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V DRUŽBI DOBRIH LJUDI

European Beekeeping Association

CHOOSE

LOCAL BEE PRODUCTS, BEE PRODUCTS FROM YOUR OWN COUNTRY OR, AT THE VERY LEAST, BEE PRODUCTS OF EUROPEAN ORIGIN!

European Beekeeping Association: "Choose local bee products, bee products from your own country or, at the very least, bee products of European origin!"

Despite the hot summer, the leadership of the European Beekeeping Association (EBA) is not resting. The second issue of the EBA magazine is finally in front of you and we are already working on upcoming publications. All EBA members and beekeeping professionals are kindly asked to submit their contributions on a regular basis, so that together we can disseminate information about the work of beekeeping organisations across Europe and the work of professional and scientific services in the field of beekeeping. I would like to thank the editor of the EBA magazine, Rodoljub Živadinović, for his highly professional work and at the same time ask you to send your contributions to his e-mail address at apikult@gmail.com.



EBA's real work will not begin until the autumn. The Association was founded for the sole purpose of helping bees, beekeepers and consumers. It is therefore not essential for me or for the EBA management to obtain various European projects (read funding) for our work that would fund our organisation as well as other institutions and experts. We only had one purpose in mind - to achieve the main objectives of the EBA, namely to raise awareness about counterfeit bee products on the market, unfair competition for beekeepers and the misleading (or even deceiving) of consumers. We want beekeepers to receive financial support as payment for the pollination service they provide and we want to provide bees with an environment in which they can survive. Achieving these goals will be the biggest reward, much more valuable than any monetary prize!

We have invited all active beekeeping associations and institutions to participate, but so far, the response has been extremely modest. Unfortunately, we feel some envy and even opposition to the work of the EBA. With that being said, I wonder whether we do not all share the common goal of helping the beekeeping sector? The EBA is not, and does not intend to be, in competition with anyone, so the concern that the EBA will be competing in tenders and thus taking money from others is unnecessary. No, EBA was not established on the basis of financial motivation and interests. We even cancelled the membership fee because we want to prove that our first and only concern is the realisation of our goals.

The EBA is rapidly preparing for the organisation of a conference on bee products, which we will organise in the European Parliament. The Scientific Committee on the Quality and Safety of Bee Products, headed by Dr Urška Ratajc, has been entrusted with the substantive and technical part of the project. On this occasion, I would like to thank all the experts on this committee for their exceptional readiness and active work. I also want the EBA to be present at as many professional beekeeping events across Europe as possible, so that we can highlight key problems and especially SOLUTIONS in modern beekeeping. The first EBA cooperation will already take place in Vranje, Serbia, and in 2025 we will be part of the ApiSlovenia event in Celje. The aim is for the EBA to participate in at least three events each year and to ensure the participation of at least one top expert working in the field of the EBA's main objectives at these events. All event organisers who want to cooperate with EBA can contact us via e-mail at eba@ebaeurope.eu.

In the second half of the year, we will continue to raise public awareness of the benefits of eating local bee products, reinforcing our slogan





"Choose local bee products, bee products from your own country or, at the very least, bee products of European origin!".

Food security in Europe can only be ensured by European farmers. HONEY (most of which is not even real honey) can be imported, but we cannot do the same with the pollination service that bees provide! The presence of bees and other pollinators is therefore essential for the production of food in Europe; beekeeping and bees can be supported most effectively by purchasing local bee products, which have also been proven to best strengthen our health!

The EBA is aware of the need for coexistence and, above all, cooperation between agriculture and beekeeping, as we depend on each other. We will do everything to find as many common paths to a common goal – providing quality food throughout Europe!

I invite everyone to join us, because together we will be stronger! At the same time, I invite you to send proposals for the operation of EBA to the e-mail address eba@ebaeurope.eu.

Boštjan Noč

President of the European Beekeeping Association and President of the Slovenian Beekeepers' Association







The recently amended Honey Directive (Council Directive 2001/110/EC, and Directive 2024/1438) came into force on 13 June 2024. It mandates more accurate labelling of honey origin and includes several features that still need to be implemented in the coming years.

Most importantly, the new Honey Directive predicts an establishment of the Honey Platform, which should include authorities in charge of the national implementation of the Honey Directive, organisations representing relevant stakeholders in the honey supply chain (beekeepers, honey packers/blenders, trade operators, logistics, retailers, consumers, etc.), organisations representing civil society, and experts from the private sector or academia.

The Platform's objectives are laid down in Article 4b of the Directive, which states that the expert group will provide recommendations on methods to improve authenticity control of honey





and on the establishment of a traceability system to trace the honey back to the beekeeper or importer. Additionally, they will advise on the update of composition and other quality parameters, and the establishment of a reference laboratory.

On June 13th, the European Commission opened a call for applications for setting up the Honey Platform. The deadline was July 15th and the EBA Executive Board and Scientific Committee decided to apply as an international organisation representing beekeepers and advocating for consumer rights. We submitted our application on time, and we hope to offer our expertise and support the Honey Platform in achieving its goals.

The Scientific Committee unanimously voted to propose Prof. Dr. Andreas Thrasyvoulou from Greece as our expert representative. Prof. Dr. Thrasyvoulou has extensive knowledge and experience in the field of honey quality.

Depending on the meeting agenda of the group, EBA may designate another member of the Scientific Committee or Executive Board to join the meetings.

Prof. Dr. Thrasyvoulou is Professor Emeritus at AUTH, with a degree in Agriculture from AUTH, and a master's degree and a PhD in Entomology from Penn State University, USA. He was the coordinator of European scientists for legislation concerning beekeeping and the monofloral categories of honey.

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He also served as president of the International Federation of Beekeeping (Apimondia) in organic beekeeping. You can read his commentary on the pitfalls of the modified Honey directive in the previous issue of the EBA Magazine.

As a member in the Honey Platform we would have a vote in crucial decisions regarding the European honey market. The EBA's priority in the Honey Platform will be addressing issues regarding the lack of harmonised methods for authenticity testing, botanical and geographical origin of honey, and testing compliance with other quality parameters. Additionaly, we are convinced that we can significantly contribute to the field of traceability with proposals from EBA beekeepers and researchers. We will advocate for higher order in the honey trade and for ensuring that the Directive distinguishes domestic from imported honey, minimizing loopholes that could allow the introduction of lower quality honey and counterfeit products into the European honey market. Since combating counterfeits is one of the EBA's main objectives, we will speak out strongly for improving these regulations and controls.

> Dr. Urška Ratajc Head of the EBA Scientific Committee

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FIRST MEETING OF THE EBA SCIENTIFIC COMMITTEE

The Scientific Committee's members were confirmed by the Executive Board on the 3rd of July. The EBA Scientific Committee unites esteemed professionals and researchers from across Europe who provide scientific counsel, focusing primarily on the safety and quality of bee products. Currently, it consists of 14 experts from 11 countries with expertise in honey authenticity testing, characterisation of bee products, honey quality and safety, development of new analytical methods, etc.

The EBA Scientific Committee's first meeting was held online on the 10th of July. The Committee discussed the preparation of a survey »European Beekeepers' Insights: Addressing Challenges and Finding Solutions« and the organisation of a conference about the quality and safety of bee products in Brussels. Two working groups were formed to address these tasks. However, the most urgent point on the agenda was the Call for applications for members of the Honey Platform expert group.

Members of the Scientific Committee (in July 2024):

dr. Soraia Falcão, Portugal prof. dr. Hrisula Kiprijanovska, N. Macedonia dr. Nataša Lilek, Slovenia dr. Dražen Lušić, Croatia dr. Juraj Majtan, Slovakia Olha Martynenko, Ukraine dr. Daniel Christian Popovici, Romania dr. Azra Sinanović, Bosnia and Herzegovina Ivan Smajlović, Serbia dr. Roxana Spulber, Romania ass. prof. dr. Lidija Svečnjak, Croatia dr. Alvija Šalaševičienė, Lithuania prof. dr. Andreas Thrasyvoulou, Greece dr. Dmytro Yanovych, Ukraine

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ESTABLISHMENT OF THE SCIENTIFIC COMMITTEE FOR THE PROTECTION OF BEE HEALTH

At the meeting of the EBA Executive Board on July 20, 2024, two important decisions were made:

1. Establishment of the Scientific Committee for the Protection of Bee Health;

2. Preparation and Announcement of Invitations:

The head of the Scientific Committee of the EBA, Dr. Urška Ratajc, will prepare and an-

nounce invitations for the selection of candidates for the Scientific Committee for the Protection of Bee Health. This process should be completed by August 15, 2024, and the candidates should be selected by October 1, 2024.

The invitation to join the EBA Scientific Committee for the Protection of Bee Health will be published in the next issue of our magazine.

> Boštjan Noč President of EBA

EBA MADE A FLAG

EBA made flags. You can buy it for 50 euros + shipping. Orders to ines.zunic@czs.si



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News from the German beekeeping association Deutscher Imkerbund

CONFUSION ABOUT A DECISION OF THE PARLIAMENT

Last month we received several messages also from abroad asking about the implementation of the EU Honey Directive in Germany. Unfortunately, some people sent out very misleading information about a decision by the German parliament. Among other things, it sounded as if there will be no precise labelling of the origin of honey in Germany. However, the misinformation was based on a lack of technical knowledge of both the EU Honey Directive, which had already been published in the Official Journal of the European Union, and the legal procedures. Without going into further detail, we can inform you that Germany will transpose the amendments to the EU Honey Directive into national law in the same way as other Member States. However, we would like to use this annoying matter to call for calm and objective work. It is not about being the first and the loudest. Activism gets us nowhere.

Another issue of concern is the Asian hornet Vespa velutina. Germany is going to abolish the eradication obligation. We are in discussion with the authorities about how to continue to control the species. We have also raised the issue that there are no approved pesticides to destroy the nests. Although there are still a few approved existing active substances, they are very toxic and must not be allowed to remain in the environment. The situation in other Member States is not much better. We therefore urgently need to develop sustainable control measures. This problem will affect all EU countries. We would therefore like to encourage our colleagues in countries that are not yet affected to prepare for this situation as soon as possible. We are happy to use our experience to provide you information on this invasive species, the problems it causes and possible control measures. So, don't hesitate to contact us.

Another threat to beekeeping is the Tropilaelaps mite. This parasitic mite has already been confirmed in the Krasnodar region of Russia. It is suspected that it has also been introduced into the Russian-occupied part of Ukraine. However, this has not yet been confirmed. Contrary to previous assumptions, Tropilaelaps can establish itself in temperate regions with a breeding pause. In Asia, it is sometimes considered a greater threat to Western honeybees than Varroa. We also need to be prepared for this pest. Fortunately, we have not yet had any practical experience with it, but we have informed us using the scientific literature and experience from researchers in other countries.

Finally, we would like to remind you of our position paper on the EU Honey Platform. We wrote it together with the Austrian beekeeping association BieneÖsterreich and the French association SNA. It has now been endorsed by twelve other associations. The paper can be used for national work. We also sent it to the EU Commission on the occasion of the call for the EU Honey Platform. It is also available in English on our website:

https://deutscherimkerbund.de/597-Kompromiss_EU_Honigrichtlinie



JOIN THE EBA VIBER AND WHATSAPP GROUPS FOR FREE

Anyone interested can join the EBA Viber or WhatsApp groups free of charge to inform beekeepers about EBA activities, regardless of whether you are an EBA member or not, so that you can be timely and properly informed about everything important in EBA and European beekeeping.

If you have the Viber or WhatsApp application installed, in order to join the EBA groups, you just need to click on the following link:

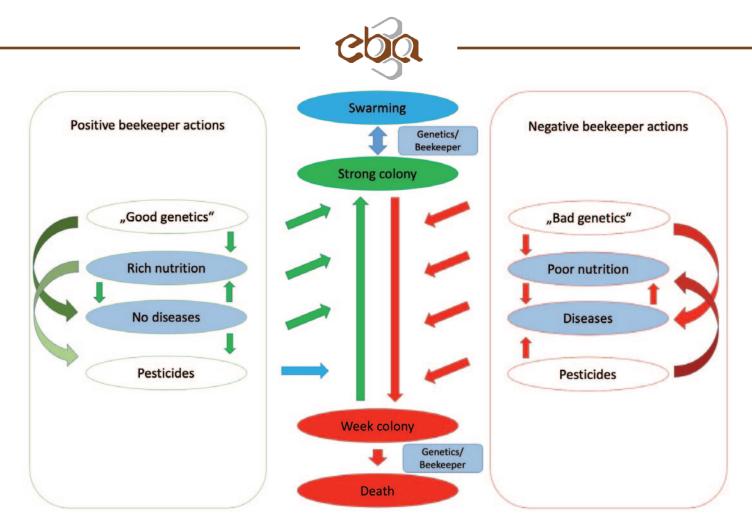
For Viber: https://ebaeurope.eu/join-the-eba-viber-group-for-free For WhatsApp: https://ebaeurope.eu/join-the-eba-whatsapp-group-for-free



RELATIONSHIP BETWEEN THE MAIN FACTORS INFLUENCING THE BEE COLONY

In this article, I will try to address questions that are important for understanding of biological processes in the bee colony, the relationships with the environment in which the bees live, and above all their interdependence. Due to these interactions honey bees (Apis mellifera) cannot be forced to do what we want, however their natural behavior can be employed to some extent for the benefit of beekeeper. The beekeeper must by no means neglect the needs of the bee colony, because any excessive depletion of the colony resources will soon result in its weakening or death. Everything written here, as well as in other places, should be taken with some reserve. Professor Jovan Kulinčević often says that bees do not read books and I am pretty sure of that too.





However, It should be noted that understanding bee biology is a major prerequisite for successful beekeeping.

The most important factors for successful beekeeping are: Abundant and high quality nutrition (good location, migration of hives and feeding), "genetically good" bees (good hereditary traits of bees for beekeepers), prevention and control of diseases and the amount of pesticides present; For successful beekeeping, in terms of profitability, much more is needed. Climatic conditions are also very important, but since we cannot directly influence them, I will not consider them separately.

Here I propose a division: Factors that can be affected by the beekeeper, such as diet, "bee genetics", Varroa destructor control, caution (disease prevention) and factors that can't, or can only partially, be affected by the beekeeper. These are primarily climatic factors and pesticides. The beekeeper can positively influence the first mentioned ones and thus accelerate progress and increase the number of bee colonies. The beekeeper can also have a negative effect, with consequential weakening and collapse of bee colonies. Scheme 1. is a simplified representation of the factors that affect the bee colony. These factors affect the bee colony positively or negatively, they are interdependent and can be present in different proportions. The sum of all the shown impactors leads either towards multiplication or towards death of bee colonies. They are all represented differently in each hive even in the same apiary.

In the **scheme**, positive influences are marked in green, and negative ones in red. At all times, all these factors are present in different combinations and to a different extent. The scheme is divided into two sections, solely for clear explanation and easier tracking of the text.

Just as beekeepers differ from each other, so is every bee colony different from others. One of the differences between beekeepers, important for successful beekeeping, is the difference in perception of a bee colony. That is why standards are important. Since I use LR hives, the sizes related to the strength of a colony will be expressed in the "LR system". For some beekeepers, a colony is "strong" if it has 10 frames of bees, and for some if it has 15 frames. Evaluation also has a time dimension, i.e. it depends on the season. In early spring, three frames of brood can be a lot, but in June it is certainly not much. Due to a wrong assessment of colony strength, as well as



of forage conditions, big mistakes can easily be made. Especially if we want to try out some new procedure in the apiary. It is always best to ask yourself why bees behave in a certain way, or why bees would need some procedure. The methods of work of other beekeepers should not be accepted uncritically. They may be keeping bees in very different conditions, so it is safer to try something new on a smaller number of hives.

Beekeepers are often interested in whether it is better to use LR, DB or some other type of hive. But for the successful survival and multiplication of bee colonies, the choice of the type of hive is the least important. All other factors shown in Scheme 1 are far more influential. The choice of type of hive is only important for the beekeeper, therefore when choosing a hive, he/she should make sure that it is well-adapted to his/her intentions (e.g. desired products) and possibilities (e.g. physical strength). All assessments related to the "strength" of a colony depend on the type of hive and the experience of the beekeeper. This can be avoided to some extent by calculating the total number of bees. For example in our country a colony (Carnicas) that has about 35,000 bees is very strong, which is about 15 LR frames completely covered with bees on all sides when they reach their population peak. If a colony has successfully overwintered, it usually comes out of this period with about 15,000 bees or 5-6 completely full frames of bees. Weather, season, forage and other circumstances should be considered separately for each hive, e.g. the quantity of bees and brood, food reserves, quality of the gueen, and based on all that, it can be decided which operation to perform. In order to facilitate and speed up work in the apiary, we should seek for equal colony strength. But by no means equally average or equally weak. The uniform strength of colonies enables easier planning and faster work, especially in a large apiary. Of course, planning depends on the purpose we have determined for the hives, i.e. the product we want to make. Only by looking at all important factors, can the job be properly planned and done.



How good surroundings, "good" bee traits and positive action of the beekeeper are interconnected

How is "good genetics" related to good nutrition? "Good genetics" is written with quotation marks because traits that are good for bees are often not good for the beekeeper and vice versa.

- Some bees hereditarily hoard more honey and / or pollen than others. This means that some bees are more productive.

- Longer-lived bees have more time to collect food, thus colonies have more worker bees available. Longevity is partly determined by genetics.

- Bees more resistant to diseases live longer. Resistance to various diseases is inheritable. In addition, due to the absence of some (primarily brood) diseases, bees have more food available.

Good genetics → higher productivity + longer worker life → Better nutrition

The longevity of the queen and of the worker bee are closely related. Long-lived queens have a greater chance to produce long-lived workers. Longer-lived workers allow the queen to maintain the same colony strength while laying a smaller number of eggs.

The queen can thus preserve vitality for a long time. In addition, the lower mortality of

brood, which is present to some extent in every colony, also allows the queen to lay a smaller number of eggs. Brood mortality is the difference between the number of laid eggs and the number of bees, workers and drones, which successfully emerge from the brood, i.e. develop into adults. Brood mortality, which is partly determined by genetics, is also influenced by other factors, such as the amount of food available, the

presence of disease, temperature and others.

The higher the mortality of the brood, the more eggs the queen must lay to make up for the loss.

Finally, longevity also depends on the nutrition of the queen before eclosion from the queen cell and the quality and number of drones with which she mated. Longevity of the queen = "genetic predisposition" + number of eggs laid (the less per season the better)

The Italian breed of bees in the USA, that was used for package bees production, are bees that are short-lived as well as their queens, because they were selected to give as many brood and bees as possible. A large amount of brood is not a good trait, especially when there is a shortage of food, because these two factors are usually associated with a shorter lifespan of bees emerged from such a brood. Such colonies can only be successful in conditions when there is abundant forage almost all year round, i.e. in warmer regions. It should be noted that these bees in the United States are genetically guite different from Italian bees in Europe or other parts of the world. This is a consequence of selection (when we are selecting for some desirable traits, at the same time we are, mostly unconsciously, selecting for some undesirable traits), but also of mixing with other sub-species.

Introduction of other breeds of bees into a country should be considered carefully. In addition to the fact that it can be illegal and dangerous due to the possibility of disease transmission, it can permanently disrupt the genetic structure of the indigenous breed of bees by introducing a series of negative traits. There is no justification

> for such action in our country (Serbia), since the Carnica breed of bees is probably the best for commercial beekeeping, especially in continental climate conditions. More specifically, the Buckfast hybrid that has been illegally imported into Serbia is genetically most similar to Italian bees, it can be good for beekeeping in Britain and other coastal regions where winters are relatively mild. Similar to Italian bees, it has a lot of bees and

brood, so it consumes much more food, and bees live shorter. For the successful survival of long and cold winters, it is important for the bee colony that worker bees live as long as possible.

The Buckfast bee was produced after the mass extinction of British indigenous bees, which was caused by the introduction of tracheal mite (Acarapis woodi) through Italian bees. Our bees are genetically resistant to this mite, so there is





no reason for us to import it. Since the Buckfast is a hybrid, its relatively good traits will last only as long as you have the original queen, bought from a reliable breeder. Although maintaining of this hybrid in Serbia will probably be abandoned quickly due to weaker performance, that will not prevent those bees from reproducing in the meantime and introducing bad traits that will occasionally manifest themselves.

It can be said that, the more brood there is, the poorer the nutrition of the larvae, and the shorter the lives of the queen and workers consequently. For quality nutrition of larvae, it is necessary to have the ratio between the bees that feed the brood and the amount of brood as high as possible.

How is "good genetics" related to the absence of disease? I use the term absence of disease instead of health, because a colony can appear healthy but still carry many pathogens. When a colony is fighting against some pathogen it can compensate for loss of brood or workers by additional effort and look healthy, while in fact it is depleting its resources. When it loses potential to compensate for loss the disease becomes apparant, and the health of the colony quickly deteriorates. However, this doesn't mean that a healthy hive does not contain some pathogen. It could be said that absence of disease means that the colony effortlessly maintains pathogens at a harmless level.

