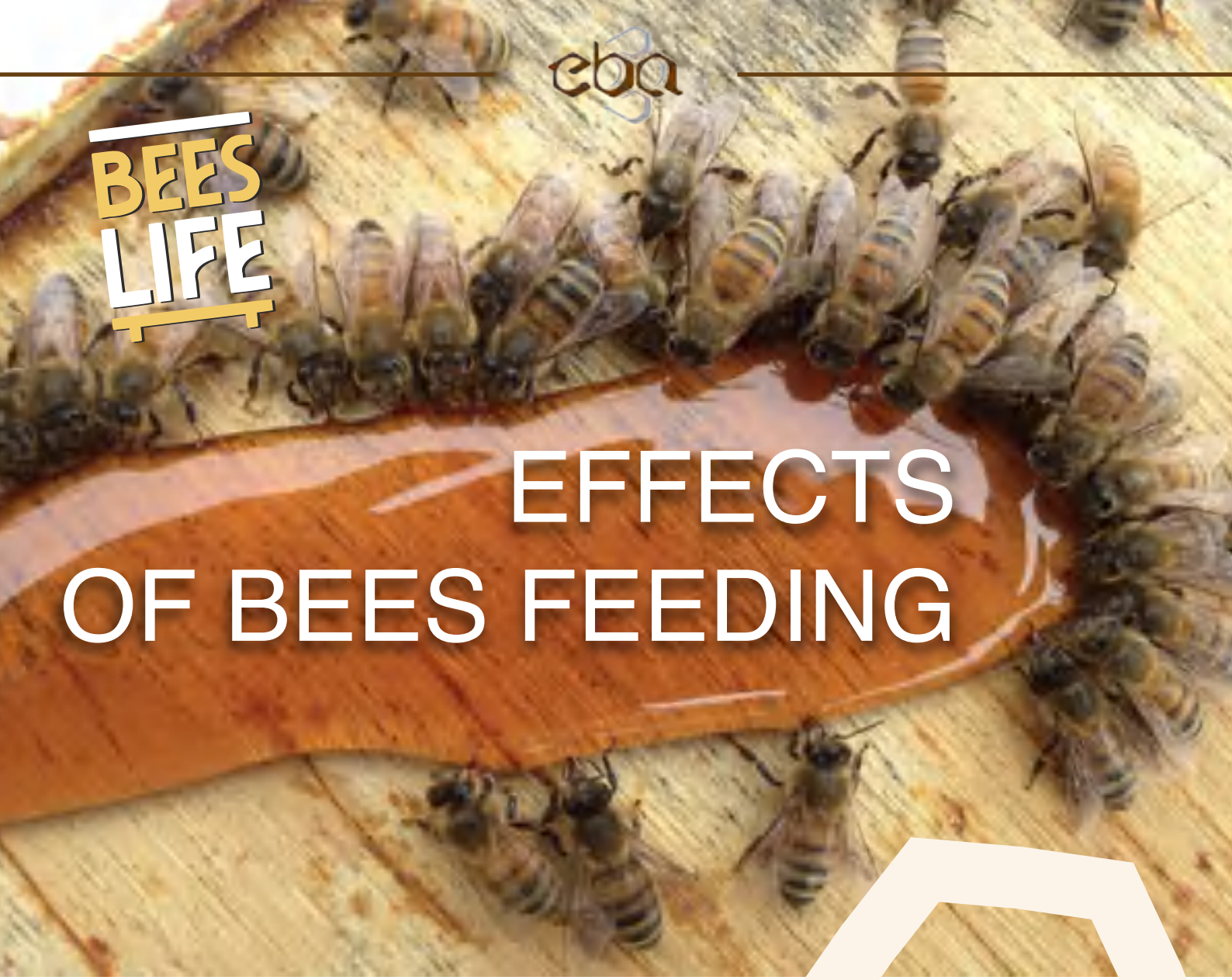
BEES LIFE



BEES LIFE

EFFECTS OF BEES FEEDING

Bees feeding

The practice of beekeeping aims to manage bees with the intention of producing beekeeping products. Given the efforts to maximize the production and combined with increasingly unfavorable conditions, the supply of food to bees, in various cases of beekeeping practice, often becomes necessary. These feeds aim to supplement the carbohydrate - energy and protein needs of the bees. In particular, the most common supplementary feedings used by beekeepers are the following (fig. 1).

Carbohydrated feeding

These feedings given to meet the bee's carbohydrate requirements as these substances are the primary source of energy for their works inside and outside of the hives. Carbohydrate supplementary feeds that are usually provided to bees are:

A. Syrups

The liquid feeds given to the bees either using specialized feeders, or bottles or even with other containers (jars, plates, etc.). They can be aqueous solutions of sugar (derived from sugar beet or cane sugar), inverted syrups and syrups from other cellulosic materials such as rice and palm.

B. Sugar pastes

They are solid feeds prepared from powdered sugar, syrup and honey. They can be homemade, but they are also available in various

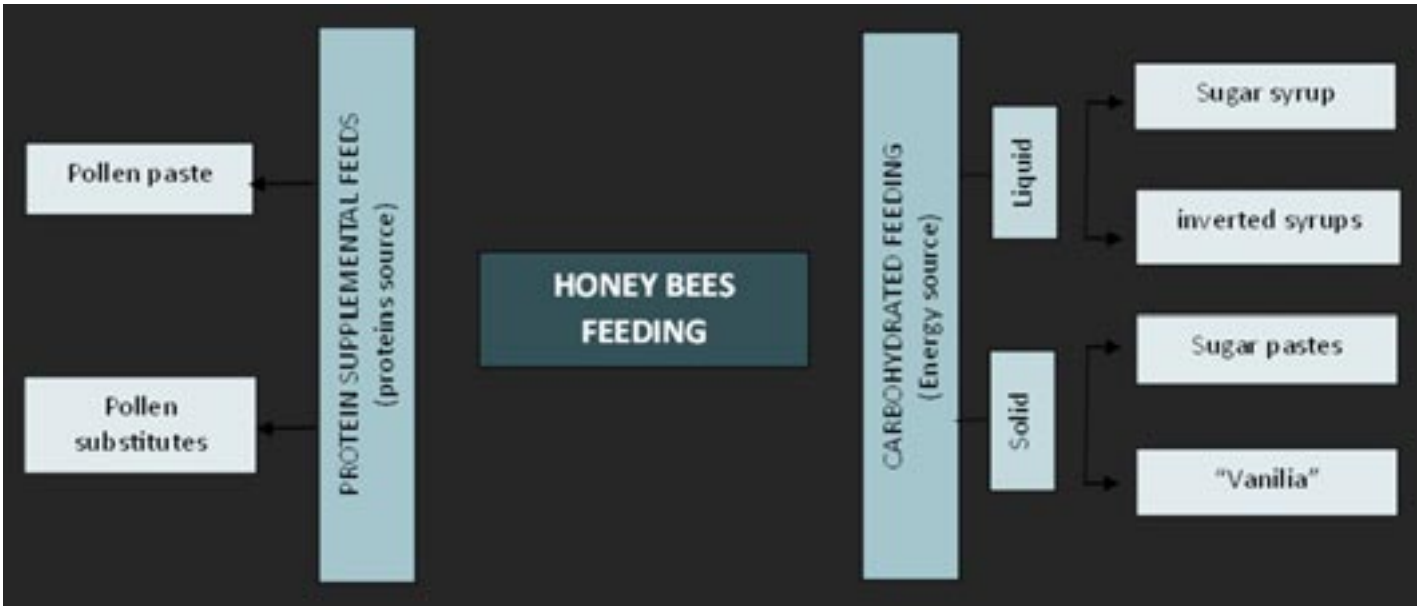


Figure 1: Types of supplementary bee feeding

types with additional ingredients (vitamins, amino acids, etc.) in commercial forms. They usually are provided to the bees in periods of low temperatures, but also during the summer flowering gaps.

C. "Vanilia"

Vanilia is produced from boiling sugar syrup that is cooled while stirring to give a thick white mixture that when cooled is almost solid.

D. Honey

The best feed for bees is their natural product, the honey. The supply is made either by stored honey frames, or by mixing honey with water.

Protein supplemental feeds

A. Pollen paste

A mixture of powdered sugar with honey or syrup that also contains pollen. It is a protein feed that is provided to bees for the brood rearing.

B. Pollen substitutes

They are like pollen paste, where the pollen is replaced to a very large extent by other protein materials such as soya, cereal and legume flour and sometimes by animal proteins (e.g. egg, milk).

Important points for feeding bees

Although bees in nature cover their needs from plant supplies, however, during the application of beekeeping practices, feeding in some cases is deemed necessary therefore the following should be noted.

✓ Synthetic feeds are complementary and cannot completely replace the bees' natural food which is honey and pollen.

✓ Feedings with syrups should be done in the evening hours to avoid robbing problems

✓ Honey or syrup containing honey cause a greater robbing tendency

✓ In periods of low temperatures, solid feeds are preferred.

✓ We do not use honey and pollen from sick beehives (ascospherosis, nosema, etc.) as there is a great risk of transferring the pathogen throughout the apiary.

✓ We do not use honey that has a very high HMF value or soured honey as it can be toxic to the bees or cause a reduction in their lifespan or disrupt their functions in the hive.

Providing supplementary feed to bees,

sometimes even reaching a total replacement of their natural diet, poses risks both to the bees and their products. In this article we will refer the possible effects of bees feeding on the produced honey.

A. Effect on physicochemical characteristics

Giving a large amount of liquid carbohydrate feeds results not only in immediate consumption from bees but also in syrup storage. Thus, if honey is harvested during feeding or in a short period of time, the collected product contains the concentrated syrup, affecting the composition of the honey. The problems that arise in this case concern deviations of legislated parameters, such as low diastase values (diastase values < 8 DN), high sucrose content (sucrose values > 5%) and HMF (HMF values > 40 mg/kg). In addition, exogenous enzymes or carbohydrates can be detected. High concentrations of oligosaccharides with more than five structural units are not a natural characteristic of honey but they are intermediate products of the enzymatic production of syrups from starch indicating adulteration.

B. Effect on microscopic profile

The microscopic profile of a honey collected after feeding often differs as it contains few pollen grains. In addition, starch or other characteristic particles can be detected, as remains from the plants during the production of the syrups.

C. Effect on organoleptic characteristics

The feeding of large quantities of syrup during flowering or honeydews secretion affects not only in physicochemical and microscopic parameters of honey, but also in its organoleptic characteristics. Usually, the honey produced in this way is very clear with a poor aroma profile and short-lasting taste stimuli. In corresponding research carried out at the Apiculture Laboratory of the Aristotle University of Thessaloniki, evaluating honey from hives fed with sugar syrup and from unfed hives, significant differences were found in their quality characteristics and in their organoleptic profiles. In figure 2 the profile of the two types of collected honey (a: fed honey, b: honey) were compared. A very poor profile is found in the case of the honey harvested from the fed hives. Specifically, the fed honey showed a short duration of taste retention, reduced intensity of aftertaste and aroma (sense of aroma from the inside of the oral cavity) compared to the honey of hives that bees only collected nectar.

D. Effect on biological actions

Usually, the research focus on investigating the effects of the feeding bees on the legislated

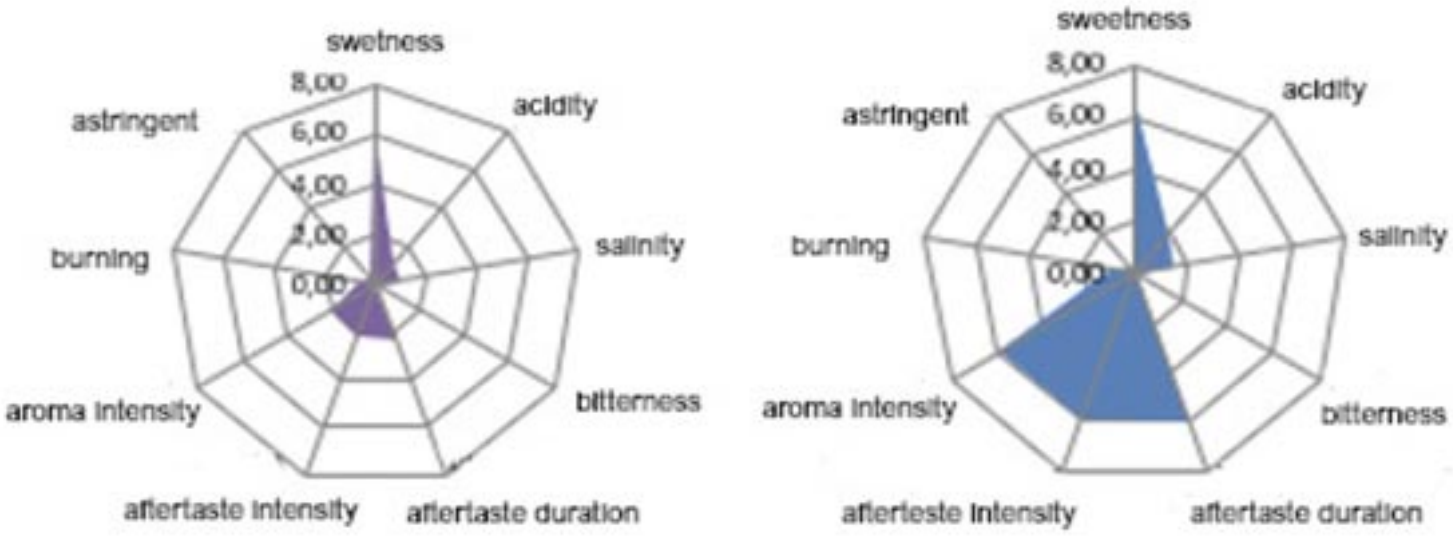


Figure 2: Organoleptic profile of (a) fed, (b) unfed honey

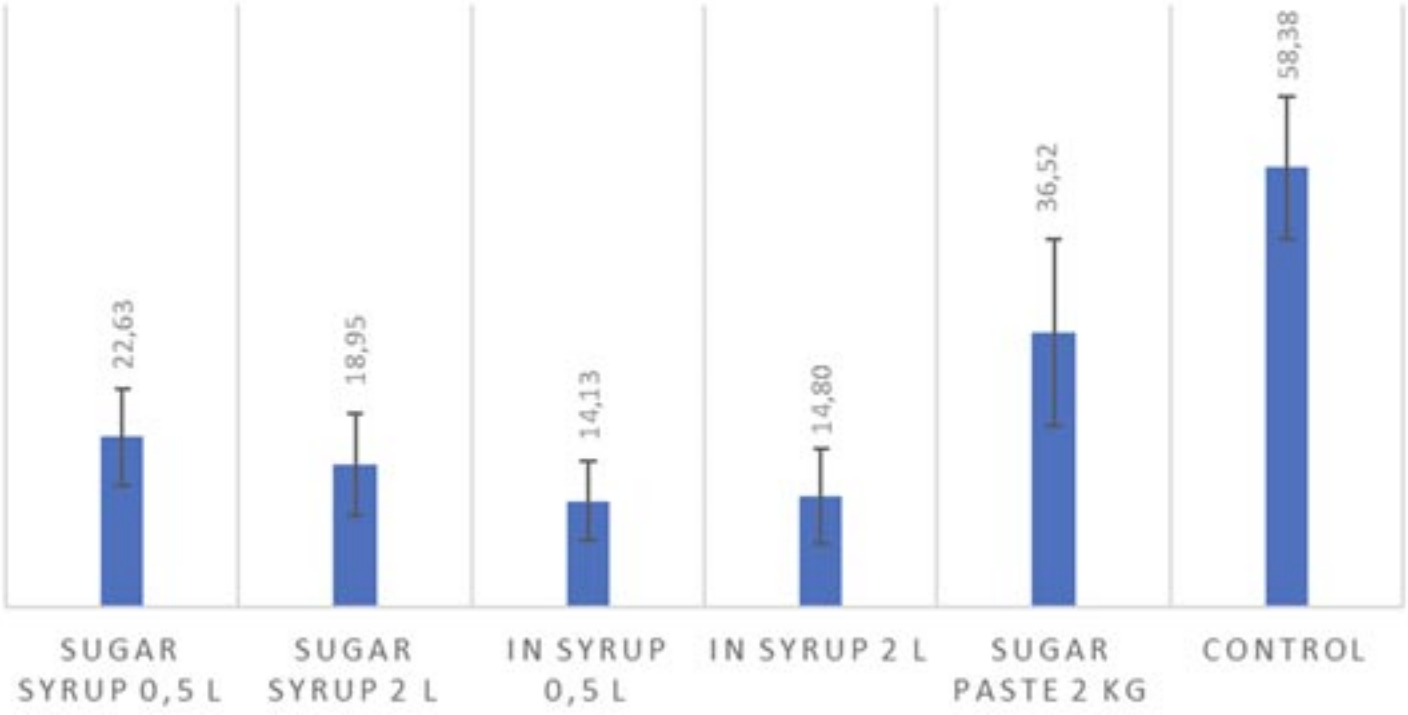
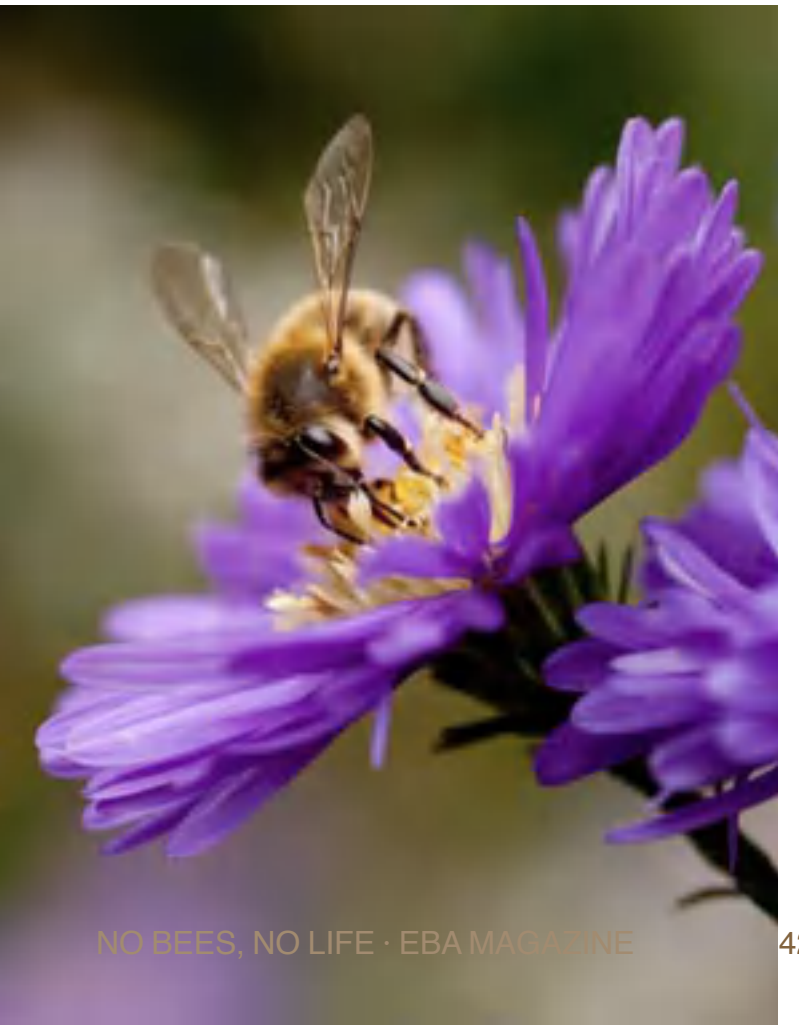