It is known that bees can be selected for almost any trait and thus for the absence of any disease. This means that disease resistance is inherited. When we compare several bee colonies, we notice that they are susceptible to different diseases and to a different level. One disease can affect another, so if bees are resistant to one disease, they can better tolerate certain other diseases. A very desirable hereditary trait of bee colonies is strong hygienic behavior. Such colonies will usually be less susceptible to American foulbrood (Paenibacillus larvae), chalkbrood, and varroa mite. This is because bees detect infected larvae earlier and remove them more efficiently. Of course, this does not mean that those bees are completely resistant to all these diseases. Or that with this good trait they



will not have any bad or a series of other weaker traits. Often, the overexpressed hygienic behavior is accompanied by lower productivity, as a consequence of the fact that a larger number of bees are engaged in hygienic work (e.g. propolis collection), which leaves a smaller number of bees available for collecting food.

Good nutrition and the absence of disease are closely related and will therefore be con-

sidered together. Good nutrition directly leads to increased resistance of the bee colony to all diseases. On the other hand, the absence of disease directly increases the amount of food collected. This creates a circular chain reaction that leads to the progress of a bee colony.

The greater the absence of disease, the better the diet, and the better the diet, the harder it is for diseases to cause serious damage to the bee colony. Of course, all this is influenced by other factors, primarily the amount of food and the amount of pathogens in the environment. Take, for example, a situation where the colony is well fed. It is known that one of the consequences of disease is that harmful organisms take away part of the nutrients from the host, i.e. the bee.

During the winter, bee colonies become infected with the microsporidian pathogen Nosema ceranae and/or N. apis. Nosema does not have fully developed mitochondrias, so it cannot produce ATP (adenosine triphosphate), a molecule that stores and transmits energy within the cell. Therefore, as a highly specialized pathogen, it takes ATP directly from the bee's midgut cell. Bees produce ATP predominantly from honey. This means that if the colony is well supplied with quality honey (not honeydew), no matter how much ATP is "stolen", the bees will be able to synthesize more for their needs.

This is where the quality of food comes into play. Bees that have more nosema need to consume more honey, for their own needs and for the needs of the growing population of nosema. This further means that, in winter, when there are no conditions for a cleansing flight, it is best for such bees to consume honey with as few indigestible particles as possible, because in that case they will not overfill the hindgut with these substances. Therefore in such conditions, when there is no brood in winter, it is best for bees to consume "honey" made from sugar syrup given to them by the beekeeper. If there is enough "quality" food, infected bees will survive till the cleansing flight, the most infected bees are the oldest-forager bees, and in spring they will bring the first pollen. Thus, before they die, they will improve the quantity and guality of food in their colonies. In addition, if they die outside the hive, they will not contribute to further spread of nosema, which would be especially the case if such bees died of starvation inside empty honeycomb cells. If infected bees do not survive the winter due to poor quality food (e.g. honeydew diet), they will not contribute to the colonies by bringing pollen in early spring. Instead, the colony will have to recruit younger bees for the first, very dangerous (due to bad weather) spring forage.

This will leave fewer bees available for work inside the hive, which will further contribute to poorer nutrition of the colony, and above all to poorer nutrition of the first spring brood. Poorer fed bees from the first brood will contribute less to their colony, which will again lead to feeding problems because those bees will have a shorter life, and they will be more susceptible to other diseases.

High-quality disease-free bees live longer and have more time to bring more food to their colonies. More food enables the rearing of bees



that will live longer on average and be more tolerant to diseases.

How is good nutrition related to the effects of pesticides? It should first be understood that the toxicity of pesticides, as well as the action of all other substances, is related to their concentration. This consideration refers to the maximum allowed concentrations of pesticides, or slightly higher and slightly lower concentrations of pesticides than those alowed. If the concentration of pesticides (especially insecticides) is much higher than the maximum allowed, it will surely kill the bee colony. However, in reality, bees are in most cases exposed to pesticides within the legally permitted concentrations or somewhat higher.

Under such conditions, good nutrition of bee colonies demonstrates its importance. Bees that are eclosed from a brood that was better fed will also have a larger mass. Higher mass in itself implies greater resistance to pesticides. If the bees are malnourished, they will start to cap the brood earlier, consequently the bees that emerge will have a smaller mass. Such bees will be more susceptible to diseases, less productive and more sensitive to pesticides. Simply put, to kill a bee weighing 110 mg you need more pesticide than to kill a bee of 90 mg.

In addition, better fed bees have more developed fat bodies. It is commonly thought that the reserves of proteins and lipids in the hive are found in bee bread. However, the most important place where these substances are stored are fat bodies. The fat body, an organ that plays a role similar to the liver, is the most important organ for breaking down and excreting pesticides from the bee body. It happens that all foragers die from poisoning, and that the house bees survive. Prior to becoming foragers, workers lose weight and fat bodies disappear, and as a result, they lose the ability to break down pesticides and excrete them from the body. Moreover, they are probably also exposed to a slightly higher concentration of toxins than house bees. The consequence of this is that at the same concentration of pesticides, better-fed colonies survive poisoning, and poorer-fed colonies fail. Like with everything, this also depends on other factors, primarily the predominant orientation of bees on different fields (differently sprayed with pesticides) and the number of bees in the colony. You can imagine there are many different shades here. For





example, a well-fed colony will lose all foragers, but still recover. A poorly fed colony will die because it will not be able to compensate for the lost foragers, because in addition to the foragers, a big part of the house bees are lost (bees with less developed fat bodies die), and there are not enough food reserves. On top of this, the presence or absence of disease will decide which colony will die and which one will survive.

Absence of disease increases the chance of a colony to survive the effects of pesticides. The absence of a disease means that the colony is better nourished, which further means that the bees have a higher mass and thus tolerate pesticides better. The influence of varroa on the action of pesticides is especially strong. Bees that are eclosed from a brood infected with varroa have a smaller mass, so they are more sensitive to pesticides. It is now known that varroa does not feed on hemolymph but on fat tissue of bees. Since the fat body is an organ that serves to break down pesticides, it becomes clear that bees that are parasitized by varroa will have a harder time withstanding the effects of pesticides, either because of their reduced mass or because of their reduced ability to degrade and excrete toxins. The higher the proportion of bees that has been exposed to varroa at any stage of life, the

greater the chance that the colony will not survive the poisoning.

"Good genetics", good nutrition and health can be directly affected by the beekeeper, while the action of pesticides is indirectly influenced by these three previously mentioned factors.

How bad surroundings, "bad" bee traits and negative action of the beekeeper are interconnected

How is "bad genetics" related to poor nutrition? It can be said that everything is very similar to when we talk about the opposite case where "good genetics" is associated with good nutrition.

- Some bees hereditarily hoard less honey and / or pollen than others. This means that some bees are less productive.

- Shorter-lived bees have less time to collect food, thus colonies have fewer workers available. Short-livedness, like longevity, is partly determined by genetics.

- Bees more susceptible to diseases are shorter-lived. Resistance to various diseases is an inheritable trait. In addition, due to the presence of some diseases bees have less food available.

"Poor genetics" → lower productivity + shorter

worker life → poorer nutrition





The short life of the queen and the worker is closely related. Short-lived queens have a greater chance to produce short-lived workers. Due to the short-lived nature of the workers, the queen must lay more eggs to maintain the same colony strength. The queen thus cannot preserve her vitality for a longer time. In addition, the higher mortality of brood, also forces the queen to lay more eggs. As it has already been said, the higher the mortality of the brood, the more eggs the queen must lay in order to compensate for the loss.

When the queen is prone to chalkbrood, and that in principle holds for all other diseases as well to a less extent, then the colony consumes more food. This is a consequence of the consumption of nutrients for the development and reproduction of pathogens. If we take e.g. that 10% of the larvae in the colony are infected with chalkbrood, this means that almost 10% of the food invested in feeding the brood is wasted. In addition, the consequence is that 10% fewer bees will eclose from the brood, so the colony will have 10% fewer workers available for all tasks, and will therefore collect less food. Depending on the climate, the food available, and the degree of inhereted hoarding, some colonies may guickly perish due to chalkbrood, while some can tolerate it quite successfully.

How is bad genetics related to the presence of disease? Disease resistance is an inherited trait. When we compare several bee colonies, we notice that they are susceptible to different diseases to a different degree. But remember, if a colony looks healthy it can be the case that bees are doing their best to fight off disease. One disease can affect another, so if bees are susceptible to one disease, they will be more exposed to a number of other diseases.

B e e s that are not tolerant to varroa will be

varroa will be more susceptible to viruses and some bacteria, because they become infected more easily. This is due to the fact that the varroa puncture the bee's protective cuticle to feed. This is also the case with the tracheal mite A. woodi when they puncture the surface of the airways (trachea), only to a lesser extent. Nosema and American foulbrood disease utilise a similar mechanism in the spread of other diseasees. Here, the fungi nosema or the bacteria P. larvae damage the surface of the gut and thus leave a breach for other pathogens, e.g. various viruses, which pass from the gut into the hemolymph ("bee's blood") and then spread throughout the body. It has been shown that it is practically impossible to infect healthy bees with viruses. Despite the incredibly high concentration of virus contained in the syrup that such bees were fed, there were no harmful consequences for the bees.

Poor nutrition and the presence of diseases are interrelated and stimulate one another. Poorly fed colonies raise low-quality short-lived bees. Such low quality bees have a lower body weight, less fat bodies and as a result they become precocious foragers. Such foragers are less efficient at collecting food. Due to all that, their lifespan is shorter, and the amount of food they collect during their lifetime is smaller. As colonies are left with ever less bees and food, if conditions do not change, this quickly and inevitably leads to colony collapse. The forage conditions in the environment must either improve, or the beekeeper must feed the colonies and / or control the pathogens.

Pesticides can further aggravate such a bad situation. Concentrations of pesticides that would not seriously harm a healthy, well-fed colony can easily destroy a diseased and malnourished colony. This is because the average weight of bees and the ability to break down pesticides in poorly fed and diseased colonies is lower.

Particularly dangerous is the use of chemical agents to control varroa, in cases when they have low efficiency. These are the chemicals to which varroa has developed some resistance. By using such chemicals, the number of varroa is not reduced sufficiently, but the bee colonies are poisoned. Especially if there is a synergistic effect of used chemical with another present substance. The same if not an even worse effect is achieved when one insists on not using pesticides without the preconditions for this decision. Beekeeping

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can be done without chemical control of varroa, but not by buying colonies and saying: "it is unnatural or unhealthy, so I do not treat them, bees will develop resistance to varroa by themselves." Such an approach will surely lead to the collapse of purchased colonies and to the spread of infection to surrounding beehives. First, a beekeeper needs to obtain bees with genetic tolerance to varroa, install an anti-varroa bottom board, regularly remove caped drone brood and similar.

Reproduction of bee colonies. When the sum of all these factors leads to a very strong colony, and the conditions in the environment are favorable, the bee colony reproduces. It then depends on the beekeeper and the genetics of the bees whether the bees will swarm and when they will do it, or the beekeeper will make splits previously.

Death of bee colonies. Conversely, when due to a combination of different factors, the colony weakens greatly, and the conditions in the environment are unfavorable, the loss of the bee colony is imminent. Then it is very important that the beekeeper does not allow it, because it leads to robbing, the spread of disease or, in the best case, the loss of honeycombs due to the wax moth. Very weak colonies are not worth saving, or it is impossible and it can be dangerous. For example if we try to syrup feed a very weak colony during a drought period it is very likely that robbing will occur. A very weak colony should be removed from the apiary before it dies.

In conclusion, it can be said that an inexperienced or careless beekeeper may have a negative effecton all these factors. First of all, not noticing and not controlling disease leads to damage both to one's own and to the surrounding apiaries. Excessive honey extraction or pollen collection can threaten the survival of strong colonies. Excessive use of pesticides to control varroa, especially ineffective ones, does more harm than good. It can easily happen that a beekeeper, due to a wrong assessment of forage and weather conditions, as well as of the strength of the colony, adds more frames with foundation (in the wrong position as well) than bees can draw, thereby separates the brood so that the bees cannot heat it enough and in that way puts the bee colony in trouble without reason.

Initially, at first contact between bees and beekeepers, the traits of one do not depend on

the traits of the other. Over time, the beekeeper improves the desired traits of the colonies by proper work, and reduces the manifestation of undesirable traits. Of course, he can also do the opposite. A clear example of this is the use of swarm cells for division of hives. Such queen cells should be taken only from a very strong colony, and only when it swarms in favorable conditions. Otherwise, by taking any swarm cell, we select bees to swarm more frequently, so swarms will start to emerge from half-empty hives.

It is certain that every beekeeper affects bee colonies both positively and negatively. He does this every time he opens the hive or does anything around the apiary or in the workshop that can affect the diet, health and behavior of the bees. In order for these actions to be as positive as possible, and negative as little as possible, it is necessary to educate oneself from reliable sources, and apply this acquired knowledge. In that way, the beekeeper improves his work skills in the apiary by practice. Objective observation of the situation in the apiary and knowledge of the mutual relations of various factors, enables the beekeeper to recognize a problem and react in the right way. It can be said that sometimes a beekeeper will succeed to get the most out of colonies with bad traits, while at another time a beekeeper will manage to ruin colonies with excellent traits. This of course can be the same beekeeper.

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WHEN IS YOUR HONEY BEE COLONY READY FOR "THE PUBERTY TALK"?

Puberty is an unlikely topic for a beekeeping journal, but it is a special time in any organism's life, and I'll do my best to keep this professional. Before puberty, an organism invests its resources in two areas: survival and growth. Once an organism begins to also invest in reproduction, puberty begins. Sure, that 13-year-old boy with a cracking voice probably isn't producing sperm and fathering children, but he certainly is on his way. It's a critical developmental transition that all organisms undertake, even colonies of honey bees.

What does puberty in a honey bee colony look like? A fully reproductive honey bee colony is a hermaphrodite, capable of reproducing by producing males (drones) or females (swarms). Mature drones are produced before swarms, so to determine the very first investment in reproduction, we have to go back to the very first stage of producing a drone. And what's that? Yes, it's our favorite comb, those large cells of drone comb. When workers build drone comb, it's the equivalent of the colony going through puberty. Even if the colony might not use the drone comb for rearing drones until the following year, the specialized cells are a sign that reproduction is somewhere on the horizon.

So, what do we already know about honey bee colonies going through puberty? Since the 80's we've known that large colonies begin to build drone comb earlier than small colonies (Lee and Winston 1985). But large colonies are differ-



ent from small colonies in almost every way: large colonies have more worker comb, more honey stores, more brood, and more workers (Rangel and Seeley, 2012). What is it about being in a large colony that triggers workers to build drone comb? This is what my colleagues and I set out to determine.

We wanted to see which colony parameter(s) would trigger workers to construct drone comb. These parameters included: number of workers, area of worker comb, area of worker brood, and area of honey stores. We needed to increase just one parameter, such as the area of worker comb, while keeping all other parameters equal. Therefore, we could see how increasing a single parameter might (if at all) increase the area of drone comb built.

Feel free to take a minute here and think about which parameter you think would trigger a colony to build drone comb. I'm sure you have lamented the zeal with which bees seem to build drone comb wherever they can, and you might have also noticed that the largest colonies built drone comb with the most fervor. But remember that "large" encompasses many parameters, so what part of "large" really matters? To be perfectly honest, any of the parameters were plausible, or even a combination of the parameters. Once we amassed the data, however, we found that increasing the number of workers was the only parameter that led to an increase in the proportion of drone comb built. Furthermore, if we increased the number of workers in all the treatment groups, there was no additional drone comb built even if the colony also had more worker comb, worker brood, or honey stores. We had a solid answer: the colony parameter that induces a colony to switch from building worker comb to drone comb is the number of adult workers (Smith et al 2014).

What does this mean? First off, it's cool! The workers are somehow detecting the number of other workers in the colony. If that number is over a size threshold, then the workers begin building drone comb. As with most research, answering one question raises more questions. Why use worker number to trigger puberty? If I can speculate a bit, this might be because the number of workers in a colony is the most reliable currency that a colony has for detecting that it can afford to invest in future reproduction. Worker number is intricately linked to other important metrics of





colony survival, such as the amount of honey a colony can store before winter.

Therefore, colonies that do not have enough workers to adequately survive and grow cannot invest in reproduction. But how exactly does one bee "know" how many other bees are in her colony? Right now, I have no idea, but investigating that mystery is the next stage of my PhD... Stay tuned.



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References:

Lee PC, Winston ML (1985) The effect of swarm size and date of issue on comb construction in newly founded colonies of honeybees (Apis mellifera L.). Can J Zool 63:524–527. doi: 10.1139/z85-077

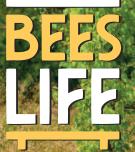
Rangel J, Seeley TD (2012) Colony fissioning in honey bees: size and significance of the swarm fraction. Insectes Soc 59:453–462. doi: 10. 1007/s00040-012-0239-5

Smith ML, Ostwald MM, Loftus JC, Seeley TD (2014) A critical number of workers in a honeybee colony triggers investment in reproduction. Naturwissenschaften 101:783–790. doi: 10.1007/s0011 4-014-1215-x

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HAVE STRONG BEE COLONIES ALL YEAR



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The strength of a bee colony depends on an incredibly large number of factors. I will try to cover the most important factors in this text, so that it becomes clear to everyone that the bee colony is actually one unit in which all gears must be ideally cast and lubricated in order to function perfectly.

Valuable data

Johann Dzierzon (1878) showed that bees live on average no longer than 6 weeks, i.e. from 4 to 7 weeks, or as he says from 30-52 days. He came to these data simply. He added an queen bee of the Italian race to his dark native bees, and observed the rate of death of the dark bees. An excellent method for amateur beekeepers.

The only thing is that Dzierzon did not emphasize whether he also removed all the brood from the old queen. Because, if not, the last of her bees hatched 21 days after the removal of

the queen, which would shorten the determined life of bees by exactly 21 days, and reduce it to one to 3-4 weeks. Personally, I cannot believe that Dzierzon did not think of something like this, but since there is no concrete data, anything is possible. If he really removed the brood as well (which is extremely likely), then he got a slightly longer life of the bees than average, because most of the workers did not grow brood and secreted royal

jelly for several days, which extended their life for a certain time.

It should be known that the actual development of a honey bee does not last 21 days, as it is written in the books, but between 19 and 23 days (Steve Taber). The average length of bee development is 20.5 days. There are also genetic differences between breeds and strains of bees, which are manifested by smaller or larger differences in the length of bee development in the brood.

Before World War II, N. Nickel and Armbruster marked bees with paint. They found that bees hatched in spring and summer do not live longer than 48 days, while half of the marked bees disappeared after only 24 days! It is also a period of intensive work and development of the bee colony, so bees are exhausted a lot and therefore live shorter lives.

However, S. A. Popravko (1982) from the former USSR, based on the analysis of experiments of a large number of researches, determined that the length of life of summer (short-lived) bees does not significantly depend on the breed and the degree of workload (just a few days more or less). The drastic influence of some factor on the length of life of bees was established by Anna Maurizio (1955) from Switzerland, because she showed that the length of life of bees is always sharply shortened when they are busy with intensive rearing of larvae (production of royal jelly) and increases when there is no such work, but, of course, with the condition that the bee colony is supplied with abundant amounts of pollen. The obvious proof of this is certainly the two to three times longer life of bees in bee colonies without a queen. The shortening of the life of brood-rearing bees is certainly re-

> lated to the consumption of lipid-protein reserves in the bees' bodies. The shortening of the life of bees is greater in bee colonies that do not have an abundance of pollen available, because the bees are then forced to use up their own reserves. That is why in some years the bee colonies enter the winter with a solid number, and come out significantly weakened, if during the previous August and September there is a

drought and therefore a lack of pollen. The period of pollen shortage can also be called the period of late winter, just before the start of the first pollen-bearing pasture, when the autumn stocks of bee bread are mostly used up, and there is still no fresh pollen. That's why it is more than important to keep bees in an area rich in pollen-bearing pasture in late summer, which is the only guarantee for securing a rich supply of pollen in combs that can meet the first spring pollen pasture.

Nature is a miracle

If we look at it from an evolutionary perspective, we will reach some interesting conclusions. I've often wondered (now I'm going to exaggerate a bit to be graphic), why bees, which have been





as they are today for 40 million years (more than twenty times longer than the existence of man), and have been developing for at least 110 million years, have not created bee colonies of several hundred thousand bees, and brought several tons of honey? The answer is difficult to give, but let's try. We will bypass the basic possible reason - an optimally developed bee colony never needed such a quantity of honey. When a bee colony "decided" to increase the amount of brood, the life of the bees was shortened, due to depletion by the secretion of royal jelly. When a colony "decided" to reduce the volume of brood, the life span of bees increased. That occasional genetic diversity and genetic "jumping out" from the usual was delayed by nature by drowning in more prevalent variants, so according to natural laws it had to be equalized at the most optimal level. This level implies that the evolutionary achievement has reached the stage that we know today in bees, which nature declared to be the most harmonious with vegetation and other factors, and which has been maintained for 40 million years, although the climate and the representation of various plants have changed significantly several times in that period. So, the bees reached their optimum as soon as they did not change anything significant for so many years. And let's look at the man! It was created about 2 million years ago, and has been constantly changing since then. It was only 50,000-60,000 years ago that it began to resemble today's people (then, at the same time, a man was born whose descendants are absolutely all people on the planet today), and only a few hundred years ago, in the Middle Ages, it had a smaller brain volume than today! And he dares to think that he can and should change the bees, instead of studying them and using their instincts. The period in which we significantly influence the tendencies of bees and their instincts is so small and insignificant that it cannot be described in words. It is high time that we sober up and realize that nature cannot be managed, but that it can only be helped or hindered. Each of our interventions must be well thought out, precise, short-term,





timely and supported by scientific knowledge, but above all it must be in accordance with natural laws.

Further research

We should also not forget that the length of life of bees is also influenced by genetics. Charles Mraz also noticed that, under the same conditions, bees live longer in some communities and shorter in others.

Based on his research, Krežak (1973) from the former Czechoslovakia claims that the length of life of bees does not depend on the amount of work performed, but on how they were cared for and fed during development, while they were larvae. This perfectly coincides with the positions taken by Anna Maurizio and S. A. Popravko. Breeders of the broods are is young bees, whose mandibular glands are maximally developed from the fifth to the fifteenth day of life, when they secrete food (royal jelly) for the larvae. The brood raiser lowers her head into the cell, performs an inspection for 2 to 20 seconds, and then uses her jaws to mix the secretions of the mandibular and hypopharyngeal glands with which she feeds the larva, i.e. deposits food on the wall or bottom of the larval cell (Lesley J. Goodman, 1998).

Vorst and Jakobs (before 1980) of Belgium found that bees lived longer if fed on bee bread than fresh pollen, confirming the value of rich reserves of bee bread in late winter and early spring. According to them, the same applies to bees infected with nosemosis. However, the bees first consume the pollen that has just been introduced, and leave the pollen from comb for situations when there is no pollen from nature. This is also confirmed by Colin G. Butler (1949) from United Kingdom, who in an independent experiment also determined that bees infected with nosemosis live longer if they are fed with bee bread, than with freshly introduced hazelnut flower pollen.

It is certainly important to take care of the bees immediately after the performance. Because, in the first three to four days of life, young bees are fed by older bees through trophylaxis (Lesley J. Goodman, 1998). Those young workers get honey, some nectar and maybe a little bit of royal jelly for the larvae, but pollen nutrition is also very important in the first seven days due to the proper development of the glands, and it decreases in quantity only from the eighth to the tenth day of life (Lesley J. Goodman, 1998). In the first five days of life, the brood nurse feeds the drones with a mixture of brood jelly, pollen and honey.

The largest number of bees are in the hive five weeks after the appearance of pollen in nature (if the climatic conditions are favorable, and on average a little later), and the amount of brood raised in that period directly corresponds to the amount of pollen in the nest.

Speaking of pollen, it should be emphasized based on all the above, that it is extremely important to find a terrain with rich pollen pasture for bees. The lack of bee bread in the comb in late winter and early spring has a very unfavorable effect on the bee colony. In the literature, information can be found that in the absence of bee bread, bees can nurture their brood using the protein reserves from their body for only 15 days.

The crucial role of pollen

According to G. D. Bilash (1990), the average bee colony consumes 20 kg of bee bread per year, and very strong colonies up to 35 kg. Thomas D. Seeley (1996) claims that the annual consumption is reduced to an average of 20 kg of pollen and about 60 kg of honey, in the conditions of the northern regions of America. The American





scientist L. Standifer (1980) claims that strong bee colonies annually consume 45.3 kg of pollen. Pollen is the main source of protein for bees. When the bee hatches from the brood, the level of protein in its organism increases, so it is considered that the increase in the level of protein indicates the growth and development of the bee, i.e. to represent a measure of growth (M. Haydak). The pharynx develops (the most developed from the 6th to the 10th day), waxy and other glands, fat tissue grows. Schtraus (1911) showed that adult bees have a higher amount of nitrogen in their bodies than newly hatched bees. M. Haydak (1933, 1934) examined changes in bee mass and nitrogen content, especially in the head, thorax and abdomen. He showed that five-day-old bees, compared to newly hatched ones, have more nitrogen in the head by 92%, in the chest by 37.5%, and in the stomach by 76%. All this is due to the optimal supply of pollen to the community. Even from the 8th to the 10th day, the bees' need for pollen did not decrease. Only carbohydrates are enough for older bees to maintain vital energy. Bees need heat above 30 °C to digest pollen. At lower temperatures, digestion is not complete (Grešnovski, 1967). In most cases, bees do not use pollen before conserving it in comb. According to F. A. Robinson, pollen contains several attractive substances for bees that collect pollen, primarily fatty acids and ether letein. N. G. Bilash (2003) and Hopkins (1969) claim that the most attractive is octa-decatrans—2-, cis—9—, cis—12—trienoic acid. This acid makes up as much as 35% of the acidic zone of fatty acids in clover pollen, which influence greater attractiveness for bees (this also includes myristic, palmitic, oleic, linoleic and linoleic acids). Myristic acid dominates in sunflower pollen (Farag, 1978). It has been definitively confirmed that the bee-attracting aroma of pollen resides in its lipid components (Dobson, 1988), and these components have been shown to have antimicrobial properties (J. Morris, 1979). The importance of attractive substances was also established by L. Standifer when experimenting with pollen substitutes. It turned out that nutritional value was much less important to the bees when consuming, than the attraction that the researcher changed by changing the proportion of pollen in the mixture with one of its substitutes.



Thus, he determined that the best results are achieved if there is at least 20% pollen in the mixture. Due to the same reason, a 3-4 times lower uptake of yeast compared to pollen was observed (Nataly Grigorevna Bilash, 2003). In any case, pollen substitutes are only added as a last resort, because all of them are actually harmful to bees in one way or another, more or less. This harmfulness is best manifested by strange abnormalities in the covered brood, but also during queen rearing, when due to the addition of soy flour, part of the queens may die in the brood boxes (Steve Taber, C. L. Farrar), but the fact remains that bee colonies that consume cakes in the spring with soy flour and pollen give fantastic results (C. L. Farrar), just keep in mind that Farrar

recommended cakes with three (!) parts of pollen and one part of soy flour. Obviously, the credit goes primarily to the pollen. Steve Taber does not recommend giving bees milk powder either. He conducted research for several years looking for an adequate substitute for pollen, but as he says, all experiments ended in failure, and he did not publish anything. Yeast is often used as a substitute for pollen, but it has been established (Nataly Grigorevna Bilash, 2003) that proteins from bee bread are used both for raising brood and for creating reserves in the body of the bee, while proteins from yeast are mostly used only for creating reserves in bees. According to Russian research. raising 10,000 bees (about 1 kg) requires

bees even 1,428 g of pollen). The average bee colony spends 16.6 kg bee bread (15-28 kg) per year for bee brood. Successful rearing of only winter bees requires an average of 5.5 kg of bee bread (3-7 kg). The strength of the bee colony in the spring and the effectiveness of using the early spring pastures increase proportionally to the stock of bee bread in the hive. C. L. Farrar (1960) recommended that a community enter the winter with at least 2.5 frames of Farrar's bee bread, or 3,000 cm2 of bee bread. Colonies with optimal bee bread stocks rear 27.4% more brood and produce up to 40% more honey than colonies without adequate stocks. If there are not enough supplies of pollen, the bees stop raising drones, because they consume five times more food for

> development than worker larvae. One kilogram of drones eats 15-20 kg of honey during their lifetime. Drones, which were deprived of protein nutrition in the first week of life due to a lack of pollen, later did not produce enough sperm. Therefore, the mass rearing of drone brood indicates a good supply of pollen to the colony. If there is not enough bee bread in the hive, workers are born with less developed hypopharyngeal glands, which causes poor quality and insufficient feeding of the larvae, but also a reduced ability to process nectar into honey, due to the earlier cessation of these glands. The amount of jelly in cells with three-dayold larvae is 308% (9.8 mg vs. 2.4 mg) higher in colonies with sufficient bee bread supplies than in communities with a deficit of bee

from 894 to 1,080 grams of pollen, which is in perfect agreement with Farrar's claim from 1966 that raising 4,500 bees requires about 500 g of pollen. Only Ladislav Sevčik (1975) claims that 1 g of pollen is needed to raise 7 bees (for 10,000 bread, the mass of three-day-old larvae is by 39.8% (14.4 mg vs. 10.3 mg), the mass of oneday-old bees by 8.6% (115.9 mg vs. 106.7 mg), and the length of the tongue by 2.8% (7.3 mm vs. 7.1 mm). Deficiency of pollen affects the development of glands that secrete wax, as well as the



development of fat tissue, which overall leads to less wax secretion. The weight of both clumps of pollen is 8–20 mg, on average 11–12 mg, and for them the bee usually visits about 500 flowers. Bees collect large lumps for 61 minutes, medium ones for 62 minutes, and small ones for 63 minutes. It means that they spend the same amount of time for each, but the efficiency is different because it depends on many factors, the amount of pollen in the flowers, its stickiness.

The size of the clumps is inversely proportional to the wind strength. One honeycomb cell contains an average of 140 mg bee bread (from 101–175 mg). A kilogram of bee bread contains about 7,000 cells. Bees never fill the cells with bee bread to the very top, but it occupies an average of 57% of the cell volume (from 36–77%). The reason lies in the fact that the bee must have a support in the cell to compact the pollen clumps.

The surface layer of the bee bread, which is being prepared for longer storage, is soaked by bees with honey. During grazing, bees like to fill such cells with honey and match. A frame with bee bread conducts heat worse than a honeycomb with honey by 40.9%, and by 59.3% than an empty honeycomb. As we already know, and bees only confirm, nothing in nature has only one role. Thus, bee bread is not only food, but also a significant insulation of the winter cluster. Most bees collect either nectar or pollen. Only on scarce pastures do bees collect both resources. The biggest incentive for bees to collect pollen is the number of cells with larvae in the brood, or in other words, the number of hungry mouths. Bees introduce 1 g of pollen per day to 62–90 larvae, an average of 77.6.

If there is not enough pollen in the colony, bees spend twice as much honey when building combs than when there is plenty of pollen. Bees that do not use optimal amounts of pollen in their diet live shorter, are less resistant to diseases, the course of the disease is more severe, and their survival at low temperatures is weaker. L. Bornus (1985) established that during the winter the first to die were those bees that did not have the remains of undigested pollen grains in their intestines. In other words, these are bees that did not have access to the bee bread supply. In a controlled experiment, it was determined that bees that were given pollen in their meals lived twice as long as bees that were completely deprived of pollen.

For years, the question of adequate pollen replacement has been raised. Anna Maurizio (1949, 1950, 1951) examined the effect of soy flour and its defatted products, powdered milk and yeast on young bees. After 25–28 days of controlled nutrition with these substances mixed with 2, 5, 10 or 20% honey dough (2 parts of





honey to 5 parts of powdered sugar), the condition of the pharyngeal glands, fat tissue and other parameters was controlled. No substitute was found to match the effect of pollen. According to this and other experiments, it was determined that bees cannot raise brood for more than two weeks only on soy flour, if they do not have pollen available. That's why Farrar conducted experiments, and found that solid results with soy flour can only be achieved if at least 25% of pollen is added to it. But much later he himself stated that soy flour can have some unwanted effects on the development of the litter. That is why it is recommended, when there is a certain natural deficit in the pollen pasture, and the reserves in the hive are exhausted, feeding with pre-collected pollen. However, this can have negative consequences (according to Steve Taber, it is possible to infect a colony with American foulbrood, if pollen is collected from colonies that have a latent or manifest disease). To make sure that you do not transmit American foulbrood with the pollen, you must expose the pollen to gamma rays before feeding the bees, which do not leave any harmful effects, but kill all forms of life, including American foulbrood spores.

Care should be taken to ensure that the pollen is well dried, because if it contains more than 6% water, it will start to ferment in storage (Campus, 2003). When the pollen is collected in the catcher, it contains 20–30% water, and therefore, as well as due to its high protein content, it is very suitable for the development of microorganisms (Stefan Bogdanov, 2003). Some beekeepers freeze it undried, believing that in this way they preserve a greater part of its nutritional value than with drying, which has not been fully confirmed, although it sounds very logical.

There is even Russian data that frozen pollen does not accelerate the development of the bee colony at all.

In addition, there are plants that do not provide enough quality pollen for bees. For example, dandelion pollen lacks two essential amino acids. Then, in the US, it was found that bees that consume only rapeseed pollen throughout their lives live 48% to 65% longer than those whose protein source was exclusively sunflower pollen, making it a deficient food for bees. From all this, it is enough to draw the conclusion that the best protein diet for bees is actually a mixture of pollen from various types of plants.

The power of colonies

The number of bees in a colony is most easily determined by weighing all the bees on a scale. We take out each frame from the hive and shake all the bees from it into a box, which we measure later. Bee mass is known to average around 100 mg, while Dr. Ralph Büchler (2003) claims that the mass is higher in bee colonies that are not depleted by artificial food and varroa destructor, where the bee hatches with 140 mg, thus citing Schneider (1987). The mass of the hatched bee also depends on the age of the comb. According to G. D. Bilash, V. I. Lebedev and N. I. Krivtsov, a young bee hatched from a black comb is 13.1% smaller than its peer hatched from a light comb. According to them, the average weight of a bee hatched from a light comb is 123 mg, and from a black comb only 106 mg. However, the bee loses mass as it ages. This means that there are about 10,000 bees in 1 kg of bees. So, we multiply the number 10,000 by the measured mass of bees expressed in kilo-





grams, and approximately get the total number of bees.

However, the most accurate way to determine the number of bees in a colony is to count them. Technically speaking, this is almost impossible to do, unless we kill the bees with poison gas. But, with the help of certain apparatus, the counting is very precise. Place the stressed bees in a box with opaque walls. The box has an opening where a device for counting the number of passes (counter) can be installed. Before shaking, we put the queen in a cage, and the cage in a box made of wire mesh, which we put against the opening. We turn on a bright light behind the queen cage, and those two stimuli will make all the bees move from the dark box to the light, near the queen. The device will do our job.

Someone will surely wonder why we should be interested in how many bees there are in colony. In the practice of professionals, it is really irrelevant, but only at first glance. They wouldn't even become professionals if they didn't know



bee biology in detail, and if they didn't understand what was happening in the hive at all times. Here are just a few examples important for practice. Slovenian master beekeeper Franz Prezelj (2003) clearly defines some relationships between the number of bees and the area of the hive. According to him, bee development is best when there are 300-400 bees per liter of hive volume. He considers a colony with 400-480 bees per liter of hive volume to be ideal for honey production. If the bee has more, it indicates that the swarming instinct may occur. The mentioned number of bees can also be expressed through the possession of frames. The bee colony has a good hive when there are an average of 125 bees on one square decimeter of the honeycomb

surface, or 1,250 bees on one side of a standard size LR frame. It is the last hour when we have to increase the volume of the hive by adding new supers (extensions), and prevent overcrowding and the possible appearance of swarming. With continuations, he recommends the following method of determining when to add new continuations. When we lift the top super (meaning the LR and Farrar hive), and see that the bees are covering the saton bearers of all the frames,

it is time to add a new extension. Mr. Franz mentions something else interesting, which may not be the place in this part of the book, but it is important to mention. He criticizes the frequent opening of the hive, noting that one inspection in the middle of heavy grazing, that day reduces the intake by at least 1 kg, but also hinders the queen from laying.

Optimum strength of the bee colony

We found that colonies are not as numerous as we have read so far in various books. However, science has gone further. It has been proven that there is an optimal strength of the bee colony at which it produces the most honey per kilogram of its bees. If the power was higher or lower than optimal, the yield per kilogram of bees would decrease. Therefore, the beekeeper must always try to have colonies of optimal strength, in order not to invest in the formation of enormously strong colonies, because in this way he would get less honey per unit mass of bees.

On this occasion, we will quote G. F. Taranov: "It is known that the interbreeding of bee colonies increases with the increase in the number of bees in the community. At the same time, the amount of collected honey per unit of live mass of bees increases. However, this increase is not unlimited. Experiments on the formation of extra strong colonies, by uniting several colonies, for example of 8-9 kg of bees, showed that they collect per unit of live mass, and often per colony, significantly less honey than strong colonies that

have reached a natural strength of 6 -7 kg of bees. In artificially formed extra strong colonies, the natural balance is disturbed. Due to the abundance of bees flying from the hive to the bee pasture, the nectar-receiving bees cannot quickly receive the offered nectar, they are forced to carry it to the nest over considerable distances. All this lowers the intensity of collection and its processing, which results in less honey. There-

fore, there is an optimal strength of bee colonies, at which per unit of live mass of bees, and for the entire colony, bees collect the largest amount of honey. Both the reduction and the increase in the strength of the colony reduce the effectiveness of the work of bees".

In the book Product of beekeeping, V. I. Lebedev says: "...it is known that when the mass of bees increases to 5 kg, the amount of collected nectar increases not only per colony (as a consequence of the greater number of bees), but also per unit of live mass of bees (as a consequence high-quality able-bodied members of colony).

With a larger amount of bees in the colony (8–10 kg), its productivity per 1 kg of bees decreases. Thus, experimentally, it was determined that compared to 1 kg of bees, the productivity of bee colonies, which had an average of 4.4 kg of bees, was 33% higher than weaker colonies (weighing 3.5 kg) and even 62% higher in relation to very strong colonies (mass 7 kg)".





Professor Slobodan Miloradović (1997) from Serbia calculated the following startling truth based on the above data. If a 7 kg colony would bring 21 kg of honey, a 4.4 kg colony would collect 21,384 kg of honey, or even slightly more than a bee colony that is 1.5 times stronger than it!

As for wintering, A.S. Ykovlev (1971) determined that the optimal strength of the harvested colony is within the limits of 9 to 11 streets of bees, in the Central Russian race of bees.

Both V. I. Lebedev and A. I. Toroptsev (1998) claim that the winter biological optimum of the Central Russian breed of bees is almost exactly that, from 9 to 12 streets of bees, and at that power the colonies consume the least amount of food per unit of bees. A decrease or increase in the number of bees leads to a sudden increase in consumption. The maximum food consumption per unit of bees is in colonies with a strength of 4–5 streets.

It is interesting that V. I. Lebedev and A. I. Toroptsev (1998) state that colonies of the Central Russian race should enter the winter with no less than 2 kg of bees, which according to them corresponds to colonies with 8–9 streets of bees. Then they state that more experienced beekeepers prepare colonies with no less than 2.5 kg of bees (10-11 hives) for the winter.

Personally, I believe, based on experience and certain knowledge, that with our Carniola breed, the orientation optimal strength during winter is somewhat lower, from 7 to 9 streets of bees (or rather, bees in 7-9 streets). But, in our conditions, only colonies that prepare for winter in the presence of solid pasture in August and early September reach this strength.

Dr. Himer (1924) from the Institute for Beekeeping in Erlangen (Germany) determined that under the conditions there, a colony of normal size occupies 5-6 streets, and that the diameter of the winter claster is about 20 cm.

It is considered that the winter death of bees in relation to the number of bees at the beginning of November should not exceed 10%.

Conclusion

According to all the above, it can be concluded that with a lot of knowledge you can go far in beekeeping, but also that not everything is as simple as it seems. Because strong colonies are created all year round. Even the smallest mistake has negative repercussions for several months to come.

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Our tomorrow depends on today's investments.

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THE TRANSFORMATIVE POWER OF CERTIFIED ORGANIC BEEKEEPING: A SUSTAINABLE FUTURE FOR APICULTURE

Certified organic beekeeping offers a transformative approach to apiculture, adhering to stringent European regulations. It bans chemical treatments, nurturing bees in a natural, unadulterated environment. Organic beekeepers prioritize colony health, employing natural feeding techniques, applying natural active ingredients and solutions for their colonies' health, and relocating hives to pristine, remote areas to avoid exposure to conventional agriculture.

Key Differences from Conventional Beekeeping:

1. Prohibition of Chemicals

Use of chemical compounds and substances to combat diseases and pests is prohibited.

2. No Antibiotics (EU Regulation)

The use of antibiotics is not permitted, in accordance with EU regulations, which also applies to conventional beekeeping.

3. Natural Feeding

Artificial feeding is not allowed. Bees are fed, if necessary, with organic self-produced honey

4. Safe Apiary Locations

Beehives are transported to regions (apiaries) in the mountains and areas sufficiently distant from conventional crops, unless they are certified organic crops.

5. Regular Inspections

Regular and ad hoc inspections are conducted during production and standardization.

6. Zero Residue Analysis

Analyses are carried out on certain characteristics of honey, with zero residue tolerance. This ensures the highest quality product.

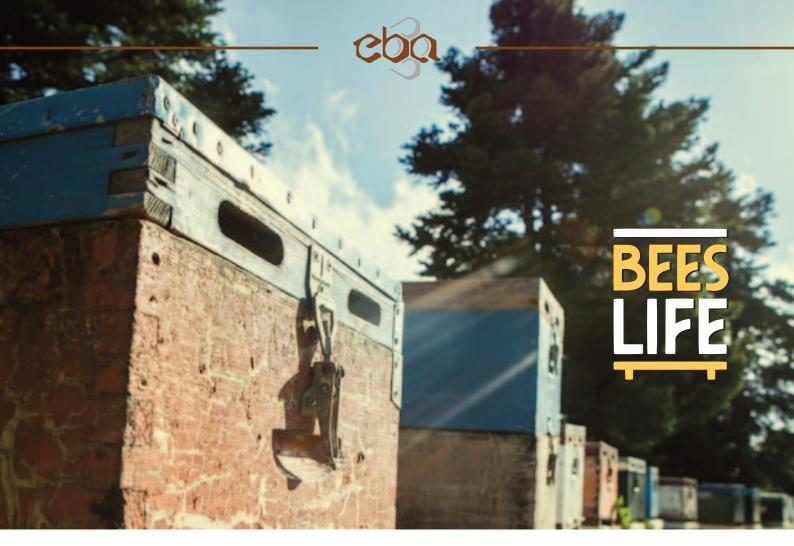
7. Accredited Standardization

Standardization/bottling is done in an accredited facility for organic product standardization, ensuring maximum safety and traceability.