Figure 3: Total phenols (mg GAE/100 g honey)

parameters or on dangerous-toxic ingredients of the produced honey. We know that the high consumption of honey is mainly due to its high biological value. But how different are the biological activities in a fed honey? The honey produced by feeding bees has been affected in its composition as the bees do not only collect plant juices, but also process a simple mixture of sugars, therefore the concentrations of its beneficial components are reduced. In research that carried out at the Apiculture Laboratory of Aristotle University of Thessaloniki, honeys produced by feeding bees with different amounts of sugar syrup and invert syrup and then apart from their physicochemical parameters, the total phenolic content was determined.



A significant reduction in total phenolic content was found in "fed honey" compared to the honey collected form the hives of the control group (beehives without feeding) (fig. 3). The diagram shows a significant reduction of total phenols in honeys obtained from the hives that fed with liquid carbohydrate food (sugar syrup, invert syrup), with the greatest effect observed in honey collected from beehives that consumed high amounts of syrup. Solid carbohydrate feeding had little effect on the studied parameter, which is due to very small storage of the food provided in this case. The highest phenolic content was found in the honeys collected from the bees that did not receive any supplementary feeding, as the nectar collected by the bees contained an abundance of phenolic components.

E. Hazardous compounds for humans

The addition of different materials to bees feed not only affect the physicochemical, microscopic and organoleptic characteristics of the produced product, but can also transfer to it components that are harmful to human health. Considering that the addition of soya flour the purpose of reducing the production costs of the pollen pastes is increasingly common and if we combine it with the introduction of genetically modified soya in crops, the question arises whether this intervention can ultimately have an impact on honey as well. In research carried out in Germany 389 commercial honey samples were collected and 5.9% of them were found by microscopic analysis to contain soya residues. Furthermore, in eleven samples, the presence of genetically modified soya was confirmed using molecular techniques. Additionally, eleven commercial pollen substitute products for bees were analyzed, and in four of them, genetically modified soya was also detected.

The incorporation of materials of animal origin such as eggs or milk into the bee feed may affect the composition of the produced honey. Evidence of the transfer of components from these materials to the honey produced is gradually being documented in the literature. In a related investigation carried out with honey samples from the United Kingdom, the European Union (EU) and outside the EU, immunochemical techniques were applied for the detection of allergenic substances. In 27.5% of the samples, the presence of gluten was found in values from 2.5 to 13.8 ppm, while in 7% of the samples the milk allergens Bos d 5 and Bos d 11 were found, proving the possibility of transferring components of animal origin from food in the produced honey. These results raise concerns about the future effect on the quality and marketing of honey after the ever-increasing use of materials of animal origin in bee feed.

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NEW TECHNOLOGY FOR OBTAINING PROPOLIS

In recent years, interest in propolis as a beekeeping product has grown significantly, especially during the COVID-19 pandemic. By the order of the Ministry of Agrarian Policy and Food of Ukraine, dated November 15, 2023, No. 1968, hygienic requirements for facilities where beekeeping products are produced and/or distributed were approved. Both globally and in Ukraine, which is aligning its legislation with EU standards, increasing attention is being paid to production conditions and hygiene. This drives the improvement of technologies and equipment for producing products, including those from beekeeping.

Propolis is a sticky, resinous substance collected by bees from buds, leaves, and stems of wild plants, which they process. It has bactericidal properties and is used to seal cracks in the hive, polish the walls of wax cells, and embalm the corpses of enemies such as mice and reptiles. In November 2023, the international stan-

dard ISO 24381:2023 "Bee propolis - Specifications" was published, defining propolis as a resinous balsamic mixture of exclusively natural and plant origin. This mixture is collected by worker bees of the species Apis mellifera from exudates of certain plants, to which bees add secretions from their mandibular and wax glands. They use it to protect the health of the bee colony.

Scientists from the USA, based on the results of many years of research, concluded that propolis plays an important role in the immunity of a bee colony. It is believed that propolis is a component of the colony's social immunity and, alongside individual immunity, helps bees overcome the negative impacts of pathogens and other pests. Honey bees cover elements of their hive with propolis, forming a propolis shell. This shell requires annual renewal and fresh propolis to maintain its effectiveness (Simone-Finstrom, Spivak, 2010).

The technology for obtaining propolis, proposed by Sadovnikov A. in July 1982, is outdated. The equipment and methods for collecting propolis using canvases are no longer used in either amateur or industrial apiaries. Beekeepers typically collect propolis in two ways: by cleaning hive parts such as the top and side bars of frames, flight holes, or ceiling boards, or by using special propolis collection devices made from artificial materials. The first method is labor-intensive and ineffective for industrial-scale propolis collection, whereas the use of nets and grates is more efficient and productive.

An important aspect of obtaining propolis is the availability of plant resin sources. In the temperate climate zone, where most of the European continent is located, the main sources of propolis are plants of the genus Populus—Populus nigra L., Populus alba L., and Populus tremula L.. Important secondary sources of propolis in the temperate climate of Europe, though with a less significant contribution, are Betula pendula Roth, Aesculus hippocastanum L., Alnus glutinosa (L.) Gaertn., Pinus sp., Salix alba L., Quercus, Fraxinus, and Picea (Warakomska & Maciejewicz, 1992; Milojković et al., 2016; Przybyłek & Karpinski, 2019). Therefore, beekeepers should ensure that the primary sources of propolis are present within the bees' flight zone. Additionally, apiary sites should be located at a distance from roads and agricultural land to reduce man-made pollution and prevent contamination of plant resin with anthropogenic emissions.

Preparation for placing propolis collection devices in honey bee nests

It is known that honey bees move wax and propolis within the nest from one location to another. Wax, like propolis, is used to seal gaps and cracks, in addition to building honeycombs. Before placing collection devices, the upper bars of the frames should be cleaned of wax and pro-

A B C

Fig. 1. Deposition of propolis by honey bees in collection devices
A – Propolis deposition in individual holes of the collection device; B – Propolis formations created by honey bees; C – The side of the collection device accessible to honey bees

polis, which will reduce their presence in these devices and stimulate the bees to bring fresh plant resin. It is especially important to clean the frame bars during manipulations with the hive bodies, as the inter-frame space is typically filled with wax by the bees. This preparation will also help reduce the content of propolis and wax contaminated with veterinary drugs in the final product.

Behavior of honey bees during propolis accumulation in collection devices

Honey bees deposit the collected propolis in collection devices in the following sequence: first, they cover the perimeters of the holes in the nets or grates, and then seal the central part (Fig. 1, A). If the bees have access to the collection devices from only one side, depressions are formed on that side, while hemispheres form on the opposite side (Fig. 1, B and B-2, respectively) (Dvykaliuk et al., 2022; Dvykaliuk, 2023).

Propolis collection devices are usually placed above the honey bee nest, on top of the upper bars of the hive frames, and can be covered with insulation, polyethylene films, or other materials commonly used in beekeeping. In this case, the beekeeper should ensure there is a 3-4 mm gap between the propolis collection device and the covering material. If the collection device is covered without providing this space, honey bees will form defective propolis deposits in the holes, significantly reducing the productivity of each hole (Fig. 1, B-1; Fig. 2.1). Conversely, if space is provided, honey bees will fill the hole with a substantial amount of propolis, forming a complete deposit (Fig. 1, B-2; Fig. 2.2, 2.3).

In order to study the behavioral characteristics of honey bees during propolis accumulation in collection devices, we conducted research in 2023. Specially designed collector tablets were constructed to allow bees sequential access to the grids for propolis deposition. Each device contained grids (220×220 mm) made of nylon, ethylene vinyl acetate, and polyethylene, with hole sizes of 0.87±0.01 mm², 3.91±0.14 mm², and 10.69±0.20 mm² (N = 3, n = 10). The collector tablets were made from pine (Pinus L.) bars measuring 30×10 mm, between which the grids were fixed. After the bees deposited propolis, part of the grid in each device was closed off to prevent access, while the other part remained accessible (after rotating the devices 180 degrees).

It was observed that when bees were given access to previously filled mesh grids where pro-

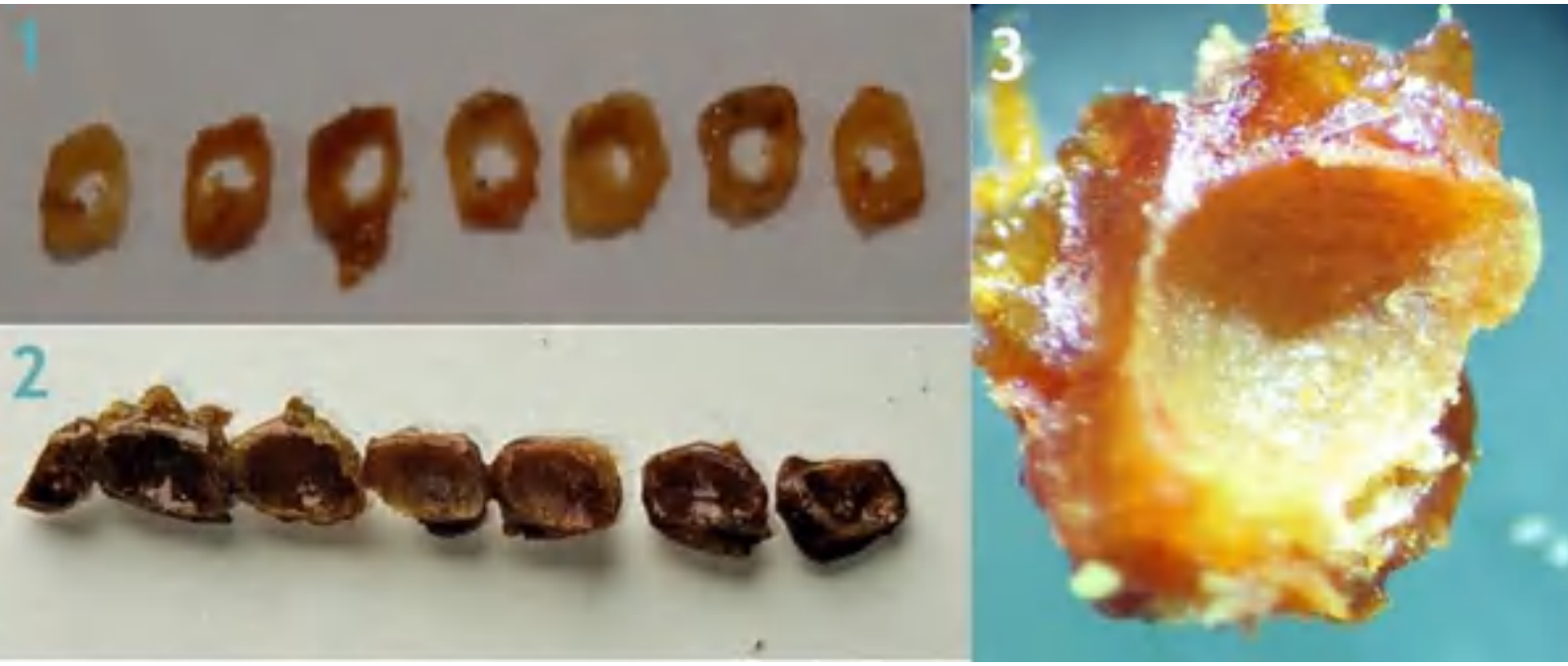

Fig. 2. Propolis formations in mesh holes created by honey bees
1 – Incomplete (with a hole) propolis formation in the mesh; 2 – Fully formed propolis formation in the mesh; 3 – Fully formed propolis formation under a microscope

polis had been deposited, they rearranged it into hemispherical shapes in the holes of the nest. Thus, bees repeatedly formed structures from propolis in the mesh holes, creating depressions on one side and hemispherical shapes on the other (Dvykaliuk, 2024). Future research is needed to determine how this behavior affects the quality of the propolis and the wax content within it.

Beekeepers should be mindful of the position of propolis collection tools during colony maintenance and manipulations. It is also important to prevent bees from accessing the grids from above, as this can lead to the transfer of previously deposited propolis and the formation of thin propolis plates in the holes instead of larger lumps (Fig. 2.2, 2.3).

Transportation and storage of uncleaned propolis collection tools

Saccardi et al. (2021) conducted several studies, including research on the loss of volatile components in propolis over time. The results showed that propolis samples lost an average of 0.9±0.3 wt.% (N=3) after seven hours at room temperature (24°C). Therefore, beekeepers should ensure proper packaging and storage of collection tools coated with propolis until they are cleaned (Fig. 3). Packaging will also protect the propolis from contamination with dust and mechanical impurities. In addition, collection tools should be cleaned as soon as possible, and the harvested propolis should be packaged to prevent damage by wax moths.

Rapid cleaning of propolis collection products will allow their reuse during the current beekeeping season, thereby increasing the profitability of the apiary.

Mechanization of the process of cleaning propolis collection products

Today, most beekeepers use modern propolis collection products, such as elastic nets and

Fig. 3. The method proposed by the author or packaging propolis collection products for transportation and temporary storage.

plastic grids. However, there are no devices available on the market for cleaning these nets and grids from propolis, which significantly complicates the production process and increases costs due to manual labor. Additionally, not all beekeeping operations maintain proper hygienic production conditions.

Based on long-term research, a device has been developed for cleaning elastic nets from propolis. It has been determined that cooling the nets to +5°C for 60 minutes is the optimal preparation for cleaning. The device consists of two pairs of specially designed shafts that rotate toward each other, drawing in and bending the elastic nets in a wave-like manner. As the nets bend, the cooled propolis falls into a tray located beneath the device. The purification level of the propolis collection materials, depending on the type and weight of the propolis, ranges from 80-95% per cycle (Dvykaliuk & Adamchuk, 2021; Dvykaliuk, 2023).

On August 6, 2024, and October 5, 2024, a serial model of the device for cleaning elastic nets from propolis, designed and manufactured in Ukraine, was presented (Fig. 4). One of these devices was installed by the author at the production facilities of Kyivoblbdzholoprom LLC (78 Kibenko St., Boyarka, Kyiv region, Ukraine). The introduction of this improved technology for obtaining propolis replaced the previous technology proposed by Sadovnikov A. in 1982. Beekeepers now have the opportunity to visit Kyivoblbdzholoprom LLC free of charge to familiarize them-

Fig. 4. The author presents the device and technology for obtaining propolis to beekeepers in Ukraine.

selves with the proposed device and, if available, to clean elastic nets from propolis.

Scan the code to view a video presentation of the propolis collection device

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EFFECTS OF DISINFECTANTS ON BACTERIUM PAENIBACILLUS LARVAE IN LABORATORY CONDITIONS

Simple Summary

American foulbrood is a highly infectious disease that can harm the beekeeping sector if it becomes clinically visible. It is caused by the bacterium Paenibacillus larvae, and its spores are resistant to various disinfectants. It is important to ensure effective final disinfection following eradication measures at apiary in order to prevent the disease from reoccurring. A study was conducted to test ten commercially available disinfectants commonly used in beekeeping, as well as those with proven efficacy in the medicinal and veterinary sectors, on different strains of P. larvae bacterium. Early diagnosis methods and proper control measures can help minimize the disease's clinical signs and its incidence.

Abstract

American foulbrood is an infectious disease of the honeybee brood that causes multiple types of damage to beekeeping. The causative agent of the disease is the bacterium Paenibacillus larvae, which forms resistant infective spores and is viable for decades. After the eradication meas-

ures have been implemented, in cases of clinically visible disease, it is necessary to conduct effective final disinfections of equipment and tools. This study aimed to determine the effect of ten commercially available and commonly used disinfectants on certified strains of P. larvae under laboratory conditions, as well as to compare the obtained results among individual genotypes of P. larvae. Selected products were tested by determining the zone of inhibition using an agar diffusion test, a suspension test for viable bacteria, a surface disinfectant test, and a sporicidal effect in the suspension test. Incidin OxyFoam S and Sekusept Aktiv are both effective against all examined genotypes of P. larvae. Despadac and Despadac Secure have a bactericidal effect, but their sporocidal effect is not as satisfactory as that of Genox. Genoll does not exhibit a sporicidal effect, and Ecocide S at 1%, Bee protect H forte, and Bee protect F did not exhibit a satisfactory sporocidal effect. Additionally, EM® PROBIOTIC FOR BEES did not exhibit any bactericidal effect. The effective application of control measures and proper application of final disinfection can reduce the reoccurrence of visible clinical signs of disease, whereas methods of early diagnosis can significantly reduce the incidence of the disease.

Keywords: Apis mellifera; American foulbrood; Paenibacillus larvae; spores; disinfectants

1. Introduction

American foulbrood (AFB) is a severe infectious disease of honeybee colonies that threatens modern beekeeping [1]. The causative agent is the Gram-positive bacterium Paenibacillus larvae [2], which forms oval infectious spores. Remains of infected dead larvae contain billions of resilient spores, which can remain viable on combs, honeybee products, equipment, tools, and the apiary environment for decades. Vegetative forms of P. larvae are sensitive to scorching, drying, and disinfectants. According to Bakonyi et al., the infectious form of the bacterium presents as spores, and bent honeybee larva become susceptible to infection at the age when they actively take food [3]. Clinical recognition of the disease is possible based on the appearance of alterations in the honeybee brood. In the case of suspected AFB, cappings are wrinkled and retracted with dark spots and holes [4,5]. A lattice brood is noticeable, and the diseased larva turns into a shapeless, brown, and viscous ropy mass [6]. In the advanced stage, the rest of the dead larva fit along the lower wall of the honeycomb cell. A honeybee colony is officially declared infected if clinical examination has established changes typical of the disease and laboratory microscopic examination of the decayed larvae allows for identifying the P. larvae spores. Eradication measures involve stamping out and burning infected colonies and associated equipment, as well as the final disinfection of equipment and tools. Burning is the fastest, best, and most expensive way to combat AFB. Sometimes it is possible to save adult bees as an artificial swarm, housed in the new or disinfected hive on comb foundations [7]; however, relapses of clinically visible disease are possible due to poor implementation of final disinfection.

Bednář et al. described various forms of physical and chemical disinfection of beekeeping tools and equipment [8]. Also, they state that the success of disinfection measures in the apiary depends on the correct choice of disinfectants, the spectrum of microorganisms, the recommended concentration of working solutions, the method of application, and exposure to the disinfectant. It is important that the material is disinfected in regard to its possible damage and the possible effect on the environment. Dobbelaere et al. refute the common opinion of beekeepers that the method of burning wooden parts of beekeeping equipment and accessories with flames is a sufficient disinfection measure [9]. Therefore, comprehensive disinfection of the wooden parts of the hive is possible by combining different methods of heat application, such as immersion of wooden parts in microcrystalline wax (150 °C, 10 min) or using high concentrations of disinfectants [10,11]. However, high concentrations of disinfectants are not economically and environmentally friendly, so implementing preventive zoo-hygienic measures and regularly replacing at least 25–30% of dark, old honeycombs is a very important way to mechanically remove P. larvae spores and other pathogens [12–17]. Sporicidal effects on P. larvae were determined for gluta-

radehyde and sodium hypochlorite, 0.5% aqueous sodium hypochlorite solution, 1.1% caustic soda solution, and gamma radiation [18–20]. Kiriamburi et al. examined the biocidal effects of two commercial disinfectants, Virkon® and Disinfection for beekeeping® [21]. Furthermore, extracts of various plants (flavonoids, alkaloids, terpenes, essential oils) exerted successful in vitro inactivation of P. larvae in several studies [22–26].

The study aimed to determine the effect of ten commercially available and commonly used disinfectants in beekeeping and veterinary medicine in general, on certified strains of P. larvae, in laboratory conditions, and to compare the obtained results among different ERIC (enterobacterial repetitive intergenic consensus classification) genotypes.

2. Materials and Methods

2.1. Selection and Cultivation of Microorganisms

The used strains of P. larvae were from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany): genotypes (DSM 7030 (ERIC I), DSM 25430a (ERIC II), LMG 16252 (ERIC III) and LMG 16247 (ERIC IV).

P. larvae strains were cultured on a solid nutrient medium Columbia sheep blood agar and in a liquid nutrient medium Brain–Heart Infusion (BHI). To prepare the liquid nutrient medium, 37 g of BHI medium, 3 g of yeast extract, and 1 L of H2O were used. The P. larvae were grown in the liquid nutrient medium on a shaker (New Brunswick Innova 4340 Incubator shaker, New Brunswick, NJ, USA) at a temperature of 37 °C and a speed of 200 rpm. The incubation period was 48 h.

2.2. Disinfectant Effect Test

Disinfectants were selected based on the recommendations of producers and beekeepers, as well as their availability on the market. The following disinfectants and food additives were used: Bee Protect products (Bee Protect H forte and Bee Protect F); (Honey Bee Pro I Agro Simpa d.o.o., Sisak, Croatia), which contains sucrose, macronutrients and organic acids; Genox and Genoll with foam (Genox Aquagen, Zagreb, Croatia), which contain hypochlorite acid, sodium chloride and hypochlorite ion; Despadac® (Laboratories Calier, Barcelona, Spain) and Despadac Secure® (Laboratories Calier, Barcelona, Spain) with active ingredients didecil-dimethyl ammonia chrysanthemum, glutaraldehyde in different ratios; Ecocid® S (Krka d.d., Novo mesto, Slovenia) with active substances of potassium

peroxymonosulfate, sodium dodecyl benzenesulfonate, and sulfamic acid; Sekusept® Aktiv (Ecolab, Zagreb, Croatia) and Incidin® Oxyfoam S (Ecolab, Zagreb, Croatia) with peracetic acid; and EM® probiotic for bees (EMRO, Okinawa, Japan), which is mixture of microbials. Selected products were tested by (1) determining the zone of inhibition in the agar diffusion test, (2) suspension tests for viable bacteria, (3) surface disinfectant tests, and (4) sporicidal effects in the suspension tests for genotypes of P. larvae (ERIC I to ERIC IV).

2.2.1. Agar Diffusion

An agar diffusion test was used to preliminarily determine which disinfectant meets the minimum criterion of reducing the number of viable bacteria. One bacterial colony was transferred from the solid nutrient medium plate to the BHI liquid nutrient medium optimized for the growth of P. larvae to obtain viable vegetative forms after 48 h of incubation with constant agitation. Then, bacterial cultures were diluted to 0.6 McFarland units determined by a nephelometer (Biosan Ltd., Riga, Latvia). The bacteria were diluted with a medium in a ratio of 1:9 and used in further experiments. In solid blood agar, two or more (depending on the expected diameter of the inhibition zone) wells were punctured, and individually tested disinfectants were added to them, i.e., phosphate–buffered saline (PBS), which in this case served as a negative control. To facilitate the diffusion of disinfectants into the agar, the plates were left for four hours at a temperature of 4 °C and subsequently moved to the incubator at a temperature of 37 °C. After incubation for 48 h, the diameters of the inhibition zones were measured. In further research, only substances (i.e., disinfectants) that showed efficiency in the preliminary agar diffusion test were used.

2.2.2. Sporocidal Effect of Disinfectants

Strains of P. larvae (ERIC I to IV) grown on solid blood agar at 30 °C were, according to the morphological peculiarities of typical colonies, suspended in 1 mL of PBS. Aliquots (0.1 mL) of bacteria were disposed of on solid blood agar plates and incubated at 30 °C for seven days to stimulate sporulation. The newly created colonies were collected with a sterile stick, suspended in PBS, and rinsed twice by centrifugation at 7500× g for 10 min. The resulting pellet was resuspended in PBS and kept at 4 °C until further use. The number of spores in the resulting suspension was determined spectrophotometrically (DEN-1, Biosan Ltd., Riga, Latvia) after heat treatment at 80 °C for 10 min (Thermomixer comfort, Eppendorf, Germany) to eliminate vegetative forms of bacteria. The number of spores was validated after plating of suspension and colony numbering. To determine the sporocidal effect, disinfectant was added at the concentration recommended in the manufacturer's instructions. Exposure times were set as 5, 15, 30, and 60 mins, except for Incidin Oxy Foam S, where a 1 min exposure was added (based on manufacturer declaration). After exposing the P. larvae spores to disinfectant, the spore suspension was filtered (Shott's bottle and Millipore vacuum pump used), and a filter (pore size of 0.45 µm) was placed in 2 mL of sterile PBS and vortexed (V-1, Biosan Ltd., Riga, Latvia) for two minutes to release spores. Then, 100 µL of suspension was seeded on Columbia sheep blood agar at 37 °C. After 48 h, colonies of P. larvae were counted, with Koch's method of counting bacterial colonies being used to determine the number of P. larvae spores that survived the disinfectant exposure, which serves to determine the number of living cells on the principle that one colony grown on a solid nutrient medium indicates one live spore. Seeding using the method of dilution of the sample enabled more accurate determination and

counting of the actual number of colonies grown, as the number of colonies grown corresponds to the number of bacterial cells, i.e., spores in the sample (the number of colonies is indicated as the number of colony-forming units (Colony Forming Units, CFUs). We mathematically obtained the exact number of viable spores as the ratio of the number of bacterial colonies divided by the volume of the planted sample and divided by the reciprocal value of the dilution planted on the substrate. The ratio of the number of bacterial colonies grown in the control and treated group creates the logarithm (Log) reduction.

2.2.3. Determination of ATP Level

P. larvae were grown in the liquid nutrient medium BHI on the shaker to ensure ideal conditions for the growth and reproduction of the bacterial population. After the cultivation step for 48 h at 37 °C with agitation, the suspension of bacteria, prepared at a concentration of 0.6 McFarland units, was used in further research in dilution 1:10.

To determine the amount of ATP, we used the EnSURE (EnSURE Multi-Parameter Luminometer, Hygiena, Germany) instrument along with the Super Snap High Sensitivity ATP test (EnSURE, Hygiena, Germany), which detects low concentrations of ATP in the tested sample. The sampling aimed to examine the number of bacteria in the control sample before and after exposure of P. larvae to a disinfectant in the time sequence, depending on the time of exposure. The procedure is very simple and fast and involves immersing the test stick in the sample. According to the manufacturer's instructions, the sample is combined with the test solution, and then placed in a luminometer where the ATP level is spectrophotometrically determined.

2.2.4. Effects of the Disinfectant on the Contaminant Surface

To determine whether the selected disinfectant works on the surface, a clean dry hard surface free from organic pollution and microorganisms was experimentally contaminated with P. larvae. After the surface was dried, it was treated with the selected disinfectant according to the manufacturer's instructions regarding the concentration and length of exposure. At the end of the required time, the stick of the Super Snap High Sensitivity ATP test (Hygiena, Potsdam, Germany) was used to swab a surface of 10 cm2. The level of ATP was determined using an EnSURE device (Hygiena, Potsdam, Germany).

In addition, the surface was sampled with a sterile test stick (10 cm2), then placed in PBS and vortexed, while 100 µL of the suspension was seeded on solid blood agar and incubated for 72 h at 37 °C. After the end of incubation time, counting of bacterial colonies was used to determine the effect of a disinfectant on P. larvae on solid surfaces.

2.2.5. Statistical Data Processing

Results were presented as the mean ± standard error of at least three repetitions of each sample and used test. As the results in the control and treated groups for all four P. larvae genotypes (ERIC I–IV) were the same, they are presented as one for each test performed, e.g., results for sporocidal effect for all four genotypes are presented in one column. Statistical analysis was conducted using GraphPad Prism 9.0 (GraphPad Software, La Jolla, CA, USA). The existence of a statistically significant difference between the results was determined using a one-way analysis of variance (one-way ANOVA) with Tukey's post hoc test.

3. Results

A summary of the results is presented in Table 1. All results were compared with the control groups.

Despadac (1% solution) shows inhibitory activity for the growth of P. larvae, and the mean diameter of the inhibition zone was 19.25 mm, while Despadac Secure (10% solution) showed an inhibition zone of 14.75 mm. These results for Despadac Secure are statistically significant

(ANOVA, F (8.32) = 68.30; $p < 0.0001$) in relation to the control group. The results show that disinfectants whose main active component is active oxygen (Ecocide S, Krka, and Sekusept Aktiv, Ecolab) have a statistically significant effect on the vegetative forms, creating a relatively wide band for an inhibition zone of bacterial growth inhibition (ANOVA, F (2.17) = 35.15; $p < 0.0001$). Ecocide S caused a mean inhibition zone diameter of 41.63 ± 7.289 mm, while Sekusept Aktiv caused the formation of an inhibition zone with a width of 44.63 ± 12.19 mm. Incidin OxyFoam S caused the formation of a relatively wide band of the P. larvae growth inhibition zone, which was 47.52 ± 12.19 mm ($p < 0.0001$). Genox, at a concentration of 10%, showed a weaker disinfecting effect than an undiluted product; however, the producer recommends dilution of a product, even at a larger scale (up to 1% which in our study shows no efficacy). The diameter of the inhibition zones caused by Genoll is significantly smaller (9.50 ± 1.04 mm). A single analysis of variance showed that Genox 100% and Genox 10% had a significant inhibitory effect on the growth of P. larvae ($p < 0.0001$) as well as Genoll ($p = 0.0088$). The application of Bee Protect F caused the formation of an inhibition zone of 24.75 ± 3.09 mm, and the application of the product Bee Protect H Forte inhibition zone of 36.75 ± 1.89 mm. Both described results compared to negative control values are statistically significant ($p < 0.0001$). The influence of effective microorganisms on the P. larvae showed that the visible zones of inhibition are only a few millimeters wide, so no additional analyses were made for the product EM® PROBIOTIC FOR BEES. These results are presented in Figure 1.