8. Certification Costs

For certification of organic production, the beekeeper pays a significant annual fee to the certifying body for regular and ad hoc inspections at all stages of production and standardization.





Organic Farming at a Glance Aims of organic farming

Organic farming is an agricultural method that aims to produce food using natural substances and processes. This means that organic farming tends to have a limited environmental impact as it encourages:

responsible use of energy and natural resources;

• maintenance of biodiversity;

 preservation of regional ecological balances;

· enhancement of soil fertility;

· maintenance of water quality.

Additionally, organic farming rules encourage a high standard of animal welfare and require farmers to meet the specific behavioural needs of animals.

European Union regulations on organic farming are designed to provide a clear structure for the production of organic goods across the whole of the EU.

This is to satisfy consumer demand for trustworthy organic products whilst providing a fair marketplace for producers, distributors and marketers.

Building trust in organic farming

In order for farmers to derive benefits from organic farming methods, consumers need to trust that the rules on organic production are being followed. Therefore, the EU maintains the following strict system of control and enforcement to guarantee that organics rules and regulations are being followed properly.

As organic farming is part of a larger supply chain which encompasses food processing, distribution and retail sectors, these are also subject to checks.

• Each EU country appoints 'control bodies or authorities' to inspect operators in the organics food chain. Producers, distributors and marketers of organic products must register with their local control body before they are allowed to market their food as organic.

• After they have been inspected and checked, they will be awarded a certificate to



confirm that their products meet organic standards.

• All operators are checked at least once a year to make sure that they are continuing to follow the rules.

• Imported organic food is also subject to control procedures to guarantee that they have also been produced and shipped in accordance with organic principles.

The organic logo

The organic logo gives a coherent visual identity to EU produced organic products sold in the EU. This makes it easier for EU based consumers to identify organic products and helps farmers to market them across all EU countries.

The organic logo can only be used on products that have been certified as organic by an authorized control agency or body. This means that they have fulfilled strict conditions on how they are produced, transported and stored.



"Experience the profound difference of certified organic beekeeping, where every jar of honey reflects a commitment to nature, sustainability, and unparalleled quality."



Certified organic beekeeping offers a revolutionary approach to apiculture, prioritizing the health of bee colonies and the environment. By banning chemicals, emphasizing natural feeding, and ensuring safe hive locations, this method not only produces superior honey but also supports biodiversity and ecosystem regeneration. Through strict adherence to European regulations and rigorous inspections, organic beekeeping stands as a sustainable and ethical alternative to conventional practices, reflecting a deep commitment to nature, sustainability, and unparalleled quality.

Support us and embrace the profound benefits of certified organic beekeeping!

Georgios T. Athanasiadis

Organic Apiarist, Physicist, Tutor MSc, MA General Secretary of the Association 'Organic Beekeeping Community' General Secretary of the Institute of Ecological Agriculture



References

Athanasiadis, G. T., & Mangana, C. E. (2024, May 01). Bee Naturalles. Retrieved from https://beenaturalles.com/

European Commission. (2024, June 17). Control and enforcement. Retrieved from AGRICULTURE AND RURAL DEVELOPMENT: https://agriculture.ec.europa.eu/farming/organic-farming/controls_en

European Commission. (2024, May 20). Organic action plan. Retrieved from AGRICULTURE AND RURAL DE-VELOPMENT: https://agriculture.ec.europa.eu/ farming/organic-farming/organic-action-plan_en

European Commission. (2024, June 18). Organic Farming Information System. Retrieved from AGRICULTURE AND RURAL DEVELOPMENT: https://ec.europa.eu/agriculture/ofis_public/index.html?lang=en

European Commission. (2024, June 02). The organic logo. Retrieved from AGRICULTURE AND RURAL DEVEL-OPMENT: https://agriculture.ec.europa.eu/farming/organicfarming/organic-logo_en

European Union. (2024, April 28). COMMISSION NO-TICE - Questions and answers on the implementation of EU rules on organic beekeeping. Official Journal of the European Union, pp. 1-3. Retrieved from https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/ ?uri=CELEX:52024XC02874

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Dear readers,

We are delighted to present the contents of our paper titled "Looking for the causes of and solutions to the issue of honey bee colony losses" which has been cited over 60 times according to the Scopus database. This paper has garnered significant interest among the beekeeping readership in Serbia, and as such, we have shared it in the specialized beekeeping journals Srpski pčelar and Beogradski pčelar.

In our paper, we thoroughly analysed various factors contributing to honeybee colony losses and offered solutions based on the latest research and practical experiences. Additionally, during the course of our research, we have identified and incorporated more recent studies, thus enriching our paper with up-to-date references. Our goal is to enhance the understanding of these issues and provide beekeepers with tools to help preserve their bee colonies.

We hope this reading will be not only interesting but also useful for your work. We are happy to answer all your questions and suggestions, which you can send to the editorial address of the journal "No bees no life".

LOOKING FOR THE CAUSES OF AND SOLUTIONS TO THE ISSUE OF HONEY BEE COLONY LOSSES

Summary

Colony losses, including those induced by the colony collapse disorder, are an urgent problem of contemporary apiculture which has been capturing the attention of both apiculturists and the research community. CCD is characterized by the absence of adult dead bees in the hive in which few workers and a queen remain, the ratio between the brood quantity and the number of workers is heavily disturbed in favour of the former, and more than enough food is present. Robbing behaviour and pests usually attacking the weakened colony do not occur. In the present paper, the causes of the emergence of this problem are discussed, as well as the measures of its prevention. The following factors, which lead to colony losses, are analyzed: shortage of highquality food (pollen and honey); infestation with parasites, primarily with Varroa destructor, and mixed virus infections; bacterial infections (American and European foulbrood), fungal infections (nosemosis and ascosphaerosis) and trypanosomal infections (lotmariosis); and, finally, general management of the apiary. Certain preventive measures are proposed: (1) providing ample high-quality forage and clean water, (2) avoiding sugarisation, i.e. superfluous use of sugar syrup, (3) meeting the nutritional needs of the colony, (4) when feeding bees, taking care of the timing and the composition of diet, avoiding pure sugar syrup which in excessive quantities may induce energetic and oxidative stress, (5) when there is



a shortage of natural feed – honey in the brood chamber – use sugar syrup with natural/artificial supplements to avoid protein starvation, (6) organized control of V. destructor in the colonies is obligatory due to its vector role, and (7) compliance with hygienic and sanitary measures and principles of good apiculture practice and management in apiaries. To conclude, all preventive measures are feasible in compliance with rules and regulations concerning regular spring and autumn bee health monitoring by licensed veterinarians, who can propose adequate treatments if necessary.

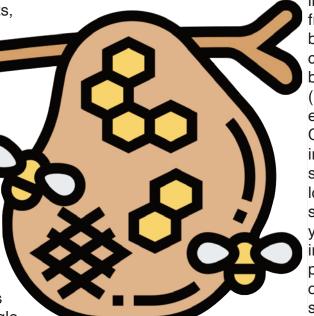
Key words: Apis mellifera, colony losses, honey bee pathogens, nutrition, pesticides

INTRODUCTION

Given their contribution to pollination, managed honey bees (Apis mellifera) are far more respected for this activity than for the production of honey and other products [1, 2]. Thus, it is understandable that substantial losses of bee colonies in the United States and Europe keep start-

ling beekeepers and scientists. especially because there is no agreement on the definite cause(s) of this syndrome. Extensive research has shown that no single factor can be accused of the losses, which, leads to seemingly the only possible conclusion that multiple stressors must be involved, such as loss of forage, pathogens, parasites, agropesticides and incorrect beekeeping practices [3-10]. Thus, it is very hard to propose a single

solution which could be most effective [11]. Historical data prove there were huge losses in the past, although they did not generate such avid interest. Even though tremendous losses do exist throughout the world, their causes vary with time and place. Many international attempts at understanding the causes and occurrence of bee losses were made. Recently, two independent groups of scientists conducted extensive studies. A two-year monitoring of nearly 6,000 apiaries in 17 European countries revealed that winter losses, ranging widely from 2% to 32%, were frequently followed by seasonal losses [12]; the CO-LOSS questionnaire filled out by 14,813 beekeepers from 27 European countries, Algeria, Israel and Mexico revealed that in winter 2016/2017 out of 425,762 colonies, 5.1% suffered unsolvable gueen problems and 14.1% failed to survive the winter [13]. Natural disasters killed another 1.6% of honey bee colonies, which added to a total of 20.9% colony losses. The losses varied between countries and were considerably higher in apiaries owned by beekeepers who had small numbers of colonies. Analysis detected that migratory beekeeping did not affect significantly the winter loss, but had some influence in several countries [13]. Because of the multifactorial nature of colony losses, it is extremely complicated to conduct controlled reproducible research on the influence of factors involved [6,180]. Despite the steep decrease in the numbers of managed bee colonies in Europe (25% in central Europe from 1985 to 2005) and



in North America (59% from 1947 to 2005), globally there was a significant rise in their number bv approximately 45% (1961-2008), owing to the increase in enormous China and Argentina, for instance [6]. Thus, it was suggested that colony losses should be considered throughout the year rather than taking into account those happening in winter only. Colony losses, in general, should not be equated specific phenomenon

known as colony collapse disorder – CCD [7,180], which was introduced and described by van Engelsdorp et al. [14] as a condition manifested in sudden bee death and the absence of adult dead bees, both inside and in front of the hive.

with

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Most frequently, there is a queen and few (a handful) workers who have survived. There is a



huge quantity of brood, which is disproportional to the number of worker bees. There are ample food reserves (honey and bee bread). It is also characteristic that in the hives robbing behaviour and invasion by common pests (wax moths and small hive beetle) appear much later [11, 14-16].

In compliance with the European Food Safety Authority – EFSA [17], the most

frequent causes of colony losses are:

• Shortage of high-quality food (pollen and honey),

• Parasitic infestations, primarily by Varroa destructor and mixed virus infections,

• Bacterial infections (American and European foulbrood), fungal (nosemosis and

ascosphaerosis) and other infections,

· General management in the apiary.

Shortage of high-quality food

Global climate changes, environmental pollution and "chemisation" in all human activities, especially in agriculture, lead to disturbances in the ecosystem, plant production, and production of high-quality food intended for bees. Global changes in the flowering dynamics and the quan-



tity and quality of pollen and nectar [18], as well as the practice of growing monocultures (corn), which are of low quality for bees, additionally increase the risk of protein starvation in bees [19-21]. In the last several decades, the diversity of melliferous plant species changed globally, and in Serbia alone Stachys annua disappeared, and some others have drastically decreased nectar and pollen production, e.g., Trifolium repens, T. pratense, Melissa officinalis, Thymus serpyllum and Mentha piperita [22]. Moreover, intensive pesticide use decreases the production of pollen, which is transformed into quality bee bread, the main protein source for 3-18-day-old bees and older open brood [18, 22, 23]. Further, a decline in the number of grazing animals (primarily sheep and goats), and, consequently, decreased manure production, leads to impoverished land, significantly lower reproduction of melliferous plants. Pollen quality changes throughout the season, the best being provided by early blooming plants: Corylus avellana, Salix alba and S. nigra, Helleborus odorus, Galanthus nivalis, Viola spp., Laminum spp., fruit trees, Taxacarum spp., Poaceae family etc. Nevertheless, the number of hives has been on the increase, owing to the needs of people to contribute to their household income, which led to the overpopulation of certain areas with hives and shortage of food for bees [10]. In attempts to gain growth in earnings, beekeepers nealect the bees' needs: they deprive them of honey even from the brood chamber, which belongs exclusively to the bees and is an ideally balanced, energy and protein-rich food, the most important factor for wintering and fast spring development of the brood and the colony as a whole. By mixing the honey from brood and honey chambers, beekeepers harm the bees and themselves. Thus, bees remain without the best energy and protein-rich food, which the beekeepers try to make up for by feeding the bees with sugar syrup. However, it provides only additional energy, negatively influences the development and survival of the colony, disturbs the structure of the winter cluster, leads to energy and oxidative stress and decline in the immune system, and intensifies pathogen development and reproduction [14, 18, 20, 24-31, 181, 189]. This leads to the disturbance of temporal polytheism and the absence of fewer sanitary bees (in-hive 12-18-day-old bees), that is, to decreased hygienic and grooming behaviour [32-39].

Temporal polytheism is of utmost importance. Worker bees specialized in performing various tasks communicate closely, which provides colony survival but also enables easier pathogen



distribution in the colony (Figure 1). In addition, in inadequately fed bee colonies, a decrease in the numbers of workers and drones and lower vitality and resistance to pathogens (notably to N. ceranae) are often noticeable [40].

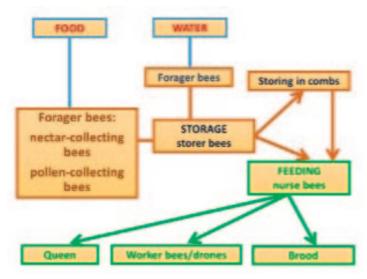


Figure 1. Flow of food and water in the honey bee colony and communication between different bee castes

Moreover, honey taken from brood chambers may contain residues of various preparations (amitraz, coumaphos, cymiazole hydrochloride, flumethrine, fluvalinate, dicyclohexylamine and fumagillin) which contaminate the honey directly (used in autumn and winter-spring treatments), or indirectly, from already contaminated wax. This honey is not eligible for human consumption because antibiotic and pesticide residues may exert various genotoxic effects [41-48].

Infestation with Varroa destructor and mixed virus infections

Varroa destructor, an obligate parasite of A. mellifera, poses the greatest threat to beekeeping. Due to the absence of an efficacious control programme, infested bee colonies collapse in a 2-to-3-year period [49]. Together with the associated bee viruses, the mite is one of the main causes of winter losses of bee colonies [7, 50-53]. Recently, it has been detected that Varroa feeds on the fat body of the larvae and adult bees [54] rather than exclusively on haemolymph, as

previously considered [49, 55, 56]. Moreover, the reproductive performance was better in mites fed on fat bodies than in those who consumed haemolymph [54, 186]. V. destructor harms the bees directly by exhausting them due to the disturbances they make in their metabolic processes, notably that of proteins [57], and indirectly, acting as a vector and/or activator of bee viruses [52, 58-60]. The mite and the Deformed Wing Virus-DWV together produce the most deleterious effect on the bees, which leads to the reduction in their lifespan and have been one of the most common causes of colony losses across the globe over the past 50 years [55, 56, 61, 62, 186]. Nevertheless, other viruses or their combination (Acute Bee Paralysis Virus - ABPV or Acute Kashmir-Israeli complex - AKI) may also cause colony losses together with Varroa mites as drivers that increase viral titters [51, 52, 63, 64].

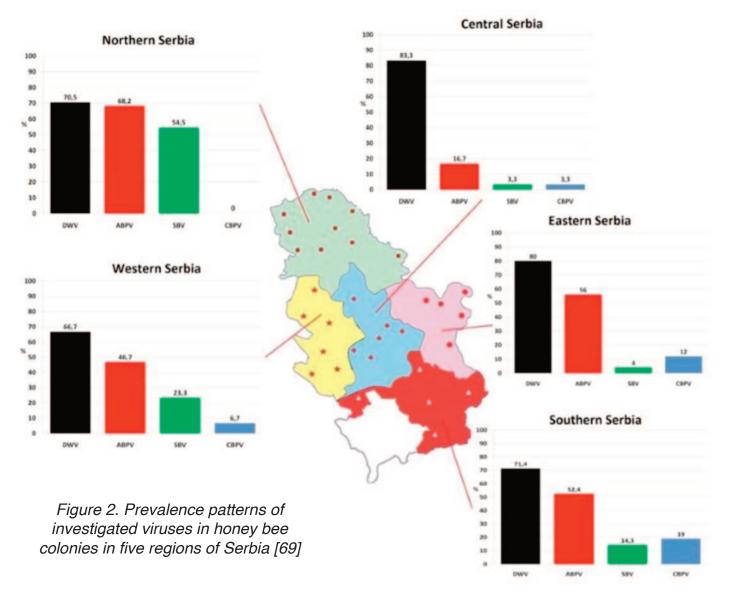
Investigating the mechanisms of synergistic actions between V. destructor and DWV, Nazzi et al. [62] proved that the mite is capable of destabilizing the dynamics of DWV development in the bees' body and leading to the transformation of the virus into a fast-replicating killer, which reaches lethal levels at the end of the season. The destabilization of a strong down-regulation of the transcription nuclear factor kappa B (NFkB) leads to immunosuppression [62] and the disturbance of various levels of immunity regulated by this factor, such as the synthesis of antimicrobial peptides, haemocyte aggregation at the sites of injuries, melanisation and antiviral mechanisms of defense [65]. It is considered that the immunosuppressive effect, primarily owing to viruses, increases the negative impact of the transcriptional profile of several immune genes in the bee. Moreover, in the absence of viruses, Varroa mites do not influence the expression of the dorsal-1A gene, an indicator of immunosuppression, unlike DWV, which produced the largest decrease in the expression of this gene [62]. This immunosuppressive activity is explained as part of the strategy used by DWV to conquer the central components of the host's antiviral immunity, thus providing conditions for covert infection.

However, any environmental stressor (e.g., pesticides or poor nutrition) may disturb the delicate, balanced relationship between the viral pathogens and the bees' defense mechanisms,



leading to the activation of the response through NF-kB and intensive virus replication in bees in which the infection was covert until then. The most common final consequence of fast virus replication is the collapse of the bee colony [61, 62, 66]. Further investigation into the mechanisms of interaction in the mite-virus complex and the induction of honey bee colony losses revealed the existence of mutualistic symbiosis between Varroa parasites and DWV, which is aimed at defeating the immune barriers of the host [55]. Additional research into the bees' response to simultaneous Varroa infestation and DWV infection proved that honey bees are able to promptly produce high immune and homeostatic response, which does not last long and is followed by downregulation of these pathways, rendering the bees susceptible to extensive virus replication [56]. Fine et al. [67] reported that even inert substances in agrochemicals may also add to some

negative effects on bees, including increased virus-induced mortality. Herbicides, although designed to inhibit weed growth, limit the availability of floral resources and adversely affect the bees` nutritional status, indirectly influencing the outcome of bee virus infections. In Serbia, the first molecular research into the presence of bee viruses was conducted on 11 apiaries [68] including 55 honey bee colonies from different regions. Real-time RT-PCR detected DWV in all apiaries and ABPV in 10 out of 11. Similar but more extensive research was done by Cirkovic et al. [69], who investigated the prevalence of DWV, Chronic Bee Paralysis Virus (CBPV), ABPV and the Sacbrood Virus (SBV) in colonies of different strengths located in five regions of Serbia (Figure 2). The analyses detected at least one virus in 87.33% of the colonies. Single infection was found in 28.67% colonies (21.33%, 4.00%, 2.67% and 0.67% in cases of DWV, ABPV, SBV





and CBPV, respectively). In the majority of them (58.66%) more than one virus was found. The most prevalent was DWV (74%), followed by ABPV, SBV and CBPV (in 49.30%, 24.00% and 6.70% colonies, respectively). Phylogenetic studies revealed that the honey bee viruses detected in Serbia were 93–99% identical with those deposited in GenBank.

Considerable numbers of beekeepers in Serbia refer to the Laboratory for genetics of domestic animals, wildlife and bees (Department of Biology, Faculty of Veterinary Medicine) each year, requesting analyses of samples taken from diseased or dead colonies. In these bees, the prevalence of viruses in the five-year period (2014-2018) was: DWV 73.12-87.16%, ABPV 61.54-81.45 and CBPV 58.82-64.22% (Figure 3). The history of diseases, based on the beekeepers' claims, most frequently pointed to: (1) inadequate anti-varroa treatment, or its absence; (2) depriving bees of large quantities of honey and feeding them on sugar, and (3) wintering bees on sunflower honey (which is often last produced in the season and contaminated with agropesticides).

It should be emphasized that the presence of virus infections of the brood and adult bees is influenced by apiculture technology (conventional vs. traditional). There are certain regions where bees are still kept in a traditional way, in primitive hives made of wicker – so-called trmka hives. Research conducted on the Pester Plateau, Serbia, showed that such beehives provide significantly better conditions for the maintenance of bee health and their resistance to pathogens [70]. Seemingly healthy colonies were kept for commercial purposes, and those in primitive hives were screened for bee brood virus (SBV) and adult bee viruses (Figure 4).

In traditional hives, SBV was detected in 33.33% of samples and in 96.67% in commercial colonies. Furthermore, the occurrence of viruses in adult bees was significantly higher in commercial colonies (Table 1). Obviously, in the brood and adult bees reared in a traditional way, in primitive hives, the prevalence of all the viruses monitored was up to 33.33%, without clinical symptoms, which is within the limits of normal distribution of viruses in bee colonies in natural conditions.



Figure 3. Presence of N. ceranae, DWV, ABPV and CBPV in bees analysed at the Laboratory for Genetics of Domestic Animals, Wildlife and Bees, Department of Biology, Faculty of Veterinary Medicine (2014-2018)

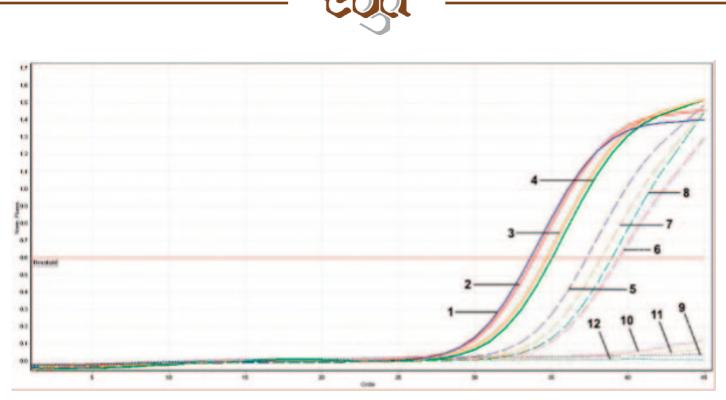


Figure 4. Amplification curves obtained by Real-Time PCR method showing detected viruses in adult bees. Lines 1-4 – positive controls: 1) Blue line – ABPV; 2) Red line – CBPV; Orange line – DWV; Green line - SBV; Dashed lines (5-8) – corresponding samples. Dotted lines (9-12) – negative controls. Individual replicates are shown for better clarity [70].

gens	Hive	Р		
gens .	Commercial ⁸ (%)	Traditional- trmka ⁹ (%)	- 1	
AFB ¹	16.67	0.00	< 0.05	
EFB ²	0.00	0.00	>0.05	
CB ³	15.83	0.00	< 0.05	
SBV^4	96.67	33.33	< 0.01	
ABPV ⁵	83.33	33.33	< 0.01	
CBPV ⁶	100.00	33.33	< 0.01	
DWV^7	100.00	33.33	< 0.01	
	EFB ² CB ³ SBV ⁴ ABPV ⁵ CBPV ⁶	ogens Commercial ⁸ (%) AFB ¹ 16.67 EFB ² 0.00 CB ³ 15.83 SBV ⁴ 96.67 ABPV ⁵ 83.33 CBPV ⁶ 100.00	CommercialTraditional- trmkaAFB116.670.00EFB20.000.00CB315.830.00SBV496.6733.33ABPV583.3333.33CBPV6100.0033.33	

Table 1. The prevalence of causative agents of bee diseases in commercial and traditionally reared bee colonies (based on the detection of their nucleic acids): 1. P. larvae, 2. M. plutonius, 3. A. apis, 4. Sacbrood virus, 5. Acute bee paralysis virus, 6. Chronic bee paralysis virus, 7. Deformed-wing virus; 8. ∑ N=120; 9. ∑ N=24 [70].

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In order to prevent huge colony losses due to mixed infections with Varroa and viruses. it is necessary to regularly control the mite, the vector of various pathogens [55, 56, 61, 62, 64, 69, 71, 72] There are various means of V. destructor control, which are successful to a varying extent. These methods are divided into biotechnical, chemical - use of synthetic 'hard' acaricides and ecological - use of 'soft' acaricides [49, 73], but may also be used in combination [74]. The longlasting work of Stanimirović et al. [75] resulted in the development of the Varroa-control strategy applicable in the Balkans (Figure 5). Biotechnical methods are time-consuming and insufficiently efficacious [74-77]. The use of 'hard' acaricides are followed by the following issues: (1) emergence of resistant mites, mainly to pyrethroids - fluvalinate and flumethrin [78-80], and (2) residues in all hive products: highest concentrations were proven in wax and propolis, lower in pollen and bee bread, and lowest in honey [49, 79, 81-84]. The most often detected varroacides in beeswax, pollen and bee bread are fluvalinate, coumaphos, amitraz and bromopropylate [84-89] and chlorfenvinphos in wax and bee bread samples from Spain [90]. Given that wax is a hive constituent which takes longest to be renewed, pesticides which remain in the hive may lead to the socalled 'toxic home syndrome'. This problem cannot be solved by wax replacement because residues of lipophilic acaricides remain in beeswax even after recycling [89]. Finally, synthetic acaricides may be harmful to bees and affect their reproductive traits and behavior, if not used correctly [43, 46, 48, 91, 92]. As a consequence of all the diagnosed problems arising from the use of synthetic acaricides, those which are natural-product-based, for example, organic acids and plant extracts, have come into widespread use [49, 73, 93, 183]. Their efficacy has been tested for more than two decades [73, 93-96]. Formic, oxalic and lactic acids are organic acids (OA) in use for the control of Varroa mites. Among plant extracts, essential oils (EO) and their components are by far the most studied for varroacide activity [75, 84]. General advantages of natural compounds, both OA and EO, are a low risk of residues and accumulation in bee products, and the low probability of eliciting resistance after repeated treatments [49]. If used

properly, their residues are low and the image of honey and other bee products as natural, healthy and clean remain untarnished. However, OA and EO have significant disadvantages: their efficacy is insufficient and depends on climatic and in-hive conditions, and means of application [49, 75, 93]. Some natural preparations exert side effects on bees and/or their brood [95, 97]. Luckily, some plant-derived formulations, rather effective, but without unwanted effects on colony development and productivity have been used recently [73, 96]. To sum up, neither of the methods of mite control, which has been used and investigated until now, can meet all these criteria: be safe for bees, highly efficacious against Varroa mites and easy to use. However, successful Varroa control is achievable if done in compliance with regulations concerning regular spring and autumn bee health monitoring by licensed veterinarians, who can propose adequate treatments.

VARROA CO	NTROL - GENERAL STRATEGY IN SERBIA
March	6-8 weeks before forage: treatment with formic acid (organic beekeeping) Flumetrin, fluvalinat or amitraz (conventional beekeeping)
April	Biotechnical methods
Мау	and
June	mite death of natural causes ("mite drop")
July	2 short or 1 long-lasting treatment with formic acid, or
August	a 3-week treatment with thymol (organic beekeeping)
September	Lithium citrate, amitraz, flumetrin or fluvalinat (conventional beekeeping)
October	
November	Winter treatment of bee colonies without brood Oxalic or lactic acid (organic beekeeping) Perizin or Apitol (conventional beekeeping)

Figure 5. Varroa control – general strategy in Serbia

Recently, in scientific circles, the idea was pushed forward that various lithium compounds could be used in Varroa control. Research carried out by Ziegelmann et al. [98] suggests that lithium salts may produce a marked acaricide effect. Our recent research proved the efficacy of lithiumsalt-based supplements (Figure 6A), especially those containing lithium citrate in various concentrations. Results showed that concentrations of 5 and 7.5 mM exerted a powerful acaricide effect, not affecting bee mortality (Figure 6A). In field experiments, the tested concentration (7.5 mM) had a satisfactory acaricide effectiveness (Figures 6B and 6C).

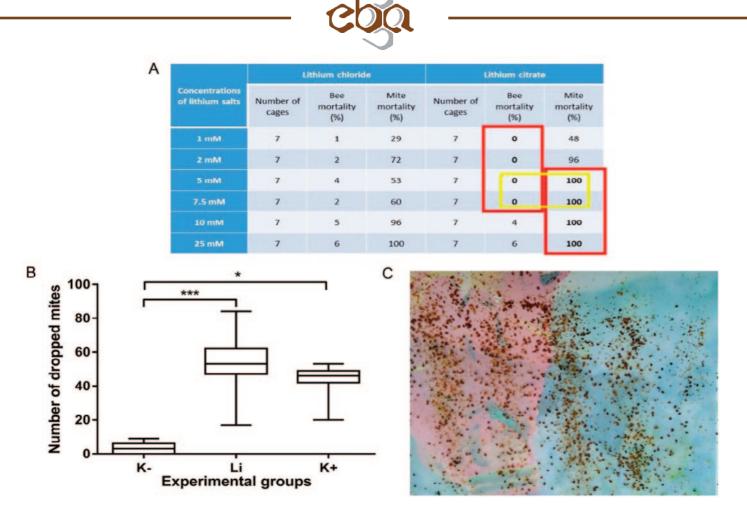


Figure 6. A) Anti-varroa effect of different lithium chloride and lithium citrate concentrations in a seven-day cage experiment; B) Anti-varroa effects of 7.5 mM lithium citrate. K- Negative control group (non-treated); Li – Group treated with lithium citrate; K+ Positive control (amitraz treated); C) Mites fallen following lithium citrate treatment.

Bacterial infections (American and European foulbrood) and fungal diseases (ascosphaerosis and nosemosis)

Bacterial diseases of special interest for the honey bee brood are American and European foulbrood [99]. Besides these bacterial diseases, an invasive and destructive mycosis caused by Ascosphaera apis should be considered as a contributor to honey bee health weakness [100]. Aspergillus spp., a cosmopolitan fungus, deserves greater attention because of its high virulence towards honey bee larvae and the ubiquity of its spores [101].

American foulbrood (AFB) is considered to be a fatal bee brood disease [50]. In some coun-

tries, it is frequent (e.g. 5-10% of bee colonies in Germany were found to be infected without symptoms) and causes considerable economic losses to the beekeeping industry. Clinical signs and the course of AFB disease vary, depending on the Paenibacillus larvae genotype, and the strength and behavioral defense mechanisms of bee colonies. The identification of P. larvae genotypes (made with rep-PCR) is important because of the differences in virulence and prognosis: genotypes ERIC I and II do not kill larvae in the early stage, but only after the comb cells are sealed, which is why hygienic bees cannot clean the diseased brood effectively, and the disease outbreaks, i.e. clinical symptoms become visible [102]; genotypes ERIC III and IV are highly virulent towards larvae, which is why the majority of them die before the cells are sealed, and hygienic bees clean the detritus and the typical disease symptoms are most often missing. These P. larvae genotypes differ in germination ability and resistance to different temperature treatments and



storage, which is why their genotyping should be checked as part of standard laboratory protocols [103].

Generally, European foulbrood (EFB) is spread in honey bees worldwide, that causes serious losses of brood and colony collapse [104]. For example, in Switzerland and Great Britain, EFB has posed great problems [105, 106] due to the failure of the sanitary measures applied, which led to the necessity of solving the problem of the spreading of M. plutonius [50]. Apart from classical microbiological methods of detection, certain molecular analyses for the conformation of M. plutonius have been developed based on PCR techniques [70, 106, 107].

Chalkbrood disease is often neglected when looking for the causes of colony losses, owing to the fact that the presence of A. apis is easily recognized and the infection diagnosed [108]. A. apis causes high brood mortality and significant decline in bee population in the hive. Strong colonies solve the problem by recruiting high numbers of hygienic worker bees, which easily recognize and get rid of "calcified" larvae, so the majority of these colonies heal in a short period of time [109]. However, chalkbrood disease may contribute to colony losses, especially those compromised by various etiological agents. The emergence of these diseases depends also on beekeeping techniques [12, 70].

Research was conducted on the Pester Plateau, Serbia [70] on seemingly healthy colonies kept in a traditional way, in primitive hives, and those in commercial hives. The colonies were screened for P. larvae, M. plutonius and A. apis (Figure 8). Traditional beehives provided significantly better conditions for maintenance of bee health and their resistance to pathogens: they were free from bacterial or fungal brood pathogens. By contrast, in commercial colonies P. larvae (16.67%), A. apis (15.83%) were detected, but M. plutonius was not found. Two thirds of traditionally kept colonies were without any of the bee pathogens checked, but not any of those

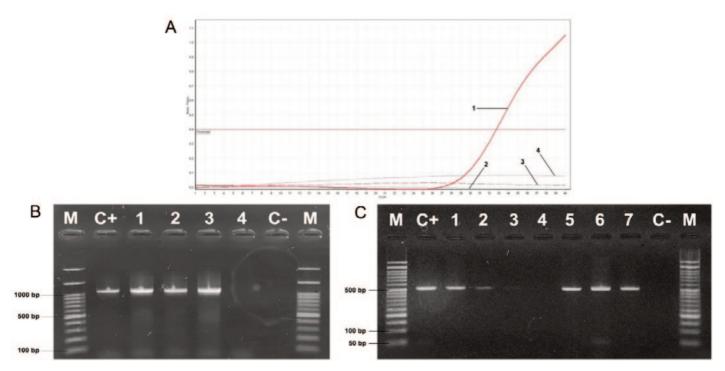


Figure 7. A) Amplification plots following real-time PCR demonstrating the detection of M. plutonius in brood samples. Continuous line (1) – positive control for M. plutonius; Dotted lines, blue and purple (2 and 4) – samples; Dashed line (3) – negative control [70]; B) Visualization of the PCR amplification products of P. larvae isolates. M – 100 bp ladder DNA marker; C+ positive control; 1-4 – samples; C- negative control. The sizes of the positive bands are indicated on the left [70]; C) Gel electrophoresis of DNA amplification products from the fungal isolates A. apis. M, 50 bp ladder DNA marker; C+, positive control; C- negative control; 1-2) samples. The sizes of the positive bands are indicated on the right [70].



kept for commercial purposes was free from all pathogens.

Nosemosis. Microsporidians and trypanosomatids and are the most abundant eukaryotic gut parasites of honey bees [110-114] and have been correlated with increased colony losses, although their role is still controversial [115-118]. There are three microsporidian species which may infect A. mellifera: Nosema ceranae, most prevalent and globally distributed [116, 119], N. apis, which prevails over N. ceranae only in cold climates [120-122], and N. neumanni, a recently described species, endemic in Uganda, causing low-level infection [123].

N. ceranae is thought to be a serious threat to the beekeeping industry, but dramatic colony losses were clearly attributed exclusively to N. ceranae infections in some regions only [124-126]. N. ceranae as a sole stressor showed suppressive effects on immune - related genes (Figure 8) in laboratory experiments [127-129]. However, in all pieces of research there was a time-dependent inconsistence in gene expression.

It has been proven by molecular diagnostics that N. ceranae is a dominant microsporidian pathogen of honey bees in Serbia [110, 111]. Among all bee samples collected from 2000 onwards, only one (originating from 2008) was proven to be infected with N. apis, whilst all other positive bees were infected with N. ceranae [110, 111]. Thus, it became clear that N. ceranae has been present in Serbia since at least 2000, and could not be regarded as an emergent pathogen in this region. Due to the absence of any molecular evidence that N. apis has ever been prevalent in Serbia, there is no ground for discussion about the replacement of N. apis with N. ceranae. As regards the prevalence of N. ceranae in Serbian

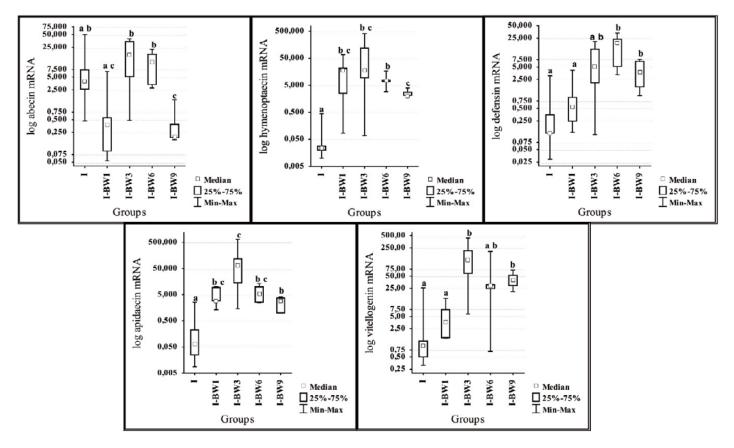


 Figure 8. Expression levels of genes for abaecin, hymenoptaecin, defensin, apidaecin and vitellogenin on day 12 after the infection with N. ceranae in groups treated with "BEEWELL AminoPlus". Groups were infected with N. ceranae spores on 3rd day after emerging and treated with "BEEWELL AminoPlus" from 1st (I-BW1), 3rd (I-BW3), 6th (I-BW6) and 9th (I-BW9) day, while the control group (I) was infected with N. ceranae but not treated. Different letters denote significant differences between groups [129].

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honey bee colonies, it was continually high, ranging from 73 to 98% (2008-2012). The highest was always recorded in March and ranged from 94 (2008) to 98% (2010). Lower, but still considerable proportions of infected colonies were detected in October (76-87%) and June (73-91%), according to Stevanovic et al. [111]. Nevertheless, the features of nosemosis type C caused by N. ceranae [130] in Spain (lack of seasonality, the absence of any clear symptoms and the inevitable collapse of infected colonies if not treated) have never been recorded in N. ceranae-infected bees in Serbia [111]. In fact, a seasonal pattern was affirmed in N. ceranae incidence in the period 2008-2011; (2) the symptoms traditionally attributed to N. apis infection (faecal marks, dead and sick crawling bees) were observed in the majority of N. ceranae-infected colonies; (3) no clear association between N. ceranae infection and colony losses was confirmed neither during the winter nor during the summer season [111]. As the symptoms of nosemosis were found in both surviving and dead colonies, being even more frequent among the former, these could not be indicative of colony losses. The same counts for some additional observations recorded in N. ceranae-infected colonies in winter: loose cluster on cold winter days (temperatures below -5°C) opposite to compact ones in non-infected colonies, and increased anxiety in bees at mild winter temperatures (0°C or slightly higher) manifested through an unusually high number of bees on the hive entrance after sound disturbing [111]. In compliance with all these is the finding that in

adult bees collected due to visible symptoms of diseases and delivered to our laboratory, the prevalence of N. ceranae in a five-year period (2014-2018) was 92.31-97.71% (Figure 3). To conclude, N. ceranae infection in Serbian bees, in the absence of other stressors, does not resemble nosemosis type C and exerts no marker indicative of colony losses. N. ceranae may be blamed for the decrease in the bees' reproductive capacities and honey production [131]. Its impact was investigated in equalized colonies headed by queens of different age (one-, two- and threeyear old) having in mind that in the queen exhausted by N. ceranae infection the renewal of the worker population might be compromised. Besides reproduction and productivity, in the three-year period (2009-2012), N. ceranae was monitored and quantified (spore load per colony). Significantly higher reproductive and productive values were recorded in colonies headed by younger queens (Table 2). This may be explained with their higher capacity to compensate the effects of N. ceranae infection, which gradually declines as they are getting older [131].

Far more frequently it was detected that N. ceranae and synergistic factors have deleterious effects on bees. For example, concurrent presence of N. ceranae and viruses have been confirmed to be capable of producing severe losses of honeybee colonies [132-134], while combinations of N. ceranae and pesticides were proved to increase the mortality of bees and alter the expression of immunity-related genes [135-138]. However, in the case of N. ceranae and thiaclo-

	ANOVA Age of the queen		Bonferroni test				
Parameter			One-year old queens/ Two-year old queens	One-year old queens/ Three-year old queens	Two-year old queens/ Three-year old queens		
	F	Р	p	p	p		
Brood size/ N. ceranae	159.67	<0.001	<0.001	<0.001	0.404		
Queen egg-laying rate /N. ceranae	106.42	<0.001	<0.001	<0.001	0.181		
Total extracted honey/N. ceranae	201.90	<0.001	<0.001	<0.001	0.007		
Winter honey stores/ N. ceranae	142.69	<0.001	<0.001	<0.001	0.266		

 Table 2. The influence of N. ceranae spore loads on parameters of reproduction and productivity in colonies with queens of different age [131]



prid combination, only the higher pesticide dose elicited significant mortality in bees, since thiacloprid showed a negative impact on N. ceranae reproduction [139].

Neonicotinoids were most frequently investigated for their influence on honey bee survival, health, behavior, immunity and reproductive and productive performances. Although numerous studies failed to provide a consistent conclusion, mostly due to the discrepancy between laboratory and field tests [140, 141], recent investigations provided the evidence that neonicotinoids exert significant negative effects on the health and survival of honey bees [142], their behavior [143], the reproductive capacity of drones [144], and that at field concentrations they may impair the immune defense [145, 146]. Comprehensive assessments of risks for bees by exposure to pesticide residues indicated the highest risk of contact exposure to pyrethroid and neonicotinoid residues via contaminated pollen. Moreover, neonicotinoids pose much higher risks in combination with ergosterol biosynthesis-inhibiting (EBI) fungicides because of their synergistic interactions [5, 147, 148], or in-hive miticides - tau-fluvalinate, coumaphos and fenpyroximate [149]. One of EBI fungicides is prochloraz, widely used in horticulture and agriculture, which has been detected in honey and pollen stored in hives [150]. It was previously found to increase almost 1,000-fold the toxicity of tau-fluvalinate, and more than 20-fold that of coumaphos and fenpyroximate [149]. Moreover, prochloraz altered the immune-gene expression in honey bees used alone and in combination with coumaphos [63, 151]. Recently, Glavinic et al. [138] monitored the expression of 15 immune-related genes in adult honey bees, and found that it may be affected

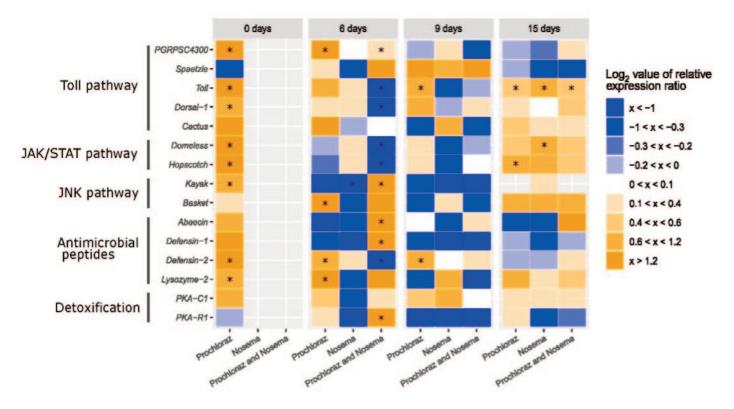


Figure 9. Heatmap immune-related genes in adult honey bee at different ages (0-, 6-, 9- and 15-days after honey bee emergence). The colors indicate the average mRNA levels compared to average levels of mRNA in control groups: blue indicates lower and yellow higher levels. Range log2 value of relative expression ratio is indicated in the legend on the right. Each row corresponds to one gene transcript and each column, to the expression profile of treatment. The gene names and the corresponding pathway are indicated on left side. Treatments are indicated in the scale at the bottom of the graph (Nosema-infected, CN; Prochloraz-treated, P; Prochloraz treated and Nosema-infected, PN). Control group (C) was used for normalization. Boxes marked with an asterisk show statistically significant effects of the treatment on gene expression, when p-value was equal or less than 0.05 [138].



when food contaminated with prochloraz was consumed by bee larvae (Figure 9). The results were obtained using a combination of a field and laboratory experiment which simulated the conditions where N. ceranae-infected and uninfected bee colonies are close enough to crop fields treated with prochloraz. Therefore, a great probability of crop contamination and, consequently, of the brood intended to produce the population of winter bees is likely. This new experimental approach introduced by Glavinic et al. [138] enables the insight into the realistic situation when beekeepers move their hives to sunflower forage, which is frequently the last one, so the bees are wintered on sunflower-derived food. In the control of Nosema infection, the introduction of good apitechnical practice is proposed, which includes: replacement of the queen with a new, young, promiscuous and healthy one [152, 187], feeding colonies with high-quality food (honey and bee bread), additional feeding with sugar syrup enriched with pollen and/or supplements, disinfection of hives and other equipment, sterilization of combs and removal of excess humidity from the hives. In addition, healthy and diseased hives should never be joined. In some countries, the antibiotic fumagillin is still used for Nosema control. The effectiveness of fumagillin was recently re-evaluated and proven in both laboratory and field conditions [153, 154], but may depend on storage, treatment preparation and the quantity consumed by bees etc. [153]. In Serbia, like in the majority of the world, the use of fumagillin is not recommended due to its adverse effects on bees [154, 155], the quality of bee products and consumer health, which result from its inadequate use [44, 45, 47, 156, 157].