In the suspension test with the vegetative form of P. larvae, the effectiveness of the disinfectant during exposure following the standards for bactericidal action was determined. The results show that both Despadac products show statistically significant bactericidal actions on P. larvae (indirectly measured by the amount of ATP in suspension) (ANOVA, F (8.31) = 26.74; $p < 0.0001$). More precisely, after 15 min of exposure

Bacterium *Paenibacillus larvae*		Method for Determination of Disinfectant Effects									
Disinfectant product	Inhibition zone diameter (mm)	Suspension test for viable bacteria				Surface disinfectant test				Sporicidal suspension test	
		Determination of the amount of ATP (ATP units)								Logarithms of reducing spores (log 10)	
		5	15	30	60	5	15	30	60	30	60
		Exposure to disinfectant (min)									
Bee Protect H forte	36.75	-	-	-	172.00	-	-	-	-	-	<1
Bee Protect F	24.75	-	-	-	102.20	-	-	-	-	-	<1
Genox 100%	30.10	71.80	333.50	483.50	513.50	76.00	102.10	481.80	440.40	3	3
Genox 10%	23.20	64.20	90.40	151.20	175.60	61.10	120.40	154.30	170.20	1	1
Genoll 10%	9.50	72.33	93.20	101.60	107.50	68.00	94.20	103.40	120.30	-	-
Despadac	19.25	52.40	247.50	775.50	-	84.20	182.20	543.00	-	2	-
Despadac Secure	14.75	98.80	275.50	755.00	-	102.60	192.40	490.00	-	1	-
Ecocid S	41.63	80.20	346.50	539.80	838.50	50.80	340.00	542.80	840.20		6
Sekusept aktiv 2%	44.63	893.80	942.40	930.00	875.50	843.20	896.80	860.00	864.20	6	5
Sekusept aktiv 1%	-	75.20	102.00	297.50	880.00	52.40	68.20	360.40	875.60	3	1
Incidin Oxyfoam S	47.52	830.70	801.10	-	-	826.60	836.40	-	-	6	6
EM® probiotic for bees	-	-				-				-	

Table 1. Summary of results on disinfectant effects of ten commercially available products on bacterium P. larvae for all examined genotypes (ERIC I–ERIC IV)

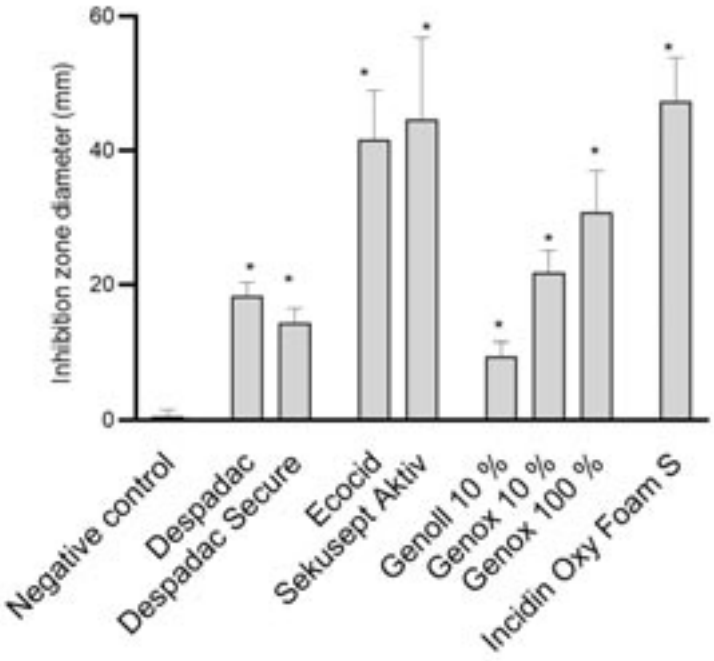


*Figure 1. Summary of the diameter of the inhibition zone caused by examined disinfectant products on the growth of P. larvae bacteria. The results are shown as the mean ± standard error of at least three repetitions for each genotype. All data from the control group and for specific disinfectants are presented in one column, as the values for all genotypes (ERIC I–ERIC IV) were similar. For the sake of clarity, BeeProtect products and EM are not presented as they are not effective; * p < 0.0001*

of the bacteria to Despadac disinfectant, the value of the relative units of ATP increased to 247.50 ± 29.55, reaching 775.50 ± 110.70 after 30 min of exposure. After exposure to Despadac Secure for 15 min, an increase in relative ATP units to 275.50 ± 22.16 was observed, reaching 755.0 ± 132.60 after 30 min. The results show that active oxygen-based products have a statistically significant bactericidal effect on P. larvae (ANOVA, F (12.44) = 46.18, p < 0.0001) depending on the time of exposure. In Sekusept Aktiv assets at 2% concentration, the effect was significant after 5 min of exposure (893.80 ± 68.66 RU, p < 0.0001) and in 1% concentration after 30 min (297.50 RU ± 28.10; p < 0.0001). The action of Ecocid S was observed after 15 min (346.50 ± 33.98) and after 30 min (539.80 ± 83.54) (p < 0.0001). After 60 min of exposure to vegetative forms of P. larvae at both applied concentrations of Sekusept Aktiv, as well as Ecocide S, a very similar number of relative units was determined, which indicates their significant bactericidal action (values of relative units as follows: 875.50 ± 84.95, 880.0 ± 97.21 and 838.50 ± 69.65; p < 0.0001). A statistically significant effect was caused by the disinfectant Sekusept Aktiv in 2% concentration after 5 min of exposure (p < 0.0001), while Sekusept Aktiv in 1% concentration and Ecocid S showed a statistically significant effect after 30 min (p < 0.0001). For Incidin OxyFoam S, the measured value after 1 min (exposure time referred by manufacturer) was 830.70 ± 61.39 (p < 0.0001), then 830.70 ± 83.70 after 5 min, while, after 15 min, a significant increase was observed to 801.10 ± 81.21 (ANOVA F (3.20) = 18.05, p < 0.0001). Genoll did not affect P. larvae, and the values of the relative units in the time frame of treatment were as follows: 72.33 ± 12.82; 93.20 ± 19.02; 101.60 ± 12.25 and 107.50 ± 15.47, respectively. Similarly, Genox's 10% was efficient at the longest period of exposure (191.30 ± 10.33, p = 0.03). On the other hand, undiluted Genox significantly affected bacteria after 15 min (333.50 ± 10.24; p < 0.0001), 30 min (483.50 ± 56.76; p < 0.0001) and after 60 min of exposure (513.50 ± 54.94; p < 0.0001). The results of the action of Bee Protect H Forte on the vegetative form of P. larvae showed that there was a significant increase in the amount of free ATP, which is an indicator of the decomposition of P. larvae. Consequently, there was a statistically significant decrease in the number of bacteria (ANOVA, F (2.15) = 13.79; p = 0.0004). Results are presented in Figure 2.

A statistically significant difference between the experimental and control group was determined after surface treatment for 30 min with Despadac (543.0 ± 67.77) and Despadac Secure (490.00 ± 66.71) (ANOVA, p < 0.0001; for both groups) for all genotypes of P. larvae. A significant decrease in the number of P. larvae after surface treatment with Ecocid S was observed after 15 min (340.00 ± 32.90; p = 0.0006), and after 30 and 60 min of exposure, respectively, an even more significant increase in the number of relative ATP units was observed (542.80 ± 82.94; 840.20 ± 68.55). Sekusept Aktiv disinfectant (1%) significantly reduces the number of viable P. larvae after 60 min (875.50 ± 84.95; p < 0.0001). After one minute of surface treatment with Incidin

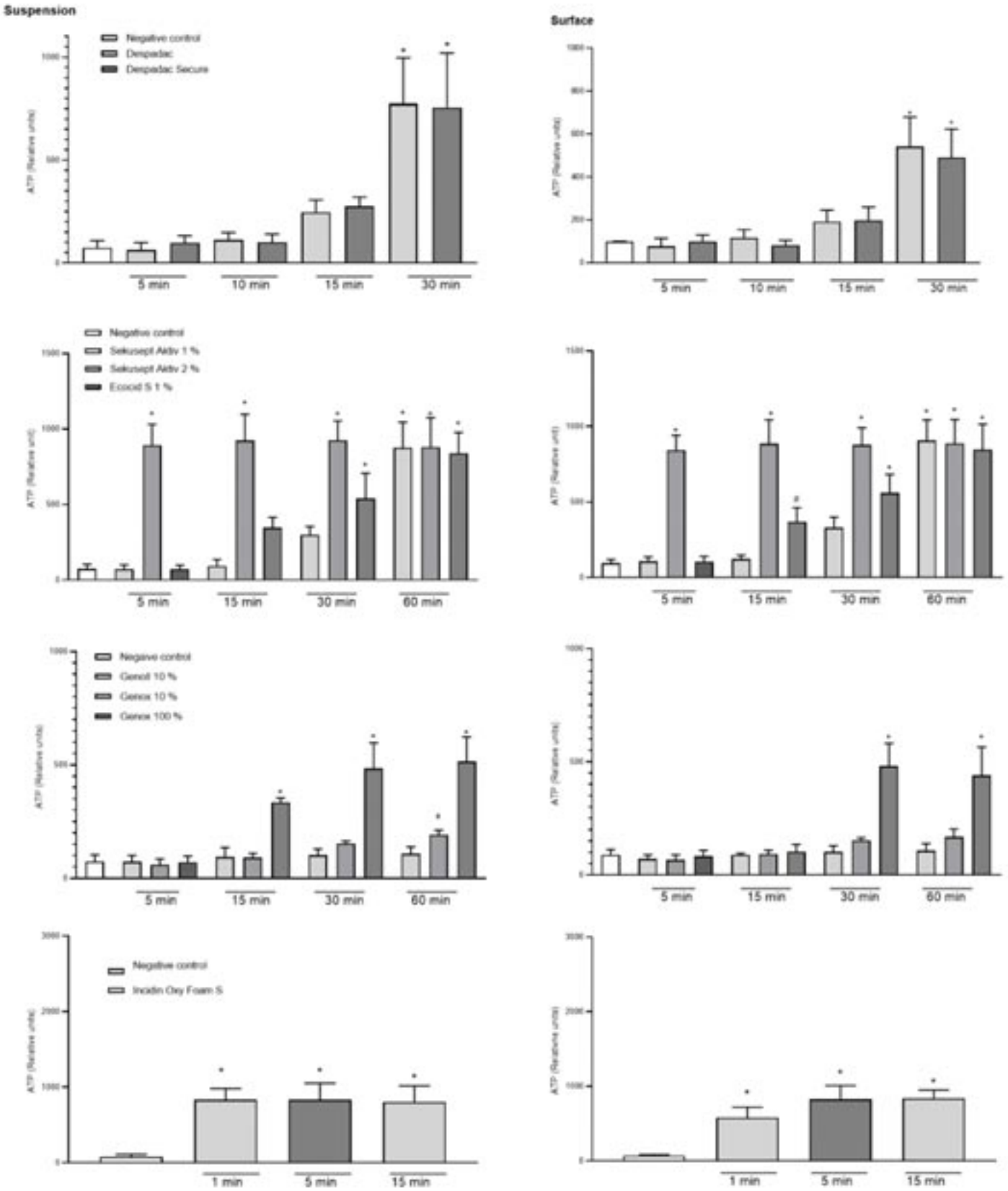


Figure 2.
*Summary of the relative units of ATP caused by disinfectant products Despadac, Sekusept Aktiv, Ecocid S, Genox and Genoll, and Incidin Oxy Foam S on the growth of P. larvae bacteria. Results for all genotypes treated with disinfectant are merged into one column. The results are shown as the mean ± standard error of at least three repetitions; * p < 0.0001; # p < 0.001*

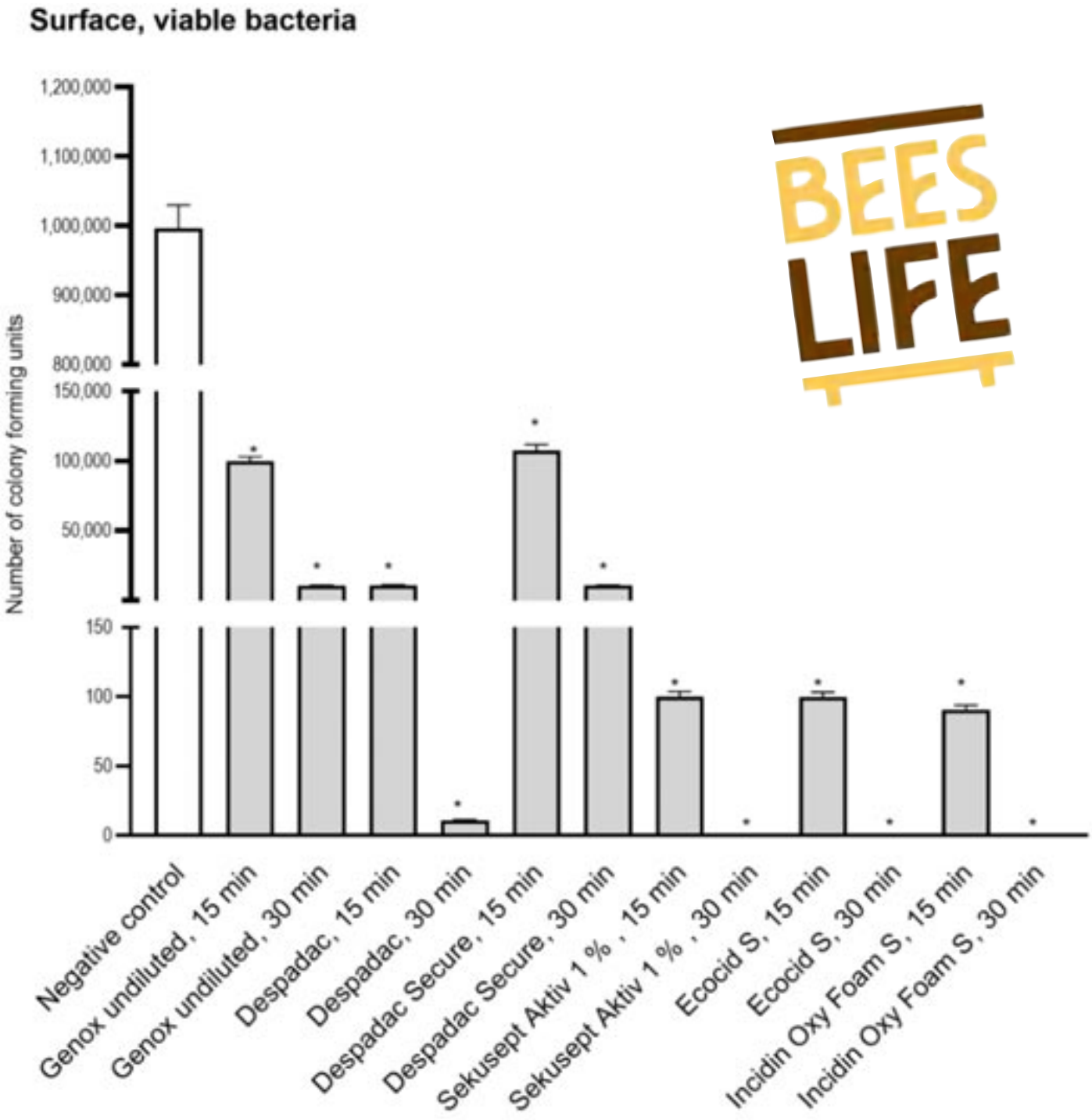


*Figure 3. Results of a number of Colony Forming Units following application of disinfectant onto the surface. For the sake of clarity, results obtained following 5 and 60 min are omitted as they are comparable to those obtained after 15 and 30 min, which are presented. All genotypes produced were susceptible to disinfectants at the same level and thus are grouped into one column. The statistically significant decrease for CFU for all groups was * $p < 0.0001$ versus control group*

OxyFoam S disinfectant, the number of P. larvae was significantly reduced (577.70 ± 57.72, $p < 0.0001$), and a further decrease was observed after 5 min (826.60 ± 68.11, $p < 0.0001$) and 15 min (836.40 ± 42.58, $p < 0.0001$), respectively. A significant effect on reducing the number of bacteria was observed by Genox only in 100% concentration after 30 min (481.80 ± 40.86; $p < 0.0001$) and 60 min of action (440.40 ± 46.95, $p < 0.0001$). After treating the surface with the Bee Protect line, even after 60 min of exposure, there was no increase in the value of the relative

number of ATP units, nor a decrease in the number of viable bacteria. Results are presented in Figure 2.

The sporicidal effect of disinfectants from the Despadac group was not observed after 10 min exposure, while, after 30 min, it amounted to a one Log reduction for Despadac Secure, i.e., a two Log reduction for Despadac. After 60 min of action of both disinfectants, the number of spores was reduced by 99%, i.e., disinfectants caused a reduction in the number of spores by two logarithms.

Disinfectants with oxygen as an active substance show a sporicidal effect after 30 min, namely a six Log reduction for Sekusept Aktiv (2%) and three Log reduction for Ecocid S (1%). After 60 min of action, both Ecocid S and Sekusept Aktiv saw a six and five Log reduction, respectively, while Sekusept Aktiv at 1% concentration reduces the number of spores only by one logarithm. After application of Incidin Oxy Foam S, an active substance hydrogen peroxide, the sporocidal effect after 30 min of exposure was six logarithms. The results showed that the disinfectant Genoll has no sporicidal effect. Genox at 10% caused a reduction in the number of spores of P. larvae by one logarithm, as well as at 100% concentration for three logarithms during exposure lasting 30 and 60 min, respectively. Bee Protect products do not show a sporicidal or negligible effect, as they also reduce the number of spores by less than one logarithm during exposure of 60 min. All four genotypes of P. larvae (ERIC I to ERIC IV) in the sporicidal suspension tests showed equal sensitivity to all the biocides tested.

We applied 1 million microorganisms onto a dry and smooth surface (square 10 cm2) and, following disinfectant action, found a reduced number of viable bacteria (using CFU method) for all disinfectants applied (Figure 3). We found the reduction to 100672 CFU for undiluted Genox (100%) after 15 min and 9976 CFU after 30 min, which was similar to the reduction achieved after application of Despadac Secure. The decreases in Despadac reached 10025 CFU and 11 CFU, respectively. Sekusept Aktiv (1%) and Ecocid s (1%) reduced the number of viable bacteria after 15 min for 4 Log (cca 100 CFU remained viable) and after 30 min for 6 Log (cca 1 CFU remained). Incidin OxyFoam S at decreased the number of CFU: after 15 min to 90.70 and after 30 min to 1, corresponding to a five and six Log reduction.