Trypanosomal infections. Of the two trypanosomatid parasites of A. mellifera, Crithidia mellificae has been known for approximately 50 years and was considered widespread until recently, when Lotmaria passim was first described. The latter turned out to be predominant when detailed genetic analyses were performed [113]. Some investigators [158-162] suggested the possible involvement of trypanosomatids in honey bee health, their immune response and in winter colony losses. Investigations into the pathological effects of C. mellificae and L. passim on honey bees require primarily molecular tools for identification of these trypanosomatids, so Stevanovic et al. [112] designed and validated primers capable of distinguishing between C. mellificae and L. passim in conventional PCR,

Year ^b	L. passim infection only	N. ceranae infection only	Co-infection	Uninfected
2007	0	5	13	0
2008	0	11	7	0
2009	0	5	13	0
2010	1	4	10	3
2011	0	3	15	0
2012	2	2	13	1
2013	0	11	7	0
2014	0	6	12	0
2015	0	10	8	0
Total	3 (1.9%)	57 (35.2%)	98 (60.5%)	4 (2.5%)

^a All samples tested negative for N. apis and C. mellificae.

^b 18 colonies were sampled each year using 60 adult bees per colony.

Table 3. Summarized annual honey bee colony sampling in Serbia and infection status with Lotmaria passim and/or Nosema ceranae from 2007 to 2015 [112].



which enables routine research on their prevalence and epizootiology [163]. Moreover, primers for a real-time PCR were designed, and the method was optimized, which allows the simultaneous detection and quantification of L. passim and its in-depth field monitoring [114]. In the first long-lasting investigation into the presence of the two Trypanosoma species in the world, archived bee samples taken in Serbia (2007-2015) were analyzed, but only one species was detected, L. passim, with an annual prevalence of 38.9-83.3%, and 62.3% on average in the nine-year period. The same samples were also checked for N. ceranae, which was found in most samples with an overall frequency of 95.7%, ranging annually from 83.3% to 100%. Only 1.9% was infected with L. passim alone, while L. passim and N. ceranae simultaneously parasitized the same host at a fairly high rate: 60.5%, (Table 3). What is more, the detection of L. passim in bees sampled in 2007 in Serbia is its oldest genetically proven conformation globally and the first one in Serbia [112].

A high positive correlation (p <0.0001) between L. passim and N. ceranae infection levels pointed to their similar annual dynamics. Significant differences (p <0.0001) in infection levels with both species between months imply the seasonal character of their prevalence. The highest parasite burdens with N. ceranae and L. passim were detected in forager bees sampled in winter and lowest in those taken in mid-summer [114].

Lower activity of superoxide dismutase (SOD), increased activities of catalase (CAT) and glutathione S-transferase (GST) and higher concentrations of malondialdehyde (MDA) in the season imply that L. passim infection induces oxidative stress, which may negatively influence the condition and productivity of bees, and, consequently, render beekeeping less economical [164, 181, 189].

General management in the apiary

Apitechnical practice today relies on depriving bees of excessive amounts of honey. The basic principles of this idea date from the 1960s, in absolutely different conditions regarding climate, floristic diversity and agrotechnique. The climate has changed globally: there is less capillary humidity in the soil due to long-lasting periods of draught and recession of ground waters, which resulted in the decline in the diversity of melliferous plants. Moreover, global agricultural chemisation and chemical apitechnical measures aimed at the control of bee pathogens in the colony result in contamination of in-hive products [84, 165, 166]. Care should be taken to place the apiaries in the areas with ample forage and clean water. The vicinity of the forage and the numbers of bee colonies in the area per square kilometre hugely influence bee health and the production and reproductive capacities of bee colonies [23]. The distance between apiaries should be at least 1.5 km, to avoid competition for food collection, regardless of the quality of the forage, which means that the overpopulation of melliferous areas with hives should be avoided [167]. Technical revolution and the development of electric and communication systems strongly influence the orientation of bees when searching forage and returning to hives, the development of brood and the colony as a whole. Thus, care must be taken when deciding where to place apiaries: they should be far from overhead power lines and base radio stations [168-170]. Inadequate bee feeding with sugar syrup in order to make up for insufficient food reserves in years of famine, may also result in compromised condition and health of bee colonies and, finally, to colony losses. This is why beekeepers should know how to prepare bee food and when to provide bees with it. Sole sugar is a "necessary evil", and literature data suggest that it is much better to prepare and apply sugar-honey syrup (e.g. 7:3 sugar-honey ratio). The presence of honey, which contains a variety of active components, helps summer bees to transform it into the form nutritionally most useful to the bees. Beekeepers should know that bee feeding is sensible at the end of summer (e.g. in Serbia 1 August - 15 September), when there are enough summer bees, which are the only ones capable of transforming syrup into a satisfactory form for bee feeding. The use of supplements with sugar syrup should not be avoided, since they provide sufficient amino acids, peptides, micro- and macroelements which are absent from pure sugar syrup [18]. The use



of supplements may prevent energetic, immune and oxidative stress in bees, and thus prevent losses in apiaries [129, 171-174, 181, 186, 188]. The presence of a young, healthy bee queen in the hive guarantees the development of healthy bee colonies and successful beekeeping [131, 175, 181-182, 186, 188]. Suitable pathogen control in hives, primarily of the bee mite V. destructor, with effective, registered varroacides is also a prerequisite for maintaining bee colonies in a good health condition. In addition, a strong link was detected between colony losses and beekeepers' education and training: professionals were capable of keeping colonies free from diseases, unlike hobbyists [12, 70]. Professionals promptly detected symptoms, especially those of American foul brood or Varroa infestation, and timely applied control measures, contributing to the survival of their colonies. This was the first time that scientists focused attention on the impact of apiculturists and beekeeping practices on colony losses. The same authors commented that the introduction of a bee killer. Varroa mite, to Europe at the beginning of 1980s, did not result in increased colony losses. This was explained by the fact that beekeepers efficiently adopted measures to combat against the mite [12].

CONCLUSIONS

Scientific consensus has been reached that colony losses (CCD) are a multifactorial issue [3, 4, 6], which follows various conditions, but, according to our observations, it develops through a sequence of steps. Firstly, various non-specifi c factors (e.g. climate changes, agrochemisation and inadequate food) decrease the strength of the colonies; apitechnical faults (depriving bees of too much honey and a consecutive addition of large quantities of sugary food, inadequate treatments of colonies mainly against V. destructor, high stress and exhausting of bees, wintering colonies on honey contaminated with pesticides sunflower honey, bad timing for wintering the colonies etc.). Such colonies easily become eligible for bacterial, microsporidial and trypanosomal infections. Manifested nosemosis combined with Lotmaria infection and latent American foulbrood infection, additionally exhaust bee colonies and

impair the immune system of the bee [176-179]. Finally, inadequate anti-varroa strategies lead to significant health problems in bees and the spread of viruses for which Varroa is a vector, and/or activator. The whole process is a path prepared for the manifestation of virus infections. This has been supported by the results of our Laboratory, which confirmed high level of viruses in samples of diseased colonies accompanied by heavy loads of N. ceranae spores. The fact that in colonies with disease symptoms more spores were detected than in dead colonies, supports our hypothesis that after the Nosema-infection peak (when the immunity is lowest) bee viruses are activated and gradual decrease in Nosema load occurs. Viruses, already present in hives in Serbia [68, 69], are "waiting for the time of decreased immunity", become the ultimate executors of bee colonies, often leading to colony losses.

Given that this is a multifactorial issue, recommended solutions to the problem consist of a sequence of activities aimed at as many as possible individual factors:

• Compliance with good beekeeping practices and hygienic measures, both in the apiary and the hives, as well as in the facilities where beekeeping material, equipment and bee products are stored

• Positioning the apiary to places with ample melliferous plants and clean water

• Avoiding areas where pesticides are used intensively, which may, if present in nectar and pollen, enter the hive and harm bees' health and leave residues in the bee products

• Breeding healthy autochthonous bee colonies with young and healthy bee queens and compliance with bee selection programmes

• Providing enough quantities of honey, pollen and bee bread for colonies during winter

• The remaining honey, pollen, bee bread from hives which suffered from colony loss is not to be used for feeding healthy colonies. If the bees do not provide enough food, the shortage should be compensated with honey-sugar syrup or sugar syrup with the addition of supplements

• Beekeeping today is impossible without acaricides to get rid of Varroa mites. Anti-varroa treatments should be done with registered preparations, applied in the adequate period of the

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year. Combinations of preparations may be used to increase the efficacy, taking into consideration the interactions between them.

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References:

1. Gallai N, Salles JM, Settele J, Vaissière BE: Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecol Econ 2009, 68:810-821.

2. Moritz RFA, de Miranda J, Fries I, Le Conte Y, Neumann P, Paxton R: Research strategies to improve honeybee health in Europe. Apidologie 2010, 41:227– 242.

3. vanEngelsdorp D, Meixner MD: A historyical review of managed honey bee populations in Europe and the United States and the factors that may affect them. J Invertebr Pathol 2010, 103:S80-S95.

 Neumann P, Carreck NL: Honey bee colony losses. J Apicult Res 2010, 49:1-5. Sanchez-Bayo F, Goka K: Pesticide residues and bees - a risk assessment. PLoS ONE 2014, 9:e94482.

 Goulson D, Nicholls E, Botías C, Rotheray EL: Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science 2015, 347:1255957.

7. McMenamin AJ, Genersch E: Honey bee colony losses and associated viruses. Curr Opin Insect Sci, 2015, 8:121–129.

8. Sanchez-Bayo F, Goulson D, Pennacchio F, Nazzi F, Goka K, Desneux N: Are bee diseases linked to pesticides? - A brief review. Environ Int 2016, 89:7-11.

9. FAO - Food and Agriculture Organization of the United Nations: Pollinators vital to our food supply under threat. 2016, http://www.fao.org/news/story/en/item/384726/icode/.

10. Kulhanek K, Steinhauer N, Rennich K, Caron DM, Sagili RR, Pettis JS, Ellis JD, Wilson ME, Wilkes JT, Tarpy DR, Rose R, Lee K, Rangel J, vanEngelsdorp D. A national survey of managed honey bee 2015–2016 annual colony losses in the USA. J Apicult Res 2017, 56:328-340.

11. Ellis JD, Evans JD, Pettis J: Colony losses, managed colony population decline, and colony collapse disorder in the United States. J Apicult Res 2010, 49:134-136.

12. Jacques A, Laurent M, EPILOBEE Consortium, Ribière-Chabert M, Saussac M, Bougeard S, Budge GE, Hendrikx P, Chauzat M: A pan-European epidemiological study reveals honey bee colony survival depends on beekeeper education and disease control. PLoS ONE 2017, 12:e0172591.

13. Brodschneider R, Gray A, Adjlane N, Ballis A, Brusbardis V, Charrière JD, Chlebo R, Coffey MF, Dahle B, de Graaf DC, Dražić MM, Evans G, Fedoriak M, Forsythe I, Gregorc A, Grzęda U, Hetzroni A, Kauko L, Kristiansen P, Martikkala M, Martín-Hernández R, Medina-Flores CA, Mutinelli F, Raudmets A, Ryzhikov VA, Simon-Delso N, Stevanovic J, Uzunov A, Vejsnæs F, Wöhl S, Zammit-Mangion M, Danihlík J: Multi-country loss rates of honey bee colonies during winter 2016/2017 from the COLOSS survey. J Apicult Res 2018, 57:452-457.

 vanEngelsdorp D, Evans JD, Saegerman C, Mullin C, Haubruge E, Nguyen BK, Frazier M, Frazier J, Cox-Foster D, Chen Y, Underwood R, Tarpy DR, Pettis JS: Colony collapse disorder: a descriptive study. PLoS ONE 2009, 4:e6481.
 Cox-Foster DL, Conlan S, Holmes EC, Palacios G, Evans JD, Moran NA. Quan PL, Briese T, Hornig M, Geiser DM, Martinson V, vanEngelsdorp D, Kalkstein AL, Drysdale A, Hui J, Zhai J, Cui L, Hutchison SK, Simons JF, Egholm M, Pettis JS, Lipkin WI: A metagenomic survey of microbes in honey bee colony collapse disorder. Science 2007, 318:283-287.

16. Oldroyd BP: What's killing American honey bees? PLoS Biol 2007, 5:e168.

17. EFSA (European Food Safety Authority): Towards holistic approaches to the risk assessment of multiple stressors in bees. EFSA Scientifi c Colloquium Summary Report, 15–16 May 2013, Parma, Italy.

18. Lipinski Z, Honey bee nutrition and feeding, in the temperate/continental climate of the northen hemisphere, OZGraf S.A., Olsztyn – A.D., 2018, 1-430.

19. Vanbergen AJ, Insect Pollinators Initiative. Threats to an ecosystem service: pressures on pollinators. Front Ecol Environ 2013, 11:251-259.

20. Di Pasquale G, Salignon M, Le Conte Y, Belzunces LP, Decourtye A, Kretzschmar A, Suchail, S, Brunet JL, Alaux C. Infl uence of pollen nutrition on honey bee health: do pollen quality and diversity matter? PLoS ONE 2013, 8:e72016.

21. Asensio I, Vicente-Rubiano M, Muñoz MJ, Fernández-Carrión E, Sánchez-Vizcaíno JM, Carballo M: Importance of ecological factors and colony handling for optimizing health status of apiaries in Mediterranean ecosystems. PLoS ONE 2016, 11:e0164205.

22. Stanimirović Z, Soldatović B, Vučinić M: Honey bee – Biology of the bees [in Serbian] Beograd, Srbija: Medicinska knjiga – Medicinske komunikacije 2000, 1-375.

23. Cirkovic D, Stanimirovic Z: Beekeeping manual [in Serbian] Državni univerzitet u Novom Pazaru, Naučna KMD, Beograd, 2018, 1-241.

24. Williams GR, Tarpy DR, vanEngelsdorp D, Chauzat MP, Cox-Foster DL, Delaplane KS, Neumann P, Pettis JS, Rogers ELR, Shutler D: Colony collapse disorder in context. Bioessays 2010, 32:845-846.

25. Stanimirović Z, Ljubenković J, Stanimirović M, Stevanović J: Eneregetski stres kod medonosne pčele izazvan infekcijom Nosema ceranae. Drugi državni pčelarski sajam, 2010a. Savez Pčelarskih Organizacija Srbije, 41-48.

26. DeGrandi-Hoffman G, Yanping Chen, Huang E, Huang MH. The effect of diet on protein concentration, hypopharyngeal gland development and virus load in worker honey bees (Apis mellifera L.). J Insect Physiol 2010, 56:1184-1191.

27. Chensheng, LU, Warchol, KM, Callahan RA: Sub-lethal exposure to neonicotinoids impaired honey bee's winterization before proceeding to colony collapse disorder. B Insectol 2014, 67:125-130.

28. Wheeler MM, Robinson GE: Diet-dependent gene expression in honey bees: honey vs. sucrose or high fructose corn syrup. Sci Rep 2014, 4:5726.

29. Nikolić T, Purać J, Orčić S, Kojić D, Vujanović D, Stanimirović Z, Gržetić I, Ilijević K, Šikoparija B, Blagojević D: Environmental effects on superoxide dismutase and catalase activity and expression in honey bee. Arch Insect Biochem Physiol 2015, 90:181-194.

30. DeGrandi-Hoffman G, Chen Y: Nutrition, immunity and viral infections in honey bees. Curr Opin Insect Sci 2015, 10:170-176.

31. Orcic S, Nikolic T, Purac J, Sikoparija B, Blagojević P D, Vukasinovic E, Plavsa N, Stevanovic J, Kojic D: Seasonal variations in the activity of selected antioxidant enzymes and malondialdehyde level in worker honey bees. Entomol Exp Appl 2017, 165:120–128.

 Stanimirovic Z, Pejovic D, Stevanovic J, Vucinic M, Mirilovic M: Investigations of hygienic behaviour and disease resistance in organic beekeeping of two honeybee ecogeographic varieties from Serbia. Acta Vet–Beograd 2002, 52:169-180.

33. Stanimirovic Z, Stevanovic J, Cirkovic D: Behavioural defenses of the honey bee ecotype from Sjenica – Pester against Varroa destructor. Acta Vet-Beograd 2005a, 55:69-82.

34. Stanimirovic Z, Stevanovic J, Mirilovic M, Stojic V Heritability of hygienic behaviour in grey honey bees (Apis mellifera carnica). Acta Vet-Beograd 2008, 58:593-601.

35. Stanimirovic Z, Stevanovic J, Aleksic N, Stojic V: Heritability of grooming behaviour in grey honey bees (Apis mellifera carnica). Acta Vet-Beograd 2010b; 60:313-323.

36. Stanimirović Z, Aleksic N, Stevanovic J, Cirkovic D, Mirilovic M, Djelic N, Stojic V: The infl uence of pulverised sugar dusting on the degree of infestation of honey bee colonies with Varroa destructor. Acta Vet-Beograd 2011, 61:309-325.

37. Stevanovic J: Ecological-ethological defence mechanisms of Apis mellifera carnica against ectoparasite Varroa destructor on the territory of Serbia, PhD dissertation, University of Belgrade, 2007.

38. Pejovic D: Investigation of the heritability of hygienic and grooming behaviour of grey honey bee Apis mellifera carnica and the influence of these features on reproductive and productive traits of honey bee colonies, PhD dissertation, University of Belgrade, 2008.

39. Cirkovic D: The investigation on the effects of pulverised sucrose on the degree of honeybee infestation with Varroa destructor, PhD dissertation, University of Belgrade, 2011.

40. Goblirsch M, Huang ZY, Spivak M: Physiological and behavioral changes in honey bees (Apis mellifera) induced by Nosema ceranae infection. PLoS ONE 2013, 8:e58165.

41. Stanimirovic Z, Fister S, Stevanovic J: Analysis of sister chromatid exchanges in cultured human lymphocytes treated with cymiazole hydrochloride. Acta Vet-Beograd 2003a, 53:419-425.

42. Stanimirovic Z, Todorovic D, Stevanovic J, Mladenovic M, Jankovic Lj, Djorđevic M: Infl uence of cymiazole hydrochloride on mitotic and proliferative activities of cultured human lymphocytes. Acta Vet-Beograd 2003b, 53:47-55.

43. Stanimirovic Z, Stevanovic J, Jovanovic S, Andjelkovic M: Evaluation of genotoxic effects of Apitol® (cymiazole hydrochloride) in vitro by measurement of sister chromatid exchange. Mutat Res-Gen Tox En 2005b, 588:152-157.



44. Stanimirovic Z, Stevanovic J, Bajic V, Radovic I: Evaluation of genotoxic effects of fumagillin by cytogenetic tests in vivo. Mutat Res-Gen Tox En 2007a, 628:1-10.

45. Stanimirovic Z, Pejin II, Kulisic Z, Djiporovic M: Evaluation of genotoxic effects of fumagillin by sister chromatide exchange and chromosomal aberration tests in human cell cultures. Acta Vet-Beograd 2007b, 57:257-273.

 Pejin II, Stanimirović Z, Stevanović J, Kulišić Z: Evaluation of genotoxic potential of amitraz by cytogenetic test in vivo. Veterinarski glasnik 2006, 60:163-173.

47. Stevanovic J, Stanimirovic Z, Radakovic M, Stojic V: In vitro evaluation of the clastogenicity of fumagillin. Environ Mol Mutagen 2008, 49:594-601.

48. Radakovic M, Stevanovic J, Djelic N, Lakic N, Knezevic-Vukcevic J, Vukovic-Gacic B, Stanimirovic Z: Evaluation of the DNA damaging effects of amitraz on human lymphocytes in the Comet assay. J Biosciences 2013, 38:53-62.

49. Rosenkranz P, Aumeier P, Ziegelmann B: Biology and control of Varroa destructor. J Invertebr Pathol 2010, 103:S96–S119.

50. Genersch E: Honey bee pathology: current threats to honey bees and beekeeping. Appl Microbiol Biotechnol 2010, 87:87-97.

51. Genersch E, von der Ohe W, Kaatz H, Schroeder A, Otten C, Büchler R, Berg S, Ritter W, Mühlen W, Gisder S, Meixner M, Liebig G, Rosenkranz P: The German bee monitoring project: a long-term study to understand periodically high winter losses of honey bee colonies. Apidologie 2010, 41:332-352.

52. Genersch E, Aubert M: Emerging and re-emerging viruses of the honey bee (Apis mellifera L.). Vet. Res. 2010 41:54.

53. Guzmán-Novoa E, Eccles L, Calvete Y, Mcgowan J, Kelly PG, Correa-Benítez A: Varroa destructor is the main culprit for the death and reduced populations of overwintered honey bee (Apis mellifera) colonies in Ontario, Canada. Apidologie 2010, 41:443-450.

54. Ramsey SD, Ochoa R, Bauchan G, Gulbronson C, Mowery JD, Cohen A, Lim D, Joklika J, Cicero JM, Ellis JD, Hawthorne D, vanEngelsdorp D: Varroa destructor feeds primarily on honey bee fat body tissue and not hemolymph. P Natl Acad Sci USA 2019, 116:1792-1801.

55. Di Prisco G, Annoscia D, Margiotta M, Ferrara R, Varricchio P, Zanni V, Caprio E, Nazzi F, Pennacchio F: A mutualistic symbiosis between a parasitic mite and a pathogenic virus undermines honey bee immunity and health. P Natl Acad Sci USA, 2016, 113:3203-3208.

56. Zhao Y, Heerman M, Peng W, Evans JD, Rose R, DeGrandi-Hoffman G, Simone-Finstrom M, Li J, Li Z, Cook SC, Su S, Rodríguez-García C, Banmeke O, Hamilton M, Chen Y: The dynamics of deformed wing virus concentration and host defensive gene expression after Varroa mite parasitism in honey bees, Apis mellifera. Insects 2019, 10:16.

57. Alaux C, Dantec C., Parrinello H, Le Conte Y: Nutrigenomics in honey bees: digital gene expression analysis of pollen's nutritive effects on healthy and varroa-parasitized bees. BMC Genomics 2011, 12:496.

58. Shen M, Yang X, Cox-Foster D, Cui L: The role of varroa mites in infections of Kashmir bee virus (KBV) and deformed wing virus (DWV) in honey bees. Virology 2005a, 342:141- 149.

59. Shen M, Cui L, Ostiguy N, Cox-Foster D: Intricate transmission routes and interactions between picorna-like viruses (Kashmir bee virus and sacbrood virus) with the honeybee host and the parasitic varroa mite. J Gen Virol 2005b, 86:2281-2289.

60. Berényi O, Bakonyi T, Derakhshifar I, Köglberger H, Nowotny N: Occurrence of six honeybee viruses in diseased Austrian apiaries. Appl Environ Microbiol 2006, 72:2414- 2420.

61. Martin SJ, Highfi eld AC, Brettell L, Villalobos EM, Budge GE, Powell M, Nikaido S, Schroeder DC: Global honey bee viral landscape altered by a parasitic mite. Science 2012, 336:1304–1306.

62. Nazzi F, Brown SP, Annoscia D, Del Piccolo F, Di Prisco G, Varricchio P, Della Vedova G, Cattonaro F, Caprio E, Pennacchio F: Synergistic parasite-pathogen interactions mediated by host immunity can drive the collapse of honeybee colonies. PLoS Pathog 2012, 8:e1002735.

63. Gregorc A, Evans JD, Scharf M, Ellis JD: Gene expression in honey bee (Apis mellifera) larvae exposed to pesticides and Varroa mites (Varroa destructor). J Insect Physiol 2012, 58:1042-1049.

64. Francis RM, Nielsen SL, Kryger P: Varroa-virus interaction in collapsing honey bee colonies. PLoS ONE, 2013, 8:e57540.

65. Sabin LR, Hanna SL, Cherry S: Innate antiviral immunity in Drosophila. Curr Opin Immunol 2010, 22:4–9.

66. Nazzi F, Pennacchio F: Disentangling multiple interactions in the hive ecosystem. Trends Parasitol 2014, 30:556–561.

67. Fine JD, Cox-Foster DL, Mullin CA: An inert pesticide adjuvant synergizes viral pathogenicity and mortality in honey bee larvae. Sci Rep-UK 2017, 7:40499.

68. Simeunovic P, Stevanovic J, Vidanovic D, Nisavic J, Radovic D, Stanisic Lj, Stanimirovic Z: A survey of deformed wing virus and acute bee paralysis virus in honey bee colonies from Serbia using real-time RT-PCR. Acta Vet-Beograd 2014a, 64:81-92.

69. Cirkovic D, Stevanovic J, Glavinic U, Aleksic N, Djuric S, Aleksic J, Stanimirovic Z: Honey bee viruses in Serbian colonies of different strength. PeerJ 2018, 6:e5887.

70. Taric E, Glavinic U, Stevanovic J, Vejnovic B, Aleksic N, Dimitrijevic V, Stanimirovic Z: Occurrence of honey bee (Apis mellifera L.) pathogens in commercial and traditional hives. J Apicult Res 2019, DOI: 10.1080/00218839.2018.1554231.

71. Glavinic U, Stevanovic J, Gajic B, Simeunovic P, Đuric S, Vejnovic B, Stanimirovic Z: Nosema ceranae DNA in honey bee haemolymph and honey bee mite Varroa destructor. Acta Vet-Beograd 2014, 64:349-357.

72. Grozinger CM, Flenniken ML: Bee Viruses: Ecology, Pathogenicity, and Impacts. Annu Rev Entomol 2019, 64:205-226.

73. Stanimirović Z, Glavinic U, Lakic N, Radovic D, Ristanic M, Taric E, Stevanovic J: Efficacy of plant-derived formulation "Argus Ras" in Varroa destructor control. Acta Vet-Beograd 2017, 67:191-200.

74. Giacomelli A, Pietropaoli M, Carvelli A, Iacoponi F, Formato G: Combination of thymol treatment (Apiguard®) and caging the queen technique to fight Varroa destructor. Apidologie 2016, 47:606-616.

75. Stanimirović Z, Ćirković D, Pejin II, Pejović D: Strategy for ecologic control in fighting Varroa destructor. Veterinarski glasnik 2007c, 61:11-35.

76. Stevanovic J, Stanimirovic Z, Lakic N, Aleksic N, Simeunovic P, Kulisic Z: Safety assessment of sugar dusting treatments by analysis of hygienic behavior in honey bee colonies. Arch Biol Sci 2011a, 63:1199-1207.

77. Stevanovic J, Stanimirovic Z, Lakic N, Djelic N, Radovic I: Stimulating effect of sugar dusting on honey bee grooming behaviour. Entomol Exp Appl 2012a, 143:23-30.

78. Lodesani M, Costa M: Limits of chemotherapy in beekeeping: development of resistance and the problem of residues. Bee World 2005, 86:102–109.

79. Loucif-Ayad W, Aribi N, Smagghe G, Soltani N: Comparative effectiveness of some acaricides used to control Varroa destructor (Mesostigmata: Varroidae) in Algeria. Afr Entomol 2010, 18:259–266.

80. Kanga LHB, Adamczyk J, Marshall K, Cox R: Monitoring for resistance to organophosphorus and pyrethroid insecticides in Varroa mite populations. J Econ Entomol 2010, 103:1797- 1802.

81. Bogdanov S: Contaminants of bee products. Apidologie 2006, 37:1-18.

82. Adamczyk S, Lázaro R, Pérez-Arquillué C, Herrera Ā: Determination of synthetic acaricides residues in beeswax by high-performance liquid chromatography with photodiode array detector. Anal Chim Acta 2007, 581:95–101.

83. Adamczyk S, Lázaro R, Pérez-Arquillué C, Bayarri S, Herrera A: Impact of the use of fl uvalinate on different types of beeswax from Spanish hives. Arch Environ Con Tox 2010, 58:733–739.

84. Stevanovic J, Stanimirovic Z, Aleksic N, Simeunovic P, Vucicevic M: The infl uence of natural and synthetic substances applied in honey bee health care on the quality of bee products (invited paper). Proceedings of the Apimondia Symposium "APIECOTECH SERBIA 2012", Feb 18-19, 2012b, Belgrade, Serbia, pp 9-34.

85. Bogdanov S, Kolchenmann V, Imdorf A: Acaricide residues in some bee products. J Apicult Res 1998, 37:57-67.

86. Chauzat MP, Faucon JP, Martel AC, Lachaize J, Cougoule N, Aubert M: A survey of pesticide residues in pollen loads collected by honey bees in France. J Econ Entomol 2006, 99:253–262.

87. Chauzat MP, Faucon JP: Pesticides residues in beeswax samples collected from honey-bee colonies (Apis mellifera) in France. Pest Manag Sci 2007, 63:1100–1106.

88. Frazier M, Mullin C, Frazier J, Ashcraft S: What have pesticides got to do with it? Am Bee J 2008, 148:521–523.

89. Mullin CA, Frazier M, Frazier JL, Ashcraft S, Simonds R, vanEngelsdorp D, Pettis JS: High levels of miticides and agrochemicals in North American apiaries: Implications for honey bee health. PLoS ONE 2010, 5:e9754.

90. Orantes-Bermejo FJ, Pajuelo AG, Megías MM, Fernández-Píñar C: Pesticide residues in beeswax and beebread samples collected from honey bee colonies (Apis mellifera L.) in Spain. Possible implications for bee losses. J Apicult Res 2010, 48:243-250.

91. Chauzat MP, Carpentier P, Martel AC, Bougeard S, Cougoule N, Porta P, Lachaize J, Madec F, Aubert M, Faucon JP: Influence of pesticide residues on honey bee (Hymenoptera: Apidae) colony health in France. Environ Entomol 2009, 38:514–523.

92. Bevk D, Kralj J, Čokl A: Coumaphos affects food transfer between workers of honeybee Apis mellifera. Apidologie 2012, 43:465–470.

93. Umpiérrez ML, Santos E, González A, Rossini C: Plant essential oils as potential control agents of varroatosis. Phytochem Rev 2011, 10:227-244.

94. Satta A, Floris I, Eguaras M, Cabras P, Garau VL, Melis M: Formic acidbased treatments for control of Varroa destructor in a Mediterranean area. J Econ Entomol 2005, 98:267-273.

95. Damiani N, Gende LB, Bailac P, Marcangeli JA, Eguaras MJ: Acaricidal and insecticidal activity of essential oils on Varroa destructor (Acari: Varroidae) and Apis mellifera (Hymenoptera: Apidae). Parasitol Res 2009, 106:145–152.

96. DeGrandi-Hoffman G, Ahumada F, Probasco G, Schantz, L: The effects of beta acids from hops (Humulus lupulus) on mortality of Varroa destructor (Acari: Varroidae). Exp Appl Acarol 2012, 58:407-421.

97. Ebert TA, Kevan PG, Bishop BL, Kevan SD, Downer RA: Oral toxicity of essential oils and organic acids fed to honey bees (Apis mellifera). J Apicult Res 2007, 46:220–224.

98. Ziegelmann B, Abele E, Hannus S, Beitzinger M, Berg S, Rosenkranz P: Lithium chloride effectively kills the honey bee parasite Varroa destructor by a systemic mode of action. Sci Rep-UK 2018, 8:683.

99. Forsgren E: European foulbrood in honey bees, J Invertebr Pathol 2010, 103:5-9.

100. Vojvodic S, Boomsma JJ, Eilenberg J, Jensen AB: Virulence of mixed fungal infections in honey bee brood. Front Zool 2012, 9:5.

101. Jensen AB, Aronstein K, Flores JM, Vojvodic S, Palacio MA, Spivak M: Standard methods for fungal brood disease research. In: Dietemann V, Ellis JD and Neumann P (Eds.) The COLOSS BEEBOOK, Volume II: Standard methods for Apis mellifera research. J Apicult Res 2013, 52 (1) DOI: 10.3896/IBRA.1.52.1.13.

102. Ashiralieva A, Genersch E: Reclassifi cation, genotypes and virulence of Paenibacillus larvae, the etiological agent of American foulbrood in honeybees – a review. Apidologie 2006, 37:411-420.

103. Forsgren E, Stevanovic J, Fries I: Variability in germination and in temperature and storage resistance among Paenibacillus larvae genotype. Vet. Microbiol



2008, 129:342-349.

104. Forsgren E, Budge GE, Charrière JD, Hornitzky MA: Standard methods for European foulbrood research. In: Dietemann V, Ellis JD and Neumann P (Eds.) The COLOSS BEEBOOK, Volume II: Standard methods for Apis mellifera research. J Apicult Res 2013, 52.

105. Wilkins S, Brown M, Andrew A, Cuthbertson GS: The incidence of honey bee pests and diseases in England and Wales. Pest Manag Sci 2007, 63:1062–1068.

106. Roetschi A, Berthoud H, Kuhn R, Imdorf A: Infection rate based on quantitative real-time PCR of Melissococcus plutonius, the causal agent of European foulbrood, in honeybee colonies before and after apiary sanitation. Apidologie 2008, 39:362–371.

107. Forsgren E, Lundhagen AC, Imdorf A, Fries I: Distribution of Melissococcus plutonius in honeybee colonies with and without symptoms of European foulbrood. Microbial Ecol 2005, 50:369–374.

108. Hedtke K, Jensen PM, Jensen AB, Genersch E: Evidence for emerging parasites and pathogens influencing outbreaks of stress-related diseases like chalk-brood. J Invertebr Pathol 2011, 108:167-173.

109. Aronstein K, Murray KD: Chalkbrood disease in honey bees. J Invertebr Pathol 2010, 103:S20–S29.

110. Stevanovic J, Stanimirovic Z, Genersch E, Kovacevic RS, Ljubenkovic J, Radakovic M, Aleksic N. Dominance of Nosema ceranae in honey bees in the Balkan countries in the absence of symptoms of colony collapse disorder. Apidologie 2011b, 41:49-58.

111. Stevanovic J, Simeunovic P, Gajic B, Lakic N, Radovic D, Fries I, Stanimirovic Z: Characteristics of Nosema ceranae infection in Serbian honey bee colonies. Apidologie 2013, 44:522-536.

112. Stevanovic J, Schwarz RS, Vejnovic B, Evans JD, Irwin RE, Glavinic U, Stanimirovic Z. Species-specifi c diagnostics of Apis mellifera trypanosomatids: a nine-year survey (2007- 2015) for trypanosomatids and microsporidians in Serbian honey bees. J Invertebr Pathol 2016, 139:6-11.

113. Schwarz RS, Bauchan GR, Murphy C, Ravoet J, de Graaf DC, Evans JD: Characterization of two species of Trypanosomatidae from the honey bee Apis mellifera: Crithidia mellificae Langridge and McGhee, and Lotmaria passim. J Eukaryot Microbiol 2015a, 62:567-583.

114. Vejnovic B, Stevanovic J, Schwarz RS, Aleksic N, Mirilovic M, Jovanovic NM, Stanimirovic Z: Quantitative PCR assessment of Lotmaria passim in Apis mellifera colonies co-infected naturally with Nosema ceranae. J Invertebr Pathol 2018, 151:76-81.

115. Martín-Hernández, R, Botías, C, Bailón, E G, Martínez-Salvador, A, Prieto, L, Meana, A, Higes, M: Microsporidia infecting Apis mellifera: coexistence or competition. Is Nosema ceranae replacing Nosema apis?. Environ Microbiol 2012, 14:2127-2138.

116. Higes M, Meana A, Bartolomé C, Botías C, Martín-Hernández R: Nosema ceranae (Microsporidia), a controversial 21st century honey bee pathogen. Env Microbiol Rep 2013, 5:17–29.

117. Schwarz RS, Huang Q, Evans JD: Hologenome theory and the honey bee pathosphere. Curr Opin Insect Sci 2015b, 10:1-7.

118. Martín-Hernández, R., Bartolomé, C., Chejanovsky, N., Le Conte, Y., Dalmon, A., Dussaubat, C., García-Palencia, P., Meana, A., Pinto, M.A., Soroker, V. and Higes, M: Nosema ceranae in Apis mellifera: a 12 years postdetection perspective. Environ Microbiol 2018, 20:1302-1329.

119. Fries I: Nosema ceranae in European honey bees (Apis mellifera). J Invertebr Pathol 2010, 103:S73–S79.

120. Gisder S, Hedtke K, Möckel N, Frielitz MC, Linde A, Genersch E: Fiveyear cohort study of Nosema spp. in Germany: does climate shape virulence and assertiveness of Nosema ceranae? Appl Environ Microb 2010, 76:3032–3038.

121. Gisder S, Schüler V, Horchler LL, Groth D, Genersch E: Long-term temporal trends of Nosema spp. infection prevalence in Northeast Germany: Continuous spread of N. ceranae, an emerging pathogen of honey bees (Apis mellifera), but no general replacement of N. apis. Front Cell Infect Microbiol 2017, 7:301.

122. Forsgren E, Fries I: Temporal study of Nosema spp. in a cold climate. Environ Microbiol Rep 2013, 5:78–82.

123. Chemurot M, De Smet L, Brunain M, De Rycke R, de Graaf DC: Nosema neumanni n. sp. (Microsporidia, Nosematidae), a new microsporidian parasite of honeybees, Apis mellifera in Uganda. Eur J Protistol 2017, 61:13–19.

124. Martín-Hernández R, Meana A, Prieto L, Salvador AM, Garrido-Bailón E, Higes M. Outcome of colonization of Apis mellifera by Nosema ceranae, Appl Environ Microb 2007, 73:6331–6338.

125. Higes M, Martín-Hernández R, Botías C, Garrido Bailón E, González-Porto AV, Barrios L, Del Nozal MJ, Bernal JL, Jiménez JJ, Palencia PG, Meana A: How natural infection by Nosema ceranae causes honeybee colony collapse. Environ Microbiol 2008, 10:2659–2669.

126. Higes M, Martín-Hernández R, Garrido-Bailón E, González-Porto AV, García-Palencia P, Meana A, Del Nozal MJ, Mayo R, Bernal JL: Honeybee colony collapse due to Nosema ceranae in professional apiaries. Env Microbiol Rep 2009, 1:110–113.

127. Antúnez K, Martín-Hernández R, Prieto L, Meana A, Zunino P, Higes M: Immune suppression in the honey bee (Apis mellifera) following infection by Nosema ceranae (Microsporidia). Environ Microbiol 2009, 11:2284-2290.

128. Chaimanee V, Chantawannakul P, Chen Y, Evans YD, Pettis JS: Differential expression of immune genes of adult honey bee (Apis mellifera) after inoculated by Nosema ceranae. J Insect Physiol 2012, 58:1090–1095.

129. Glavinic U, Stankovic B, Draskovic V, Stevanovic J, Petrovic T, Lakic N, Stanimirovic Z: Dietary amino acid and vitamin complex protects honey bee from immunosuppression caused by Nosema ceranae. PLoS ONE 2017, 12:e0187726. 130. Higes M, Martin-Hernandez R, Meana A: Nosema ceranae in Europe: an emergent type C nosemosis. Apidologie 2010, 41:375-392.

131. Simeunovic P, Stevanovic J, Cirkovic D, Radojicic S, Lakic N, Stanisic L, Stanimirovic Z: Nosema ceranae and queen age influence the reproduction and productivity of the honey bee colony. J Apicult Res 2014b, 53:545-554.

132. Bacandritsos N, Granato A, Budge G, Papanastasiou I, Roinioti E, Caldon M, Falcaro C, Gallina A, Mutinelli F: Sudden deaths and colony population decline in Greek honey bee colonies. J Invertebr Pathol 2010, 105:335–340.

133. Bromenshenk JJ, Henderson CB, Wick CH, Stanford MF, Zulich AW, Jabbour RE, Deshpande SV, McCubbin PE, Seccomb RA, Welch PM, Williams T: Iridovirus and microsporidian linked to honey bee colony decline. PLoS ONE 2010, 5:e13181.

134. Soroker V, Hetzroni A, Yakobson B, David D, David A, Voet H, Slabezki Y, Efrat H, Levski S, Kamer Y, Klinberg E, Zioni N, Inbar S, Chejanovsky N: Evaluation of colony losses in Israel in relation to the incidence of pathogens and pests. Apidologie, 2011, 42:192–199.

135. Vidau C, Diogon M, Aufauvre J, Fontbonne R, Viguès B, Brunet JL, Texier C, Biron DG, Blot N, Al Alaoui H, Belzunces LP, Delbac F: Exposure to sublethal doses of fi pronil and thiacloprid highly increases mortality of honeybees previously infected by Nosema ceranae. PLoS ONE 2011, 6:e21550.