4. Discussion

Although AFB is a notifiable disease present all over the world, till now there have been no uniform measures nor guidelines that address the effectiveness of disinfectants against bacteria P. larvae; thus, we performed a series of experiments using several products with different active ingredients to find the most appropriate for use in apiaries, keeping in mind effectiveness, user-friendly features, and the overall cost.

Hypochlorous acid is an active ingredient in sodium hypochlorite that reacts quickly to various substances, such as proteins, DNA, lipids, thiols, and disulfides. It is believed that this reaction prevents the germination of P. larvae spores by affecting the inner membrane of exposed spores [26].

This study found that two disinfectant products, Genox and Genoll, had an inhibitory effect on vegetative forms of P. larvae in an agar diffusion test. The result for Genox depended on the concentration applied. In the suspension test, undiluted Genox had a significant inhibitory effect after 15 min of administration that was dependent on the duration of exposure. A similar result was observed on surfaces after 30 min that was not dependent on contact time. It is important to note that the effect of Genox weakened slightly after 60 min, as all chlorine-based biocides have a time-limited effect due to wear during exposure to environmental factors. Hypochlorite-based disinfectants are also consumed more in the presence of organic matter, and their effectiveness is affected by the presence of proteins, nucleic acids, or other organic substances in the environment [27].

The microbicidal effect of chlorine is achieved by damaging functional molecules, such as DNA or proteins [28], and depends on the oxidative potential. A study found that dry spores of bacteria are more sensitive to NaOCl than wet spores [18] and that there are no germinating spores after 40 min of exposure in dry spores. In contrast, in a humid environment, an effective concentration of only above 0.025% of active chlorine was found. However, in a study of

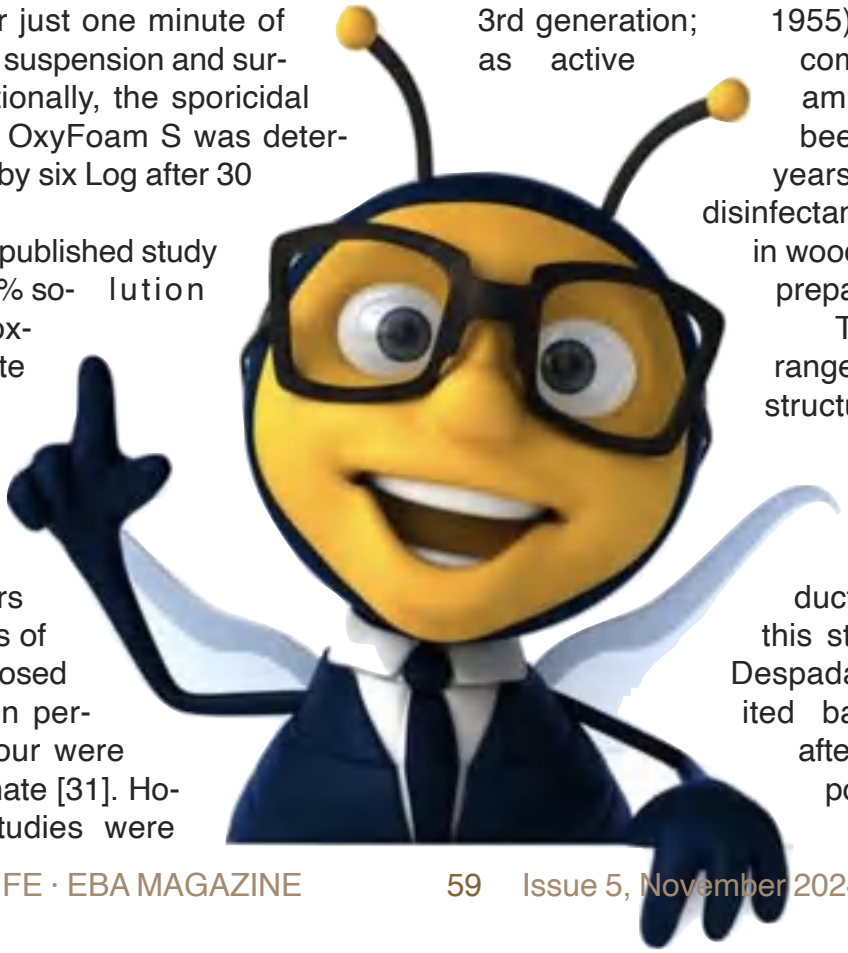
sporicidal action on P. larvae microenvironments in wet conditions, Genox 10% reduces the number of germinating spores by one logarithm, or Genox 100% for three logarithms after 30 min of contact. This disinfectant does not have a desirable sporicidal profile due to the long time it takes to achieve a sporicidal effect, which is also very limited. To achieve a good sporicidal activity, it would be expected to reduce the number of spores by a minimum of five Log reduction in real contact time. Sporicidal activity of 30 min or more requires immersion of contaminated equipment in a disinfectant solution, making disinfection difficult, time consuming, and relatively expensive.

The antimicrobial properties of hydrogen peroxide are attributed to the creation of hydroxyl radicals. When present in sufficient concentrations, hydroxyl radicals can harm nucleic acids, proteins, and lipids [29]. Killing spores with hydrogen peroxide does not involve damaging their DNA, unlike vegetative forms of bacteria. In a study of commercially available disinfectants with different chemical compositions, we selected the product Incidin OxyFoam S, as it has a declared sporicidal action and a stabilized hydrogen peroxide content of 1.5 g per 100 g of liquid. The results showed that this disinfectant product, following the manufacturer's declaration, has antimicrobial effects on vegetative forms of P. larvae after just one minute of exposure in both suspension and surface tests. Additionally, the sporicidal action of Incidin OxyFoam S was determined to reduce by six Log after 30 min of contact.

A previously published study revealed that a 7% solution of hydrogen peroxide can inactivate spores of bacteria after six hours of exposure [30]. In another study, authors found that spores of Bacillus spp. exposed to 10% hydrogen peroxide for one hour were unable to germinate [31]. However, these studies were conducted using hydrogen peroxide that was not mixed with other substances or auxiliary agents, which is different from our research. No formulations of hydrogen peroxide that are stabilized and contain additional agents for oxidation and enhanced action have been patented and proven to be more effective. They act on the proteins from spore sheaths, create hydroxyl free radicals, and oxidize membrane lipids, enabling the disinfectant to act inside the cell, affecting vegetative forms of bacteria.

Hydrogen peroxide is highly effective in killing bacteria and spores in different environments, such as hospitals and industrial settings. It is used in the form of gas through dispersing and nebulization devices, either alone or in combination with silver. Although the mechanism of inactivation of bacteria and spores by hydrogen peroxide gas is complex and not fully understood, research has shown that the gas penetrates deeper into spore structures, leading to the oxidation of essential amino acids required for spore germination [32].

In the domestic market, only one product has been declared safe for bees and used in beekeeping. This study focuses on two available products—Despadac and Despadac Secure—both of which contain didecyl-dimethyl ammonium chloride (as quaternary ammonium salts of the 3rd generation; 1955) and glutaraldehyde as active components. Quaternary ammonium salts have been in use for many years in hand antiseptics, disinfectants, and preservatives in wood processing or for the preparation of eye drops. They have a wide range of possible chemical structures that can effectively reduce the number of bacteria or inhibit and suppress their reproduction. The findings of this study indicate that the Despadac product line exhibited bactericidal properties after the bacteria were exposed for 30 min to this disinfectant.



However, there was only a slight decrease in the number of germinating spores of P. larvae, ranging from one or two logarithms. Such a result is not entirely satisfactory for disinfection purposes, but it may suggest that Despadac products could be used for sanitation. Similarly, a previous study showed that 0.06% of third-generation quartile ammonium salt in the Carrier test did not have a disinfectant effect on the spores Bacillus stearothermophilus [33]. This suggests that dodecyl-dimethyl may not be effective against spores, as the spore sheath is composed of proteins, such as keratin and biguanides, on which quartile ammonium chloride does not act [18]. Quaternary ammonium salts are used as biocides, and they work by interacting with the cytoplasmic membrane of bacteria. This interaction results in a range of processes, such as adsorption on the cell wall, penetration into the cell, reaction with the cytoplasmic membrane (lipid or protein), and disorganization of the membrane. These processes lead to leakage of intracellular material, degradation of proteins and nucleic acids, and ultimately, cell wall lysis caused by autolytic enzymes [34]. It has been shown that the selection of formulations and methods of application of disinfection affects the effectiveness of quaternary ammonium salts, and relatively few studies have been conducted in which their effectiveness is evaluated in practical conditions. In addition, we should consider the possible determination of chemical residues (ecological dynamics) and the emergence of resistance to repeatedly applied disinfectants [35]. However, the research objectives did not meaningfully confirm the possible occurrence of resistance to the action of quaternary ammonium salts because they do not have fully comparable data, as quaternary ammonium salts exist today in seven generations and, in combination with other substances (commercial preparations), can have a changed effect.

According to Regulation (EC) No 396/2005 of the European Parliament and the Council [36] (ANON, 2005), didecyldimethylammonium-chloride has been authorized as an active substance in plant protection products for use only on ornamental plants. However, its authorization has been revoked, as its use may lead to the appearance of residues in food for humans and animals. As it has been observed that their use in plant protection products leads to the appearance of residues in food, minimum residue levels have been proposed. Considering that disinfectant products used in beekeeping must be safe because honeybee colonies produce food that must not contain residues of a chemical origin, we believe that Despadac products are not a suitable choice for disinfection in beekeeping. Moreover, we have not found any record of these products being registered as biocides in the Republic of Croatia, raising questions about their regular distribution that require further investigation. In addition, this active substance is not listed in the regulation (EU) No 528/2012 of the European Parliament and the Council [37] concerning making biocidal products available on the market.

The study indicates that disinfectants containing peracetic acid are the most effective against vegetative forms and spores of P. larvae bacteria. These disinfectants have been tested in independent microbiological laboratories and have shown bactericidal properties compared to rod bacteria, as well as sporicidal action in relatively short exposures. Peracetic acid is a potent biocide even at low concentrations ranging from 0.0001% to 0.2%.

Peracetic acid shows an advantage over other types of active substance disinfectants because it remains effective even in the presence of organic residues and is broken down into nontoxic and non-mutagenic substances—acetic acid and oxygen. Thus, it ensures a high degree of disinfection effect in a short contact period. We used these desirable properties of disinfectants, along with the stability of the prepared solution over a long period, to investigate the effect of two commercial products on the vegetative forms of P. larvae and their spores. Notably, researchers found that the action of peracetic acid as a disinfectant is not dependent on the temperature of the environment, although, at higher temperatures, the results are manifested by weaker antimicrobial activity [38]. The results from the same study showed that, during the entire experimental period of 24 days, Sekusept Aktiv solution had the same disinfection potential for at least four consecutive days. Studies have also shown that the effectiveness of peracetic acid is different depending on whether the microorganisms are in

suspension or on the surface, which was not the case in our research. Kunigk et al. (2001) determined that the kinetics of bacterial cell destruction are twofold and may correspond to the contact time of peracetic acid from 2 to 25 min, and the other 25 to 35 min of contact [39]. Similar to ours is a study in which the effectiveness of peracetic acid was shown, which was constant in the first 30 min of contact of disinfectant with bacteria, but subsequently increased [40].

Sekusept Aktiv is a powder used in human medicine to disinfect instruments; however, in veterinary medicine, it is not yet widely used. Unlike this product, and the same active component, Ecocid S, produced by the Slovenian company Krka, has been present on the market for many years, and as such it is relatively often used as a disinfectant in veterinary medicine. Research shows that bactericidal concentrations of Ecocid S disinfectant cause the destruction of the cell wall and cytoplasmic membrane of mycobacteria, as synonymous with a microorganism with very high resistance to disinfectants and external factors. The results also showed that Ecocid S causes decay of the granular components of the cytoplasm with the formation of fine granular inclusions and vacuoles. These irreversible changes in the structural elements of mycobacteria led to the deterioration of bacterial cells [41].

The study of Kiriamburi et al. aimed to examine and compare the biocidal effect of two disinfectant products: “Disinfection for beekeeping” (DFB) (Swienty, Denmark) and Virkon S (Lanxess, Germany) on P. larvae spores [22]. Previous studies have shown that the 1% solution of Virkon (active ingredient potassium peroxymonosulfate) has a bactericidal effect on Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Enterococcus hirae and Mycobacterium smegmatis in suspension tests and Carrier tests on P. aeruginosa, E. coli, S. aureus and E. hirae. However, this same concentration of Virkon did not show an inhibitory effect on spores and fungi, i.e., research has shown that the concentration and required time are not following the guidelines for sporicidal and fungicidal action [42]. In their paper, the same authors concluded that 1% Virkon is effective only against vegetative bacteria, yeasts, and viruses, and should therefore be considered a low-level disinfectant [42]. The im-

proved formulation of Virkon S has been shown in studies to kill up to 80% of P. larvae spores [6]; however, in the Kiriamburi et al. study, the biocidal effect ranged from 88.6 to 96.8% after 30 min of treatment duration [22].

The suspension test showed that Bee protect H forte had a bactericidal effect, but the test on surfaces did not show the same effect. Additionally, the Bee protect products did not meet the set standards for an overall sporicidal effect in the suspension test. Although these products can increase the effect with the time of exposure of bacteria to disinfectant, they are not recommended for use in the final disinfection of equipment, accessories, and apiary after the sanitation of clinically visible AFB. However, they can be used as an aid in disinfection despite being declared as food supplements for bees.

It should be emphasized that, in disinfecting terms, reducing the number of spores of P. larvae by two logarithms (99%) does not represent a sufficient degree of disinfection due to the presence of a large number of spores. In addition, by reducing their number, it may be possible to reduce the extent of infection and/or the rate of development of the disease within one honeybee colony, but it certainly does not lead to a significant reduction in the incidence of the disease.

Effective microorganisms (EMRO, Japan) contain dozens of strains of microorganisms (bacteria, fungi, and mold) that are considered both beneficial for the physiological functioning of the organism and achieving balance in the environment and nature. Effective microorganisms are used in agriculture, forestry, animal husbandry, aquaculture, beekeeping, environmental protection and medicine [43–46]. The benefit of microorganisms in water purification have been documented for many years [43], as well as in the reduction in unpleasant odors on farms [47]. It has also been shown that this formulation of microorganisms applied in tumor cell culture leads to cell apoptosis [48]. In this study, the effect was investigated by using a dietary supplement for bees EM® PROBIOTIC FOR BEES. The achieved inhibitory effect was minimal, and therefore this product cannot be considered to be a disinfectant or biocide. However, the action of effective microorganisms in a living organism is multifaceted, as this product works to preserve normal microflora in the gastrointestinal system of mammals and insects based on the exclusion of pathogenic microorganisms and competitive antagonistic action [49] by changing metabolic pathways through enhancing the action of digestive enzymes while reducing the activity of bacterial enzymes and ammonia formation [50] and having positive effects on the immune system and gut microbiome of bees [51].

5. Conclusions

The products Incidin OxyFoam S and Sekusept Aktiv (when used at a concentration of 2%) have demonstrated a satisfactory sporicidal effect on all four genotypes (ERIC I to ERIC IV) of P. larvae. However, Despadac and Despadac Secure showed a bactericidal effect, but their sporicidal effect is not as satisfactory as that of Genox. On the other hand, the product Genoll with foam does not exhibit any sporicidal effect; additionally, the products Ecocide S at a concentration of 1%, as well as Bee protect H forte and Bee protect F, did not exhibit a satisfactory sporicidal effect on P. larvae. The food additive EM® PROBIOTIC FOR BEES did not exhibit a bactericidal effect.

Therefore, we strongly recommend considering the effectiveness of a disinfectant before use and choosing an appropriate option to reduce the re-occurrence of a disease in apiary.

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QUALITY ASSESSMENT, ANTIMICROBIAL ACTIVITY ORGANIC SUNFLOWER HONEY AND USE OF MALDI-TOF MASS SPECTROMETRY FOR THE IDENTIFICATION BACTERIA ISOLATED FROM HONEY

Summary

The aim of this study was to investigate the physicochemical parameters of quality, microbiological safety and antimicrobial potential of four samples of organic sunflower honey from the Banat area (northeastern Serbia). Humidity, ash, pH, free acidity, electrical conductivity, hydroxymethylfurfural content, sugar content and diastase activity were measured. Microbiological analysis revealed the presence of total aerobic mesophilic bacteria, coliforms, aerobic endospores, lactic acid bacteria (LAB) and a good number of molds and yeasts. The isolate identification was carried out using Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The antimicrobial effect of organic sunflower honey was investigated on five ATCC strains of bacteria: Staphilococcus aureus ATCC 29213; Escherichia coli ATCC 25922; Salmonella enterica ATCC 10708; Yersinia enterocolitica ATCC 23715; and Bacillus subtilis ATCC 23857. The results of the study showed that all honey samples meet international quality standards for all physicochemical parameters. Microbiological analysis of Sunflower honey confirmed the total bacterial counts for all samples ranged from1.80 to 1.85 x10-2cfu/g-1, whereby no presence Clostridium spp., coliform bacteria, as well as molds was detected. Investigation of the antimicrobial activity of honey samples revealed that all bacteria showed clear zones of inhibition in honey concentrations of 40-100%, which is a satisfactory result for flower honey.

Key words: Antimicrobial activity, physicochemical properties, honey, microbiological safety.

Introduction

Honey is a sweet and aromatic substance produced by honeybees from the nectar of plant flowers and honeydew that is consumed as food of a high nutritional value (1). White, (1978) (2) indicates that honey is a mixture of carbohydrates (85-95%) of which are fructose and glucose), or-

ganic and amino acids, proteins, minerals, vitamins, and lipids, with the physio-chemical composition. Color, aroma, and taste of honey differ according to the micro-climatic conditions of the environmentweather, vegetation or plant species that bees feed on, processing, manipulation, packaging and storage time which directly affects the characteristics of honey (3-4). Useful characteristics of honey are rapid absorption, antioxidant and antimicrobial factors, exceptional therapeutic properties in colds, skin wounds and burns, various gastrointestinal diseases, and oncological conditions. Furthermore, the antioxidant and antimicrobial properties of honey are due to the synergistic effect of its polyphenols, amino acids, vitamins, and enzymes (5). In recent years, a branch of alternative medicine called apitherapy has been developed, which offers treatments based on honey and other bee products against many diseases including bacterial infections. Honey can be contaminated just like any other natural food, e.g., heavy metals, pesticides, and antibiotics. Today, special attention is being paid to organic honey due to increasing pollution and numerous poisons, which has led to a certain demand for certified organic honey (6). Certified organic honey is described as lacking any chemical pollution including that related to honeybee migration in search of good blossoms not directly controlled by beekeepers. Chemical contamination during the final packaging process and storage process results in the deterioration of the quality of the products (7).

Honey contains certain microorganisms that can tolerate high concentrations of sugar and acidity, and which originate from the digestive system of honeybees, from pollen, dust, air and plants (8). The most common honey organisms are yeasts, moulds (Penicillium and Aspergillus genera), bacteria of Enterobacteriaceae family, and sporogenic bacteria, which defines the hygienic and sanitary status of honey (9). The presence of microorganisms in honey can sometimes affect the stability of a product and its hygienic quality. The results of many studies show that the microorganisms found in honey are not dangerous for human health, even in products from which Aspergillus flavus was isolated, the presence of aflatoxin was not detected because there were no favorable conditions for synthesis. In this paper, the organic sunflower honey (Helianthus annuus Linn.) from the Banat area (northeastern Serbia, an area known for sunflower cultivation), was studied for the first time. Sunflowers are one of the most important cultures containing oil since sunflower oil is rich in phenolic compounds (10).

In apitherapy sunflower honey is used in diseases of the heart and blood vessels, diseases of the digestive system and kidneys, diseases of the lungs, whereas with children as a stimulant of the immune system). This study aimed to characterize organic sunflower honey through the analysis of physicochemical quality parameters (moisture, ash, pH, free acidity, electrical conductivity, hydroxymethylfurfural content, sugar content and diastase activity) and microbiological safety (presence of aerobic mesophilic bacteria, coliforms, aerobic endospores, lactic acid bacteria (LABs, molds and yeasts), as well as the antimicrobial ability of organic sunflower honey. This research could contribute to the valorization of sunflower honey, which would lead to standardization and increased production of this product.

Materials and Methods

Honey samples

Four samples of sunflower organic honey were taken from four households in the Banat area in production year 2018 and stored in a refrigerator at 4°C until further analysis.

Physicochemical analyzes

The honey samples were analyzed for: reducing sugars, sucrose, water, water-insoluble substances, free acids, hydroxymethylfurfural (HMF), minerals and electrical conductivity using harmonized European Commission methods (11) for honey. Diastasis activity was analyzed by AOAC method 958.09 (12).

Determination of water content. The water content in the honey was determined by measuring the refractive index using an Abbe refractometer (Abbe refractometer, Tokyo, Japan) at 20°C.

Determination of electrical conductivity. A sample quantity equivalent to 20 g of anhydrous honey was dissolved in distilled water. The prepared solution was quantitatively transferred to a 100 mL volumetric flask and refilled to the mark with distilled water. An aliquot of 40 mL of the sample solution was transferred into a beaker and thermostated in a water bath at 20°C. Determination of free acid content. The prepared sample was triturated in the presence of phenolphthalein with a solution of 0.1 mol / l NaOH until light pink colour appeared. The free acid content, expressed in mEq of acid / kg of honey, was calculated using the following formula: Free acid content = mL 0.1 M NaOH × 10.

Determination of mineral content. 0.6-0.7 g of the sample was weighed and transferred to a polytetrafluoroethylene (PTFE) cuvette for microwave digestion. 1 ml of 30% hydrogen peroxide and 7 ml of 65 % nitric acid were added. The sample was mineralized in an Ethos 1 microwave oven, Advanced Microwave Digestion System, Milestone, Italy. Rotor: HPR-1000 / 10S high pressure rotor. Upon completion of the digestion, the sample was cooled, transferred to a normal 50 ml vessel and supplemented with bidistilled water to a final volume. The content of the elements was determined by the instrument: Thermo Scientific iCAP 6500 Duo ICP (Thermo Fisher Scientific, Cambridge, UK). Multi Element Plasma Standard Solution 4, Specpure, 1 g / l for all elements was used as the reference standard. The results are expressed as percentage of minerals per kg of honey.

Determination of the content of reducing sugars. The principle of this method is based on the reduction of the Fehling's solution by titration with a solution of reduced sugars of honey using methylene blue as an indicator.

Determination of sucrose content. The principle of this method is based on sucrose hydrolysis, reduction of the Fehling's solution by titration with reduced sugars from the hydrolyzate of honey with methylene blue. Sucrose content is calculated as the difference between the amount of invert sugar after and before hydrolysis and the difference obtained is multiplied by a factor of 0.95.

Determination of insoluble substances content in water by gravimetric method. 20 g of the sample (to the nearest ± 10 mg) were weighed out and dissolved in a specified amount of distilled water at 80°C and mixed well. The prepared sample is first filtered through a dried and measured sintered funnel, with a pore size of 15 40 mm. The sediment was washed with boiling water (80°C) to release sugar, which was determined by a Mohr test. The funnel was dried at 135°C (drying time 1 hour), cooled and measured with accuracy of 0.1 mg.

Determination of HMF content. The principle of the method is based on the reaction of 5-hydroxymethylfurfurol with barbituric acid and p-toluidine to give a compound whose absorption maximum is in the UV-Vis range at 550 nm.

Determination of diastasis activity. The principle of this method is based on the hydrolysis of a 1% solution of starch by an enzyme from 1 g of honey for one hour at a temperature of 40°C.

Isolation and identification of microorganisms from honey samples

To determine the presence of total aerobic mesophilic bacteria, coliforms, aerobic endospores, lactic acid bacteria (LAB) and the total number of molds and yeasts, 10 g of each honey sample was homogenized in 90 ml of distilled water with peptone (Torlak, Belgrade, Serbia). Decimal dilutions were made in the same dilute. Isolation of aerobic mesophilic bacteria was performed using Standard Plate Count (SPC) at 30°C incubation for 48 h. To determine the presence of clostridium, aliquots of 10, 5, 1 and 0.1 ml of the initial suspension were added to an empty tube, heattreated at 80°C for 5 minutes and covered with sulfitepolymyxin-sulfadiazine (SPS, HiMedia, Mumbai, India). agar, the tubes were incubated at 37°C for 5 days. Analysis of total coliforms bacteria was carried out on violet red were glucose agar (VRBG, HiMedia, Mumbai, India) incubated at 35°C for 24-48 h. Isolation of endospore aerobic bacteria was performed by diluting the suspension of the honey sample first by heating in boiling water for 2 minutes to remove all vegetative forms, and then the samples prepared were poured onto nutrient agar (HA, Torlak, Belgrade, Serbia) and then incubated at 30°C for 48 h. LAB isolation conducted on De Man Rogosa Sharpe agar (MRS, Torlak, Belgrade, Serbia) at 30°C for 48 h. The presence of molds and yeasts was determined using Sabouraud maltose agar (SMA, Torlak, Belgrade, Serbia), incubation was carried out at 25°C for 5-7 days. After isolation pure isolates were subjected to Gram staining, preliminary determination was carried out using standard morphological and bohemian methods according to Naseer et al., (2015) (13). The isolate identification was performed using Matrix-assisted laser desorption / ionization time-of-flight mass spectrometry (MALDI-TOF MS). Samples were prepared according to the manufacturer's instructions.

After completion of incubation, a small number of individual colonies of tested bacteria was applied with a sterile stick directly to a steel plate in 96 spots in the form of a thin film. The film-coated plate was allowed to dry at room temperature for about 1 min, and then 1 µl of VITEK MS-CHCA matrix, (BioMérieux), was applied to the plate. After adjustment and calibration of the apparatus according to the manufacturer's instructions, bacterial isolates prepared in this way were then subjected to MALDI TOF mass spectrometry. Escherichia coli strain ATCC 8739 was used to calibrate the apparatus. During the mass spectrometry process itself, under a laser effect on a thin film of bacteria and matrix, proteins are ionized and separated in an electric field, and then directed to a vacuum tube and separated according to mass and charge. In this way, the proteins arrive at the detector in sequences that are inversely proportional to their mass, creating a protein profile (mass spectral fingerprinting). The major peaks belong to ribosomal and other predominantly represented proteins, such as HSP (heat shock proteins), DNA binding proteins, and RNA chaperones. Then, the obtained profile is analyzed and compared with the database, which enables precise identification of microorganisms. The VITEK MS V2.0 Knowledge Base Industry Use database was used to read the results.

Mass spectra ranging from 2000 to 20,000 Da were obtained in a linear, extraction mode, with positive polarity. The resulting Spectrum was introduced into Biotyper software (BrukerDaltonics, Bremen, Germany), which is equipped with a nitrogen laser (337 nm) using Flexcontrol software ver. 3.1 (Bruker Daltonics). MALDI-TOF identification was performed using the corresponding values to match manufacturer-suggested results ≥ 2.00 (14).

Antimicrobial activity of honey samples

Five ATCC strains of bacteria were used in the study: Staphilococcus aureus ATCC 29213; E coli ATCC 25922; Salmonella enterica ATCC 10708; Yersinia enterocolitica ATCC 23715; and Bacillus subtilis ATCC 23857. The antimicrobial activity of organic sunflower honey was determined by a modified method of Matzen et al. (2018) (15). The preparation of the samples involved dissolving the honey in sterile distilled water under aseptic conditions to a final concentration of 80, 60, 40 and 20% (w / v). Positive (first) and negative control (second) were control discs (6 mm) impregnated with 1 µg penicillin (Jugocillin, Galenika, Belgrade, Serbia) and discs impregnated with sterilized distilled water. The incubation was carried out at 37°C for 24 h. Antimicrobial activity was detected based on the appearance of a light zone around the discs as a consequence of growth inhibition of the susceptible strain and was defined as a growth inhibition zone.

Statistical analysis

The obtained results were expressed as mean values of triplicate measurements ± standard deviation (SD). Analysis of variance (ANOVA) was performed to determine the existence of statistically significant differences in physicochemical and microbiological parameters between honeys. The ANOVA was followed by Tukey post-hoc test for multiple comparisons ($p<0.05$). Data were analyzed with SPSS 26.0 (SPSS, Inc., Chicago, IL) software.

Results and Discussion

Table 1 contains the results of the physicochemical analysis of the organic samples of sunflower honey. As expected, ANOVA showed that there were no statistically significant differences among honeys in physicochemical and microbiological parameters (p value >0.05) (table 1 and 2). In the honey samples, the moisture content (M %) ranged from 16.59 to 16.62% with an average value of 16.6 ± 0.0%, which is in accordance with the legal maximum limit of 20% for the moisture content in honey.Results show a very small difference in the water content of these samples, which may be due to the identical geographic area dominated by identical micro-climatic conditions as well as the very similar movement patterns of bees and the hives they use. Makarewicz et al. (2017) (16) in their work represent a slightly

higher percentage of water for sunflower honey, which had a value of 17.3 ± 0.2, noting that according to Council Directive (2001) (17) such values mean that the water content should not exceed 20 per cent. Investigating the physical and chemical properties of Romanian sunflower honey Oroian et al. (2017) (18) produce remarkably similar results when it comes to the percentage of water, which ranged from 15.80-19.60, pointing out that the moisture content of honey is very important information that can alert the manufacturer to how to keep and store the product. During storage, high moisture content can lead to fermentation caused by the action of osmotolerant yeast, it can also accelerate crystallization in some types of honey (19). Researchers looking at organic honey have noted that factors such as harvest season, maturity in the hive and other environmental factors affect the water content of the product (19 22). Knowledge of the water content of honey is important for improving its conservation and storage as well as preventing mold growth on its surface. Another important parameter that defines the quality of honey is certainly hydroxymethylfurfural (HMF). The HMF content for the analyzed sunflower honey ranged from 8.18 to 8.25 These results, with samples 1, 3 and 4 having almost identical HMF values. are very close to the minimum on the Gothe scale of 8 while the maximum HMF content is 40 mg / kg on the same scale. The results obtained are very similar to those presented by the authors of Kádár et al. (2010) (23) for sunflower honey from Spain, while HMF results for honey from the geographical area of the Czech Republic and Romania were slightly higher than 20 mg / kg. Vranic et al. (2017) (24) warn of the fact that high HMF content may indicate counterfeiting of honey by the addition of inverted sugar syrup, as a consequence of heating the sugar in the presence of acid to sucrose inversion. Diastase activity is another parameter that defines the quality of honey, the obtained results of analyzed honey samples had values for diastase activity from 10.8 (in samples 1 i 2) to 10.82 (in sample 4), which is within the required standard for this parameter. Data on diastase activity suggest amylase enzyme activity, as an indicator of honey freshness, indicates errors caused by heat and poor storage of honey, so it has nothing to do with the floral type of honey (25). Free acidity is inked with the natural presence of organic acids in honey, which remain in equilibrium with internal esters, lactones and some inorganic ions such as: phosphates, sulfates and chlorides (26). Free acidity in sunflower honey samples varied from 23.9 (for the sample 4) to 24.2 (for samples 1 i 3), statistically with no significant difference among analyzed samples. The slightly lower results for free acidity in sunflower honey samples were presented by the authors of Oroian et al. (2017) (18) moving in the range of 6.30-16.80. Such significant variations in free acidity results can be attributed to the different sampling season (26). Variations in the chemical composition of sunflower honey are not infrequent, so the author Dinkov (2014) (27), exploring honey from the Balkan Mountains in Bulgaria, notes that variations in the chemical composition of sunflower honey are the most common, be it changes in water content (33.33%). total acidity (6.67%), reducing sugars, sucrose or diastase activity. The sugar content of honey is defined through the content of glucose and fructose, i.e., sucrose. The analysis of sunflower honey showed results that ranged from 61 to 61.3, with a mean value of 61.15 ± 0.12 for glucose and fructose. The sucrose value was less than 0.5% (mean value

Table 1. Physicochemical parameters of honey samples.

Parameters	Honey 1	Honey 2	Honey 3	Honey 4	*p* value
Moisture (% w/w)	16.6±0.0	16.59±0.0	16.62±0.3	16.59±0.0	1.00
HMF (after White),	8.2±0.01	8.18±0.0	8.25±0.0	8.22±0.1	0.418
Diastase activity	10.8±0.2	10.8±0.1	10.82±0.0	10.81±0.1	1.0
Free acidity (meq/kg)	24.2±0.2	24.0±0.1	24.2±0.2	23.9±0.8	0.778
Glucose and fructose content (%)	61.2±1.6	61.1±2.2	61±1.7	61.3±0.4	0.996
Sucrose (%)	0.48±0.0	0.49±0.0	0.49±0.01	0.48±0.0	0.052
Ash (%)	0.01 ± 0.0	0.01 ± 0.0	0.01 ± 0.0	0.01 ± 0.0	1.00

Mean ± SD (minimum–maximum).

0.485 ± 0.0), which is in accordance with the Rules on the quality of honey and other bee products (Official Gazette of the RS No.101 / 15). The amount of sucrose in honey is usually directly related to the percentage of pollen in sunflower. Ash is one of the quality parameters related to the botanical and geographical origin of honey samples. The determination of ash represents the residual mineral in honey after incineration. As the samples of the investigated honey were taken from the same geographical area, it is not surprising that the result obtained for them had a value of 0.01% for all samples. Pita-Calvo and Va´zquez (2017) (28) point out that the ash content is a quality criterion for botanical and geographical origin of honey. Ash content of honey is usually small and depends on the nectar composition of the dominant plants in the honey composition. Ash content of honey is generally small and depends on the nectar composition of the dominant plants in the honey composition.

Microbiological analysis

The microbiota found in honey may originate from bees, pollen, or may be due to poor hygiene conditions when handling the product. The honey samples tested showed very similar microbial contamination (no statistically significant differences) with only the total number of bacteria moving in range for all honey samples from 1.80-1.85 x10-2cfu/g-1 (Table 2). Microbiological analysis of sunflower honey did not detect the presence of Clostridium spp, coliform bacteria, or mold. The microbiota that was isolated from all the samples tested consisted of: Bacillus spp. ranging from 0.49 -0.52 x102cfu/g-1, Saccharomyces spp., were detected only in the first two specimens, while in strain 4 two strains of Exiguobacterium spp. which have successfully grown on HA. MALDI TOF were confirmed in preliminary identification. B. pumilus, Exiguobacteriumacetylicum, while yeasts were identified as Saccharomyces cerevisiae.

Pajor et al. (2018) (29) found that 80.4% of isolated strains from honey samples belonged to the genus Bacillus. Exiguobacteriumacetylicum-bacteria make up the gut microbiota of bees (Apis mellifera) (Khan et al., 2017) (30). Frazie and Westhoff (1994) (31) note that the yeast in honey comes primarily from nectar and from the intestinal content of the honeybees. A study of 200 Swiss honey samples revealed the presence of S. cerevisiae whereby the cells of the detected yeast could not reproduce in a high sugar environment (32). Popa et al., (2009) (33) note that in all 10 Transylvania honey samples, the dominant microbiota is mold, while the yeasts are not isolated.

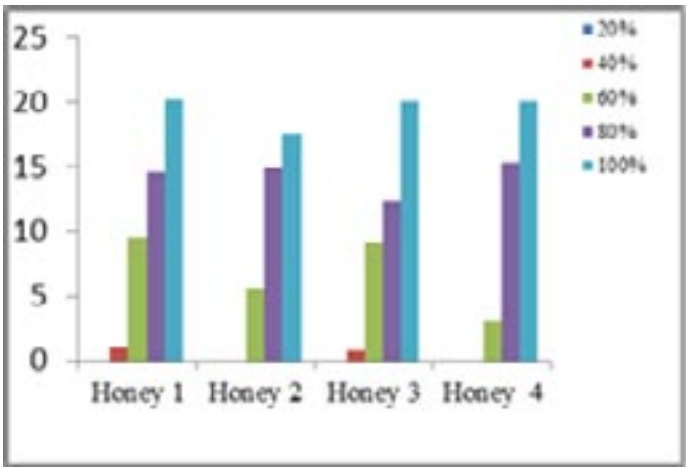
Antimicrobial activity

The antimicrobial activity of honey has long been known, and many researchers have studied and confirmed this activity of honey against a wide variety of human pathogens and bacteria of food spoilage. In the work of Wen et al. (2017) (34) point out that the animicrobic activity of honey comes from its osmotic properties, its high sugar content, its low pH, the presence of H2O2, phenolic compounds and the cationic antimicrobial peptide. Results of research on antimicrobial activity of sunflower honey samples according to St.aureus ATCC 29213, showed inhibition at concentrations of 60-100% in all tested samples, while in the sample of honey 3 and 1 at a concentration of 40%, the sizes of the inhibition zone were negligible according to the bacterium under study and ranged from 0.8-1 mm (Figure 1). However, in the case of E. coli ATCC 25922, the

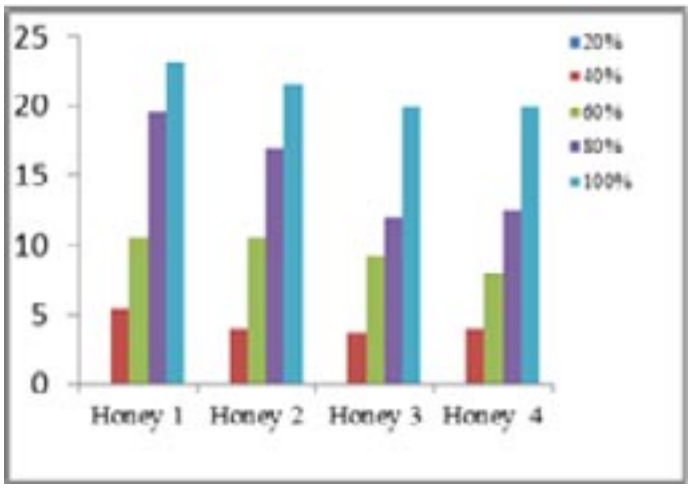
Table 2. Microbiological quality of fresh and stored honey [cfu/g^{-1}].

Total plate count	Honey 1	Honey 2	Honey 3	Honey 4	*p* value
Total bacterial count	$1.82x10^{-2}$±0.01	$1.85x10^{-2}$±0.01	$1.80x10^{-2}$±0.02	$1.85\ x10^{-2}$±0.04	0.154
Total coliforms count	-	-	-	-	-
Bacillus spp.	$0.51x10^{-2}$±0.03	$0.50x10^{-2}$±0.02	$0.49x10^{-2}$±0.00	$0.52\ x10^{-2}$±0.01	0.453
*Clostridium*spp	-	-	-	-	-
Lactic acid bacteria	-	-	-	-	-
The total fungi and yeasts	$0.28x10^{-2}$±0.01	$0.30x10^{-2}$±0.03	-	-	-

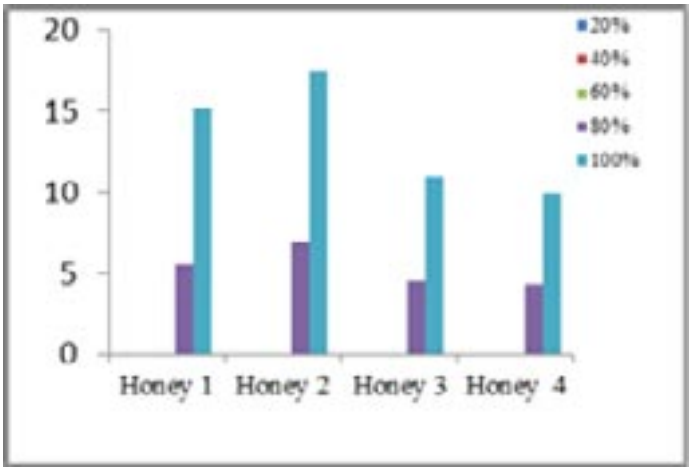
Mean ± SD (minimum–maximum).



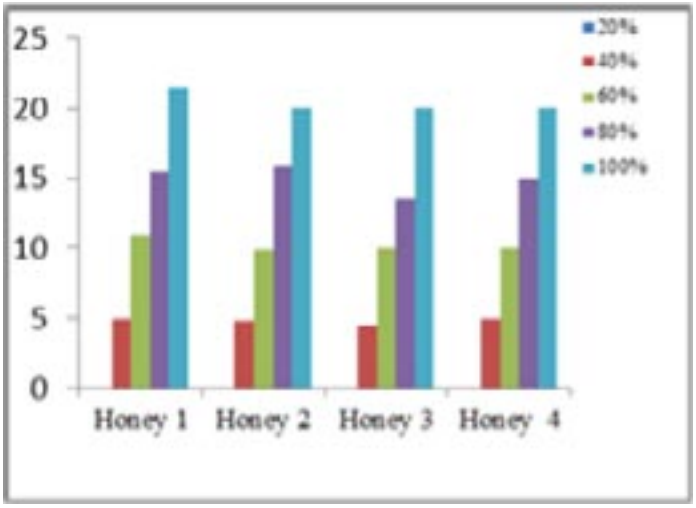
St.aureus ATCC 29213



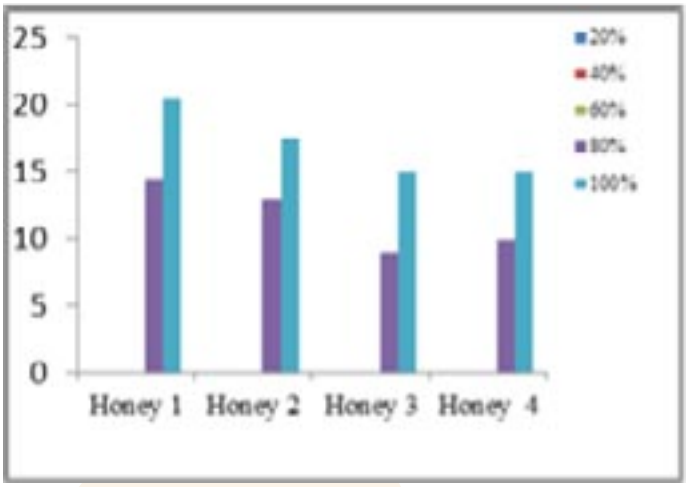
Y. enterocolitica ATCC 23715



E. coli ATCC 25922



B. subtilis ATCC 23857



Sl. enterica ATCC 10708

Figure1. The antimicrobial activity of organic sunflower honey against pathogenic bacteria (honey concentration: 20% blue stripes; 40% red stripes; 60% green stripes; 80% purple stripes; 100% light blue stripes)

honey samples tested showed no inhibition at concentrations of 20-60%, while concentrations of 80-100% showed significant antibacterial activity, with sample 2 having the highest inhibition zone of 17.5 mm, which is consistent with the results of Junie et al. (2016) (35) who showed that thetested E. coli strains were sensitive to seven honey samples whose zones of inhibition ranged from 13-14 mm. Makarewicz et al., (2017) (36) found that sunflower honey at concentrations of 75% showed remarkable antimicrobial activity against E. coli, B. subtilis, Micrococcus luteus and Proteusmyxofaciens wherein the inhibition zones range between 10,7 and 34,3 mm. The results of the animicrobic activity to Sl. enterica ATCC 10708, are very similar to the results obtained for E. coli.

The best results to Sl. enterica showed a sample of honey 1 at a concentration of 80-100%, while other samples at the same concentration showed slightly lower antimicrobial activity. Y.enterocolitica ATCC 23715 was inhibited by all tested honey samples at concentrations of 40-100%, with honey sample 1 showing the highest

inhibition zones ranging from 5.4-23.2 mm. The antimicrobial activities of the studied honey samples according to B. subtilis ATCC 23857, ranged in concentrations of 40-100%, sample 1 showed the highest inhibition zones of 5-21.5 mm. Investigation of the antimicrobial activity of organic sunflower honey samples revealed that all bacteria showed clear zones of inhibition in response to all honey samples, satisfactory results for flower honey and similar results from other authors. Junie et al. (2016) (35) in the study of antimicrobial activity of seven honey samples from the territory of Romania results in the inhibition of all honey samples for bacterial growth, where sunflower honey samples gave zones of 14-18.5 mm in diameter. Likewise, St aureus, Streptococcus pyogenes, and Serratia marcessens significantly inhibited sunflower honey at 100 per cent concentration (37). Olakunle et al. (2013) (15) while investigating antimicrobial activity of pure honey against isolated wounds, came to the results that showed all isolated microorganism shad a considerable stronger inhibition zones.

Conclusion

The quality control results of organic sunflower honey samples represent a good ratio between physicochemical and microbiological parameters and can meet international quality standards. An important aspect of the research is certainly also the antimicrobial activity, which has been proven in the research with the inhibition of pathogens and ranged at concentrations of sunflower honey samples of 40-100%. Given that honey is nowadays also used as an additive in some food products, special attention is also given to its inhibition of food spoilers. The research opens a new field of complete determination of organic honey that is gaining in importance as a functional food.

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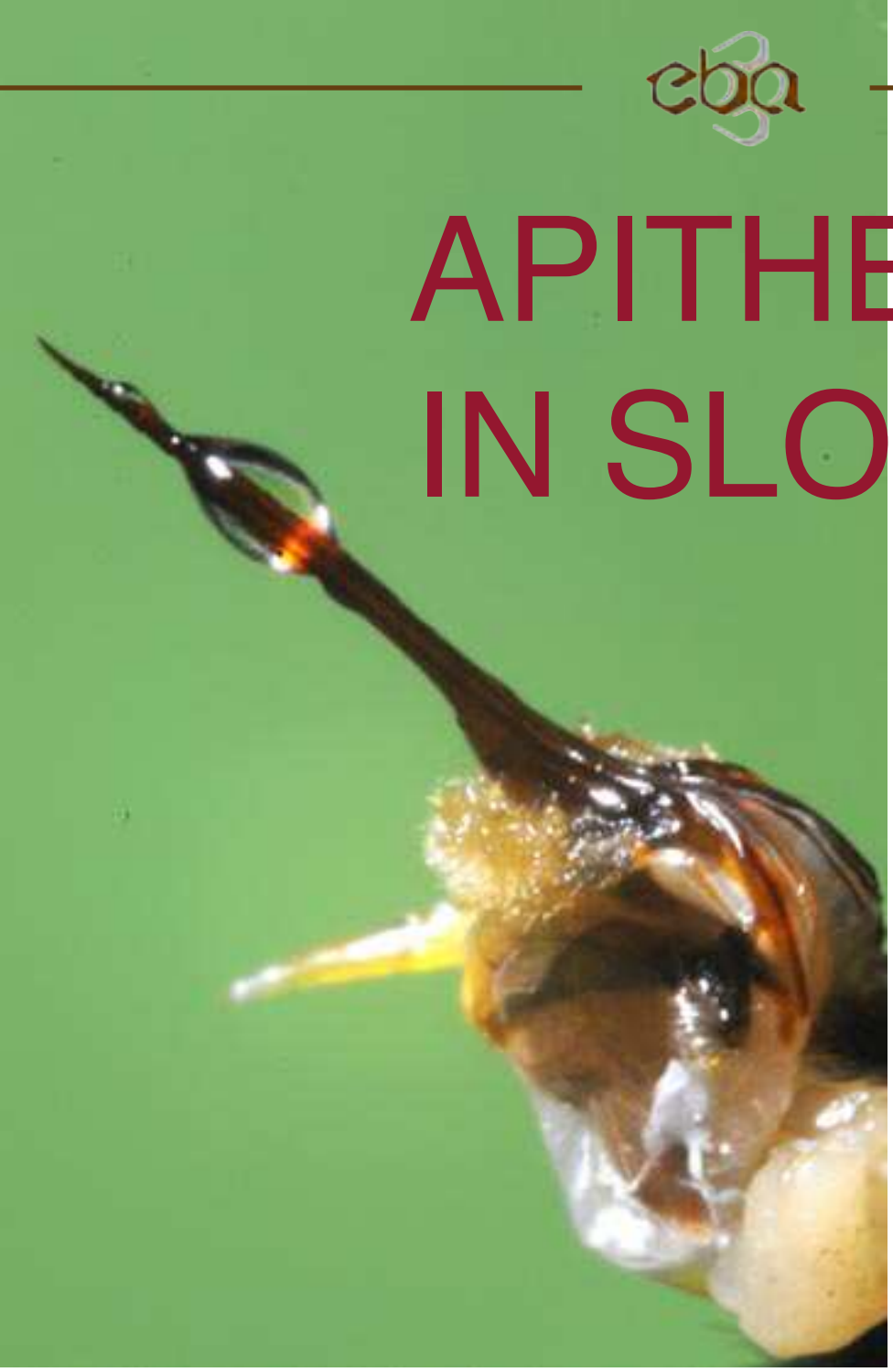
APITHERAPY IN SLOVENIA



The pioneer of treatment rheumatic diseases with bees in today's Slovenia was Dr. Filip Terč (1844 – 1917), otherwise of Czech origin, who after studying medicine in Vienna began his professional career as a surgical clinical assistant in the Vienna General Hospital, In 1875 he moved with his family to Maribor, where he devoted all his attention general medical practice and beekeeping. Something has already been written about him in our magazine, but it is right to introduce him shortly once again to our readers in this article.

Two years after arriving to the capital of Styria, he was elected president of the Maribor branch of the Styrian Beekeeping Society, and as a beekeeping expert he helped with practical beekeeping lessons at the Maribor school. As he was a rheumatologist, he soon realized when working with bees that bee stings, which he could not completely avoid despite protection, eased his rheumatic pains. This experience led him to the idea that he might be able to treat other patients with bee venom. In 1879, he began to realize his idea. By 1912, he treated a total of 660 rheumatism patients, 82 percent of whom were cured with the help of bee venom. He presented the results of his research in 1888 in the 35th issue of the medical journal Wiener medizinische Presse, but his article was met with many concerns and even ridicule from official medicine. After that, Dr. Filip Terč no longer published his articles in the medical literature. His work in the field of apitherapy remained unknown even to the wider beekeeping public for a long time. Today, medicine recognizes him as the originator of modern apitherapy, and in 2006, the world beekeeping organization Apimondia declared his birthday, March 30, the International Day of Apitherapy. His professional efforts finally received a fitting epilogue, although during his lifetime, at least within medical circles, he did not receive this confirmation.

His work is now being continued by the Apitherapy Section "dr. Filip Terča", led by apitherapist Karl Vogrinčič and operating within the framework of the Beekeeping Association of Styria.

Every year a course for new apitherapists is organized, first the theoretical part and then the practical part, which lasts a total of 181 hours. An integral part of the training are two professional excursions, usually to the clinic of an apitherapist and a three-day professional excursion to countries where the trainees get to know the work in the field of beekeeping and apitherapy, for example to Hungary, Romania, the Czech Republic, Serbia, Bulgaria... The conclusion is the creation of a seminar thesis from published topics of around 15 pages and the defense of the thesis before the committee. At the end of the training, the participant receives a certificate from the Slovenian Beekeeping Federation and a certificate from the provider of the training program. The training has been taking place since 2014 and so far more than 350 people from Slovenia and abroad have taken part in it. Upon receiving the certificate with the list of competencies, the apitherapist signs the code of ethics, with which he assumes the responsibility to observe all ethical and moral principles in his work with the client in accordance with the code. And one last piece of news: The Beekeeping Association of Styria, which celebrated 100 years of operation last year, has acquired a 10,500 square meter land near Maribor, where it plans to build a large apitherapy center in the near future.

In the continuation of my paper, I would like to describe two important studies that were carried out in 2010 with different types of Slovenian honey in order to determine which type of honey would be the most suitable for treating wounds and which for the treatment of periodontal disease. Here, I must emphasize that various aerobic bacteria are present in wounds, while anaerobic bacteria are present in plaque. The in vitro research was carried out by the Institute of Microbiology and Immunology of the Faculty of Medicine of the University of Ljubljana. It was shown that chestnut honey was the most bactericidal for bacteria in wounds, and rape honey for bacteria in plaque. Now it was necessary to carry out clinical research, which was taken over by the sanitary material company Tosama, Domžale. It turned out that Slovenian chestnut honey heal wounds also in diabetic patients, where antibiotics have failed. After disinfection with gamma rays, it became the first Slovenian medical honey

called Vivamel, which is sold in tubes of 20 and 50 grams for small wounds, but for larger wounds and burns in the form of alginate dressings under the trade name Vivamel ALGINAT.

And what was the fate of the future medical rape honey, which was supposed to treat periodontal disease? The company Tosama Domžale decided not to register this honey because it would have to pay the relatively high costs of clinical research. Therefore, rape honey is still waiting for a suitable interested party, who might find in it an interesting solution for the preventive treatment of dental plaque, before it develops into periodontal disease.

Finally, I would like to present to the readers my research on how the consumption of fresh pollen affects the prostate. It is known that the most common diseases of this part of the urogenital system are benign enlarged prostate (BEP) and prostate cancer (PC). I got the idea for the research after receiving a book in French called Ces pollens qui nous soignent by the author and professional beekeeper Patrice Percie du Sert as a gift almost 30 years ago. Four years later, this book was also published in an English translation under the title The Healing Powers of Pollen. When I read in it the chapter on the beneficial effect of pollen on the prostate, I decided to eat about 15 grams of this bee product regularly every morning (a spoonful of soup). Already in the evening, I prepare a fruit salad in a glass bowl, the basis of which is the content of one or

two persimmons, to which I add various seasonal fruits, and if I don't have it at hand, I use fruit from the freezer. Overnight, the pollen grains swell in the liquid, the skin softens and the intestinal juices can more easily use their rich contents.

In our large orchard, which is located in Goriško, near the Italian border, thanks to the Mediterranean climate, a wide selection of fruit species thrives - in addition to apples, pears, peaches, cherries, plums, walnuts, hazelnuts, raspberries, strawberries, figs even lemons . When growing fruit and vegetables, I follow the golden rule of the ancient physician Hippocrates, who says: Let your food be your medicine and your medicine your food. In the orchard and vegetable garden, I use only natural fertilizers and natural preparations to protect plants from diseases and pests.

After a few years of regularly consuming pollen, I decided to see a urologist. He first reprimanded me that I should come for a check-up right after the age of fifty, but when he saw the results of the blood and urine analysis, he asked me in amazement if I was taking any pills to alleviate the symptoms of an enlarged prostate. The value of my PSA (abbreviation for prostate specific antigen) was 0.7 nanograms per milliliter of blood, i.e. extremely low. A healthy man's value ranges between 0 and 4 nanograms. I told him that for several years I have been regularly taking a tablespoon of fresh pollen (about 15 grams) every morning with my fruit breakfast and that I consider myself to have a low PSA value probably to thank this natural remedy. He didn't quite believe me, but he strictly ordered me to come to him every year for a check-up and he also gave me the exact date and time. I now follow this order regularly. I should mention that for a while my wife also used pollen in her fruit salad for breakfast, but when the hair on her chin and under her nose started to grow, she reduced the mentioned daily dose of pollen in half and now she had no problems.

Ten years ago, I made an agreement with 20 of my friends, including two well-known doctors from Ljubljana, that we would consume one large soup spoon (about 15 grams) of fresh pollen as possible every day. We also agreed that we will regularly check the amount of PSA in the blood and copies of the laboratory results will be sent to me to keep. The experiment is still ongoing, but we already have some interesting results. In January of this year, my PSA value was 1.4, which, despite a slight increase, is still an excellent result. In all my friends, the value of this tumor marker is also falling.

The described cases prove that pollen consumption is beneficial for men over 50 who have a benign enlarged prostate. Not only because of the reduction in PSA values, but also because of a better quality of life, which is reported by all trial participants.

Franc Šivic, Slovenia

ZZZAGIMED 2024
SAMPLES CODING

Twentieth international honey evaluation Zzzagimed 2024 is well under way. This year interest has been high and Zagreb Beekeeping society has received 183 samples from all over Croatia, and neighbouring countries of Bosnia and Herzegovina and North Macedonia. The samples were sent by some participants that were part of previous Zzzagimed evaluation but there were also a good amount of new participants in this competition. On 7th. October the society conducted coding of the samples, which was held at the premises of the society. The coding was conducted by members of the Zagreb Beekeeping society and it was overseen by vice president of Society, Damir Salopek.

Coding is done in three stages that happen simultaneously as on a conveyor belt:

1. The first stage is unboxing the samples and cleaning them up if necessary, this task can be more complicated than one would think. The samples often travel quite far and competitors sometimes take ample precautions to ensure the samples don't break, so taking them out of the wrapping can take time. Despite the measure some samples do leak and unboxing can be quite the sticky business. Some samples did spill or were broken but it was all salvageable and they will all be evaluated.

2. The second stage is writing down information of each sample into the online database and assigning the number to the sample. Competitors send their names or their business names, they provide their contact info and it is also noted what type of honey they have sent (even though this can be changed at a later stage of evaluation if the test indicates that it is necessary). Few samples were not clearly declared so experienced beekeepers did their best to try and put the sample into the correct category by gathering all the provided info that can give them a clue, look-

ing at the colour and the texture of the sample and tasting it.

3. The last stage is pouring the honey from the container it was sent in to a standardised container which only has the assigned number on it, so the sample is now anonymous and only information available to the testers is declared type of honey. After the necessary amount of honey is poured into the standardised container some honey is left over and put to the side for tasting events that the society organises during the fall and winter seasons.

At these tasting events visitors can try honey and then buy the honey that they like directly from the beekeepers.

The research has shown that in the market chains and local shops quality of honey can be varied, and customers cannot be sure if they are buying adulterated honey or high quality honey.

So in this way, by connecting beekeepers directly with the customers, sale of verified high quality honey is ensured.

The coding process was done at a leisurely pace by ten members, and it took about 4 hours.

Members helping were a mix of older and newer members. It was a good opportunity for members to mingle, exchange knowledge and observe different practices of beekeepers from this region of Europe.

As per societies tradition there was also some honey rakija, other drinks and čevapčići served to the coders which made the process even merrier. Alongside the work that was being done members participating at the coding got to see and taste different types of honey, how the beekeepers pack it and what kind of labels they put on the product.

The most common types of honey sent this year were Acacia, Meadow, Floral and Chestnut honey. Amorphous honey was a bit rarer but the most uncommon were Wild Cherry of which there were only three samples, Mint which there was only one sample and Ivy which there was only one sample.

Some of these unique samples surprised even the more seasoned beekeepers present, they were unique not only in their taste but also in their texture. For instance the Ivy honey had an interesting almost dough like texture, and a unique taste.

All in all it is looking like it is going to be another excellent year for Zzzagimed international honey competition. This shows us that the beekeeping tradition is flourishing in the region.

The samples so far did surprise us in its variety, make us proud in their quality and made us excited to see what the results of evaluation will be.

Nina Rac
Member of Beekeeper Society Zagreb

A new book

EKRAMS

Reading and creating with children are powerful tools for strengthening family ties and for professional pedagogical work in promoting reading literacy and reading habits.

Spending time with books improves a child's mental, emotional, and cognitive development.

Regular reading teaches children about the world around them. Exploring stories together stimulates the child's imagination, develops his vocabulary, and stimulates the ability to concentrate.

Reading helps the child understand complex concepts, encourages critical thinking, and develops empathy as they empathize with characters and their experiences.

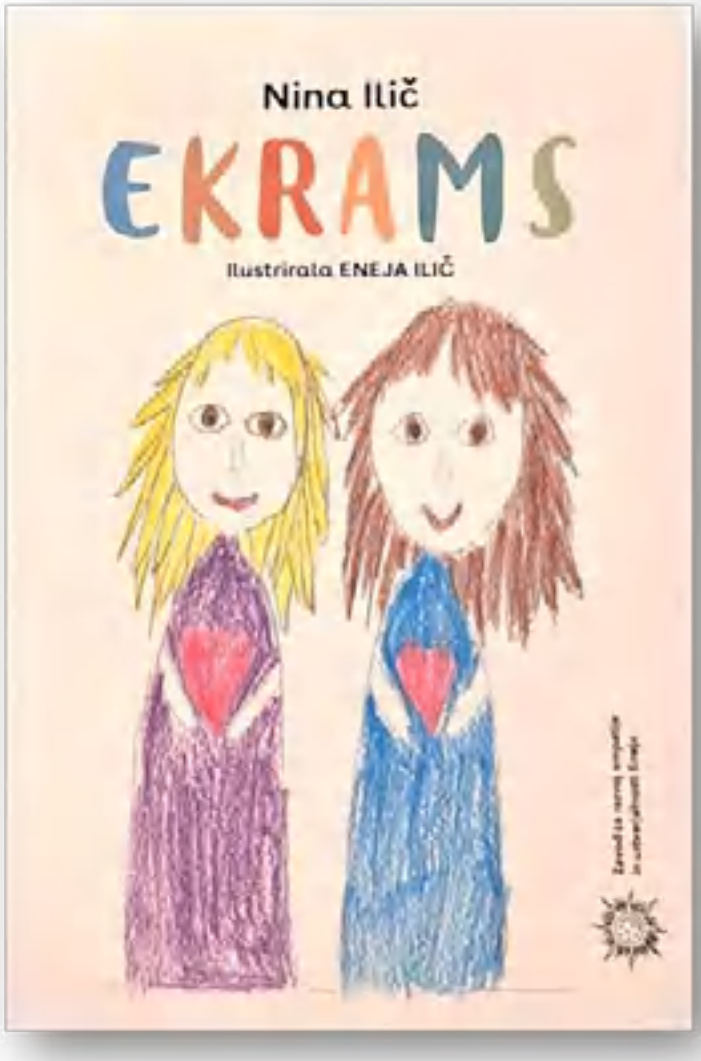
Through Ekrams story, children learn about the traditional Slovenian intangible cultural heritage of apitherapy and a new and safe recipe to help with bruises.

The name EKRAMS benefits developmentally by stimulating the child's language abilities and encouraging articulation, and the story throughout the text builds a richer vocabulary.

Joint creation stimulates the child's sense of aesthetics and utility, develops fine motor skills, and encourages creativity. Reading and creating together also encourages cooperation, communication, and problem-solving, as the child learns to express his ideas and participate in joint projects.

Therefore, the inclusion of the Ekrams fairy tale in family life and pedagogical work is an added quality of life. With an Ekrams story, we can strengthen the bond between parent and child, or child and educator while helping the child develop the key skills he needs to successfully navigate life.

All of the above and the numerous research and play opportunities embodied in the Ekrams fairy tale are why the Ekrams fairy tale is the main theme and annual program of the Apipedagogical Programs in the 2024/2025 school year.



Teacher's opinion in the book:

The fairy-tale world enriches our knowledge, in the fairy-tale world we can find recipes for our actions, fairy tales have magical powers.

Children will be able to identify with the heroine of a fairy tale, as they will believe the written story because it is true. After reading the fairy tale, we can also talk about the children's experiences and their unfortunate falls and events. We can encourage children to narrate stories, to learn new expressions, and to find solutions. By reading the Ekrams fairy tale, children will learn about the sustainable importance of beekeeping.

It is very important to protect our environment, and our nature and not focus on consumerism. We can prepare Ekrams ointment ourselves and behave sustainably. The fairy tale has healing tips, the fairy tale offers us the opportunity to explore nature and prepare Ekrams ointment. It will enrich and offer children new knowledge about how we can help each other and how we react to certain types of injuries.

The fairy tale also introduces children to the emotional reactions of the girl Eneya and her mother. With the help of various pictorial materials, children can learn about emotions, and emotional reactions and search for solutions. The content of the fairy tale can be connected to the curricular areas of work in the kindergarten, some project content, and the implementation of various activities in the natural environment. In the fairy tale, children encounter different values: friendship, health, well-being, knowledge, and family.

The fairy tale contains added value, as it was illustrated by the very main fairy tale heroine Eneya.

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Dear Exhibitors, providers of beekeeping equipment and products

You are kindly invited to become a part of the largest European beekeeping event, which promises great innovation as it expands its mission. The fair has a more than 45-year-long tradition and takes place at the Celje Showground. In cooperation with the Beekeeping Association of Slovenia, Celjski sejem has made it a part of the varied events of the March Fairs, which attract nearly 20,000 visitors every year.

The next fair will take place between 15 and 16 March 2025. This time we have set ourselves an even more ambitious goal - we are expanding the fair to the European level with European exhibitors, and have therefore renamed it APIS SlovenijaEurope. This is a groundbreaking transformation, which will help us attract even more exhibitors and visitors from all over Europe and create the largest European event in the field of beekeeping. The excellent feedback from the previous Apislovenia fairs is an excellent starting point for this new venture.

We are proud to point out that Slovenia is the home of the head office of the European Beekeeping Association, chaired by Boštjan Noč, president of the Beekeeping Association of Slovenia. This connection strengthens Slovenia's role as a globally recognized beekeeping country.

Together with you, we want to create a strong APIS SlovenijaEurope brand, so that it becomes an indispensable event for everyone who is part of the beekeeping industry. We are aware of how important it is to spread the mission that you as beekeepers carry out, which is why we are upgrading the fair with utmost pride.

President of the Beekeeping Association of Slovenia and the European Beekeeping Association, Boštjan Noč:

"On behalf of the Beekeeping Association of Slovenia and the European Beekeeping Association, I invite you to APIS SlovenijaEurope. The Slovenian central beekeeping event is being upgraded to the central professional event of the European Beekeeping Association. Saturday will be devoted to the presentation of the most current topics in beekeeping in the field of quality and safety of bee products and bee

health care. The lecturers will be from the scientific committees of the EBA (https://ebaeurope.eu/sc-on-safety-and-quality-of-bee-products/, https://ebaeurope.eu/sc-on-bee-health/). The lectures will be in English and simultaneously interpreted into Slovenian. We want the Celje event to be not merely a lecture, but also to present actual solutions for better and more successful beekeeping in Europe.

In addition to the professional part, we will also upgrade the expo exhibition with exhibitors from Europe. Let APIS SlovenijaIEurope be a day for beekeepers from all over Europe to get together, because EBA brings together everyone who lives for and with bees!"

Nina Ermenc Pangerl, president of the Management Board of Celjski sejem, emphasizes that connecting on a global scale is key:

"At Celjski sejem, we are most proud when we can introduce new content into our offer of fair events. And your content has a powerful mission. Beekeeping in Slovenia is not only a tradition, but an essential part of our cultural and natural heritage. We are a nation that understands the importance of bees, especially in terms of our future. With the APIS SlovenijaIEurope fair, we are taking a step forward – a step towards connecting and spreading the impact of this industry, not only in Slovenia, but throughout Europe. Our vision is to, together with you, put beekeeping at the forefront of global efforts for sustainability and nature conservation. With this event, we are not only keeping in step with the changes, but co-creating them. I am convinced that with your support, we will create a fair that will continue to strengthen the visibility and importance of beekeeping, not only on a European, but on a global level."

Opportunities to exchange knowledge, make new contacts and present innovations are invaluable, so we are thrilled to invite you to participate in this great trade fair event. Registrations are already open, so we encourage you to register as an exhibitor as soon as possible and become part of the inspiring story of beekeeping!

REGISTER NOW!
Link to Exhibitor portal:
Exhibitor portal
Kindest regards,
Celjski sejem Team

Celjski sejem

BEES LIFE

Ulster Beekeepers Association

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