136. Aufauvre J, Biron DG, Vidau C, Fontbonne R, Roudel M, Diogon M, Viguès B, Belzunces LP, Delbac F, Blot N: Parasite-insecticide interactions: a case study of Nosema ceranae and fipronil synergy on honeybee. Sci Rep-UK 2012, 2:326.

137. Aufauvre J, Misme-Aucouturier B, Viguès B, Texier C, Delbac F, Blot N: Transcriptome analyses of the honeybee response to Nosema ceranae and insecticides. PLoS ONE 2014, 9:e91686.

138. Glavinic U, Tesovnik T, Stevanovic J, Zorc M, Cizelj I, Stanimirovic Z, Narat M: Response of adult honey bees treated in larval stage with prochloraz to infection with Nosema ceranae. PeerJ 2019,7:e6325

139. Retschnig G, Neumann P, Williams GR: Thiacloprid–Nosema ceranae interactions in honey bees: Host survivorship but not parasite reproduction is dependent on pesticide dose. J Invertebr Pathol 2014, 118:18-19.

140. Blacquiere T, Smagghe G, van Gestel CAM, Mommaerts V: Neonicotinoids in bees: A review on concentrations, side-effects and risk assessment. Ecotoxicology 2012, 21:973– 992.

141. Van der Sluijs JP, Simon-Delso N, Goulson D, Maxim L, Bonmatin JM, Belzunces LP: Neonicotinoids, bee disorders and the sustainability of pollinator services. Curr Opin Env Sust 2013, 5:293-305.

142. Abbo PM, Kawasaki JK, Hamilton M, Cook SC, DeGrandi-Hoffman G, Li WF, Liu J, Chen YP: Effects of imidacloprid and Varroa destructor on survival and health of European honey bees, Apis mellifera. Insect Sci 2017, 24:467-477.

143. Tison L, Hahn ML, Holtz S, Rößner A, Greggers U, Bischoff G, Menzel R: Honey bees' behavior is impaired by chronic exposure to the neonicotinoid thiacloprid in the fi eld. Environ Sci Technol 2016, 50:7218-7227.

144. Straub L, Villamar-Bouza L, Bruckner S, Chantawannakul P, Gauthier L, Khongphinitbunjong K, Retschnig G, Troxler A, Vidondo B, Neumann P, Williams GR: Neonicotinoid insecticides can serve as inadvertent insect contraceptives. Proc R Soc B 2016, 283:20160506.

145. Di Prisco G, Cavaliere V, Annoscia D, Varricchio P, Caprio E, Nazzi F, Gargiulo G, Pennacchio F: Neonicotinoid clothianidin adversely affects insect immunity and promotes replication of a viral pathogen in honey bees. P Natl Acad Sci USA, 2013, 110:18466- 18471.

146. Brandt A, Gorenfl o A, Siede R, Meixner M, Büchler R: The neonicotinoids thiacloprid, imidacloprid, and clothianidin affect the immunocompetence of honey bees (Apis mellifera L.). J Insect Physiol 2016, 86:40-47.

147. Thompson HM, Fryday SL, Harkin S, Milner S: Potential impacts of synergism in honeybees (Apis mellifera) of exposure to neonicotinoids and sprayed fungicides in crops. Apidologie 2014, 45:545–553.

148. Sgolastra F, Medrzycki P, Bortolotti L, Renzi MT, Tosi S, Bogo G, Teper D, Porrini C, Molowny-Horas R, Bosch J: Synergistic mortality between a neonicotinoid insecticide and an ergosterol-biosynthesis-inhibiting fungicide in three bee species. Pest Manag Sci 2017, 73:1236-1243.

149. Johnson RM, Dahlgren L, Siegfried BD, Ellis MD: Acaricide, fungicide and drug interactions in honey bees (Apis mellifera). PLoS ONE 2013, 8:e54092.

150. Lambert O, Piroux M, Puyo S, Thorin C, L'Hostis M, Wiest L, Bulete A, Delbac F, Pouliquen H: Widespread occurrence of chemical residues in beehive matrices from apiaries located in different landscapes of Western France. PLoS ONE 2013, 8:e67007.

151. Cizelj I, Glavan G, Božič J, Oven I, Mrak V, Narat M: Prochloraz and coumaphos induce different gene expression patterns in three developmental stages of the Carniolan honey bee (Apis mellifera carnica Pollmann). Pestic Biochem Phys 2016, 128:68-75.

152. Seeley T, Tarpy D: Queen promiscuity lowers disease within honeybee colonies. Proc R Soc London Sci - Ser B 2007, 274:67–72.

153. Higes M, Nozal MJ; Alvaro A, Barrios L, Meana A, Martín-Hernández R, Bernal LJ, Bernal J: The stability and effectiveness of fumagillin in controlling Nosema ceranae (Microsporidia) infection in honey bees (Apis mellifera) under laboratory and field conditions. Apidologie 2011, 42:364-377.

154. Van den Heever JP, Thompson TS, Otto SJ, Curtis JM, Ibrahim A, Pernal SF: Evaluation of Fumagilin-B® and other potential alternative chemotherapies against Nosema ceranaeinfected honeybees (Apis mellifera) in cage trial assays. Apidologie 2016a, 47:617-630.

155. Van den Heever JP, Thompson TS, Otto SJ, Curtis JM, Ibrahim A, Pernal SF: The effect of dicyclohexylamine and fumagillin on Nosema ceranae-infected honey bee (Apis mellifera) mortality in cage trial assays. Apidologie 2016b, 47:663-670.

156. van den Heever JP, Thompson TS, Curtis JM, Pernal SF: Determination



of dicyclohexylamine and fumagillin in honey by LC-MS/MS. Food Anal Method 2015a, 8:767-777.

157. van den Heever JP, Thompson TS, Curtis JM, Pernal SF: Stability of dicyclohexylamine and fumagillin in honey. Food Chem 2015b, 179:152-158.

158. Runckel C, Flenniken ML, Engel JC, Ruby JG, Ganem D, Andino R, De-Risi JL: Temporal analysis of the honey bee microbiome reveals four novel viruses and seasonal prevalence of known viruses, Nosema, and Crithidia. PLoS ONE 2011, 6:e20656.

159. Cornman RS, Tarpy DR, Chen Y, Jeffreys L, Lopez D, Pettis JS, Evans JD: Pathogen webs in collapsing honey bee colonies. PLoS ONE 2012, 7:e43562.

160. Ravoet J, Maharramov J, Meeus I, De Smet L, Wenseleers T, Smagghe G, De Graaf DC: Comprehensive bee pathogen screening in Belgium reveals Crithidia mellificae as a new contributory factor to winter mortality. PLoS ONE 2013, 8:e72443.

161. Ravoet J, Schwarz RS, Descamp T, Yanez O, Tozkar O, Martin-Hernandez R, De Smet L, DeRisi J, Higes M, Wenseleers T, Schmid-Hempel R, Neumann P, Kadowaki T, Evans JD, de Graaf DC: Differential diagnosis of the honey bee trypanosomatids Crithidia mellificae and Lotmaria passim. J Invertebr Pathol 2015, 130:21-27.

162. Schwarz RS, Evans JD: Single and mixed-species trypanosome and microsporidia infections elicit distinct, ephemeral cellular and humoral immune responses in honeybees. Dev Comp Immunol 2013, 40:300–310.

163. Stevanovic J, Vejnovic B, Schwarz R, Aleksic N, Jovanovic N, Stanimirovic Z: Identifi cation and quantifi cation of Lotmaria passim (Trypanosomatidae) in investigation of its prevalence, annual dynamics and relationship with Nosema ceranae (Microsporidia). Book of plenary lectures, XXX Congresso SoIPA: Mutamenti Ambientali e Parassiti, June 26-29, 2018a, Milano, Italy pp. 55-57.

164. Radaković M, Vejnović B, Glavinić U, Aleksić N, Mirilović M, Stanimirović Z, Stevanović J: Oxidative stress increases in honey bees infected with Lotmaria passim. Abstract Book, 8th Congress of Apidology (EURBEE 8), Sept 18-20, 2018, pp. 108, Ghent, Belgium.

165. Aleksic N, Stanimirovic Z, Stevanovic J: External contaminants of honey bee products (invited paper). In Zivadinovic, R. (Ed), Proceedings of the Apimondia Symposium "APIECOTECH SERBIA 2012", Feb 18-19, 2012, Belgrade, Serbia, pp 46-63.

166. Calatayud-Vernich P, Calatayud F, Simó E, Picó Y: Pesticide residues in honey bees, pollen and beeswax: Assessing beehive exposure. Environmental Pollution 2018, 241:106-114.

167. Esteves RJP, Villadelrey MC, Rabajante JF: Determining the optimal distribution of bee colony locations to avoid overpopulation using mixed integer programming. Journal of Nature Studies 2010, 9:79-82.

168. Favre D: Mobile phone-induced honeybee worker piping. Apidologie 2011, 42:270-279.

169. Favre D: Disturbing honeybees' behavior with electromagnetic waves: a methodology. J Behav 2017, 2:1010.

170. Barron AB: Death of the bee hive: understanding the failure of an insect society. Curr Opin Insect Sci 2015, 10:45-50.

171. Archer CR, Köhler A, Pirk CW, Oosthuizen V, Apostolides Z, Nicolson SW: Antioxidant supplementation can reduce the survival costs of excess amino acid intake in honeybees. J Insect Physiol 2014, 71:78-86.

172. Farjan M, Łopieńska-Biernat E, Lipiński Z, Dmitryjuk M, Żółtowska K: Supplementing with vitamin C the diet of honeybees (Apis mellifera carnica) parasitized with Varroa destructor: effects on antioxidative status. Parasitology 2014, 141:770-776. 173. Ptaszyńska AA, Borsuk G, Zdybicka-Barabas A, Cytryńska M, Małek W: Are commercial probiotics and prebiotics effective in the treatment and prevention of honeybee nosemosis C?. Parasitol Res 2016, 115:397-406.

174. Stevanovic J, Stanimirovic Z, Simeunovic P, Lakic N, Radovic I, Sokovic M, Van Griensven JLD: The effect of Agaricus brasiliensis extract supplementation on honey bee colonies. An Acad Bras Cienc 2018b, 90:219-229.

175. Amiri E, Strand M, Rueppell O, Tarpy D: Queen quality and the impact of honey bee diseases on queen health: potential for interactions between two major threats to colony health. Insects 2017, 8:48.

176. Evans JD, Lopez DL: Bacterial probiotics induce an immune response in the honey bee (Hymenoptera: Apidae). J Econ Entomol 2004, 97:752-756.

177. Chan QW, Melathopoulos AP, Pernal SF, Foster LJ: The innate immune and systemic response in honey bees to a bacterial pathogen, Paenibacillus larvae. BMC Genomics 2009, 10:387.

178. Azzami K, Ritter W, Tautz J, Beier H: Infection of honey bees with acute bee paralysis virus does not trigger humoral or cellular immune responses. Arch Virol 2012, 157:689-702.

179. Gätschenberger H, Azzami K, Tautz J, Beier H: Antibacterial immune competence of honey bees (Apis mellifera) is adapted to different life stages and environmental risks. PLoS ONE 2013, 8:e66415.

180. Hristov P, Shumkova R, Palova N, Neov B: Factors associated with honey bee colony losses: A mini-review. Vet. Sci. 2020, 7:166.

181. Taric E, Glavinic U, Vejnovic B, Stanojkovic A, Aleksic N, Dimitrijevic V, Stanimirovic Z: Oxidative stress, endoparasite prevalence and social immunity in bee colonies kept traditionally vs. those kept for commercial purposes. Insects. 2020, 11:266.

182. El Agrebi N, Steinhauer N, Tosi S, Leinartz L, de Graaf DC, Saegerman C: Risk and protective indicators of beekeeping management practices. Sci. Total Environ. 2021, 799:149381.

183. Glavinic U, Stevanovic J, Ristanic M, Rajkovic M, Davitkov D, Lakic N, Stanimirovic Z: Potential of fumagillin and Agaricus blazei mushroom extract to reduce Nosema ceranae in honey bees. Insects. 2021, 12:282.

184. Jovanovic NM, Glavinic U, Delic B, Vejnovic B, Aleksic N, Mladjan V, Stanimirovic Z: Plant-based supplement containing B-complex vitamins can improve bee health and increase colony performance. Prev. Vet. Med. 2021, 190:105322.

185. Kolics É, Specziár A, Taller J, Mátyás KK, Kolics B: Lithium chloride outperformed oxalic acid sublimation in a preliminary experiment for Varroa mite control in pre-wintering honey bee colonies. Acta Vet. Hun. 2021, 68:370-3.

186. Jovanovic NM, Glavinic U, Ristanic M, Vejnovic B, Stevanovic J, Cosic M, Stanimirovic Z: Contact varroacidal efficacy of lithium citrate and its influence on viral loads, immune parameters and oxidative stress of honey bees in a field experiment. Front. Physiol. 2022, 13:1000944.

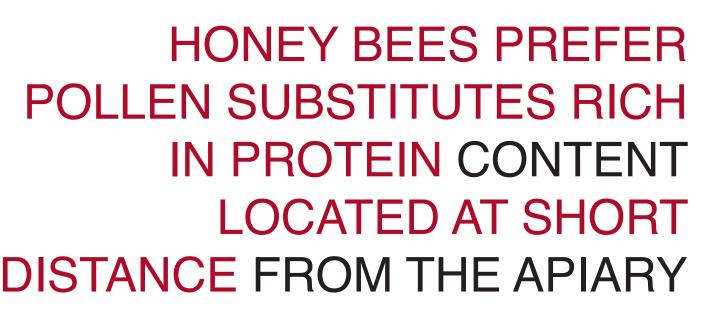
187. Glavinic U, Blagojevic J, Ristanic M, Stevanovic J, Lakic N, Mirilovic M, Stanimirovic Z: Use of thymol in Nosema ceranae control and health improvement of infected honey bees. Insects. 2022, 13:574.

188. Glavinic, U., Jovanovic, N. M., Dominikovic, N., Lakic, N., Ćosić, M., Stevanovic, J., Stanimirovic, Z. Potential of wormwood and oak bark-based supplement in health improvement of Nosema ceranae-infected honey bees. Animals, 2024, 14, 1195.

189. Mojović, M., Nakarada, D., Glavinić, U., Ristanić, M., Popović, M., Stevanović, J., Stanimirović, Z. Bridging the Buzz: In vivo EPR imaging unlocking the secrets of honey bee health. Res Sq, 2024.







Simple Summary

Pollens being the primary source of protein, lipids, vitamins, and minerals are vital for bee development and reproduction. A major issue confronting beekeeping is developing strong and healthy honey bee colonies. The possibility of prosperous honey bee colonies depends on an effective pollen substitute especially when pollen supply is scarce during the dearth period. Many beekeepers feed their bees different pollen substitutes with sufficient nutrition throughout the period of inadequate pollen quantity or quality. We delivered four different pollen substitutes (chickpea, maize, sorghum, and wheat flour) and natural pollen to honey bee colonies for comparison. Each flour was mixed with a small quantity of cinnamon powder, turmeric powder, and both powders. Further, to investigate the preferred pollen foraging distance from the hives, the best performing pollen substitutes were placed at various distances of 10, 25, and 50 m from the apiary. Chickpea flour (comparatively rich in protein content) located very close to the apiary was the best pollen substitute among the tested flours. This study is very helpful for beekeepers in supplementing their bee colonies when there is a shortage or unavailability of pollens, and it is much better to keep the food source near the apiary.

Abstract

The availability of floral resources is crucial for honey bee colonies because it allows them to obtain protein from pollen and carbohydrates from nectar; typically, they consume these nutrients in the form of bee bread, which has undergone fermentation.

However, the intensification of agriculture, urbanization, changes to the topography, and harsh



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environmental conditions are currently impacting foraging sites due to habitat loss and scarcity of food resources. Thus, this study aimed to assess honey bee preference for various pollen substitute diet compositions. Bee colonies perform poorly because of specific environmental problems, which ultimately result in pollen scarcity. Pollen substitutes located at various distance from the bee hive were also investigated in addition to determining the preferences of honey bees for various pollen substitute diets. The local honey bee (Apis mellifera jemenitica) colonies and different diets (four main treatments, namely, chickpea flour, maize flour, sorghum flour, wheat flour; each flour was further mixed with cinnamon powder, turmeric powder, flour only, flour mixed with both cinnamon and turmeric powder) were used. Bee pollen was used as a control. The best performing pollen substitutes were further placed at 10, 25, and 50 m distances from the apiary. Maximum bee visits were observed on bee pollen (210 \pm 25.96) followed by chickpea flour only (205 ± 19.32) . However, there was variability in the bee visits to the different diets (F (16,34) = 17.91; p < 0.01). In addition, a significant difference in diet consumption was observed in control (576 ± 58.85 g) followed by chickpea flour only (463.33 \pm 42.84 g), compared to rest of the diets (F (16,34) = 29.75; p < 0.01). Similarly, foraging efforts differed significantly (p < 0.01) at the observed time of 7-8 A.M., 11-12 A.M., and 4-5 P.M. at the distance of 10, 25, and 50 m away from the apiary. Honey bees preferred to visit the food source that was closest to the hive. This study should be very helpful for beekeepers in supplementing their bee colonies when there is a shortage or unavailability of pollens, and it is much better to keep the food source near the apiary. Future research needs to highlight the effect of these diets on bee health and colony development.

Keywords: honey bee; supplementary diet; pollens; foraging behavior

1. Introduction

Honey bees (Apis mellifera jemenitica) have a high commercial value for honey production [1] as well as pollination in a range of agricultural



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crops [2]. Honey bees, like other invertebrates, are poikilothermic; they cannot regulate their body temperature and must go into hibernation when the ambient temperature is too high [3]. Due to restricted foraging activity, their dietary requirements and metabolic activities are decreased during this period [3].

High summer temperatures and dry weather are the main factors contributing to honey bee mortality in Saudi Arabia. This is due to decreased plant flowering and pollen availability due to heat stress [4]. Native populations of A. mellifera jemenitica in Saudi Arabia are much more tolerant of heat than the common races. A. mellifera jemenitica exists in central Saudi Arabia, which has the warmest summer temperatures, >45 °C [5,6]. Additionally, A. mellifera jemenitica possesses a remarkable ability to hunt for pollen and possesses high fecundity [7]. The pollen quantity was discovered to have a positive correlation with temperature and a negative correlation with rainfall, relative humidity, and wind speed. The months with the highest pollen guantity (95% of all pollen) were May through September [8] in Abha Saudi Arabia, while in September and October, there was a spike in flight activity. August through September had seen the highest concentrations of pollen brought back to the hive, while November through December had seen the lowest pollen concentrations in the Al-Ahsa region of Saudi Arabia [9].



In addition, other seasonal and climatic fluctuations (precipitation, hail, etc.) produce considerable losses in floral resources throughout the year [10]. Meanwhile, flowers are essential for honey bee brood production, immunological function, and overwintering survival [11,12]. While nectar is a source of carbohydrates, pollen supplies proteins, lipids, and micronutrients [13]. When the natural flora is insufficient, the queen bee's egg-laying level decreases, resulting in a fall in the colony's population level [14]. Malnutrition reduces individual survival rates, causes larval life to cease, renders the colony prone to disease, and drives individuals to leave the colony [15,16]. Usually, a honey bee colony obtains 10–26 kg of pollen each year from flowers [17] as a rudimentary source of protein content and amino acid composition for the well-being of their colony [18]. Furthermore, appropriate protein and carbohydrate stores in the colony are suggested to aid honey bees in fighting or tolerating different stressors associated with modern apiculture [17]. Although pollen remains the most desirable and appealing protein source for honey bees, pollen replacements have advantages. Pollen introduced to the colonies from the outside is costly to get in large guantities, and it also entails the danger of introducing infections [19,20] or pesticides [21] into the colonies.

Thus, human intervention is needed to overcome these problems, particularly for disease management and additional feeding. To compensate for the lack of nutritive forage in the environment, hives are routinely given artificial "pollen substitute" diets [22]. As a result, better colony health for honey production and pollination can be maintained [23,24]. To compensate for insufficient pollen forage and boost colony vigor prior to pollination services, beekeepers provide different "pollen substitute" diets [25,26]. In order to manage honey bee colonies during a pollenscarce season, Pande and Karnatak [27] utilized germinated pulses as a substitute for pollen. For the production of four distinct diets-ger horse gram, ger chickpea, ger green gram/mungbean, and ger pea-various germinated pulse flours were used. Similarly, Kumar and Agrawal [28] made six distinct combinations of artificial food using defatted soy flour, brewer's yeast, parched gram, spirulina, skim milk powder, sugar, glu-



cose, protein hydrolysate powder, and natural pollen. This artificial diet favored the biochemical composition and net consumption, and also had a positive effect on colony parameters such as egg laying and brood production.

In addition, in another study, different supplements were used such as roasted chickpea flour, broadbean flour, maize flour, and soy flour [29], and these supplements enhanced brood production and longevity. A study conducted in India using four flours-soybean, wheat, maize, and gram-as pollen substitutes found that pollen substitute is crucial for the growth and development of bee colonies not only during times of scarcity but especially during foraging and pollination and to overcome pesticide exposures [30]. Meanwhile, in another study, six protein-rich ingredients-defatted soybean flour, chickpea flour, maize flour, wheat germ, pea flour, and dried brewer's yeast-were combined in various ratios with sugar powder, bee honey, and water to create ten diets [31], and these diets increased biological activities including diet consumption, sealed worker brood area, and pollen and honey store area.

Honey bees can visit multiple food sources at once and travel up to 11 km to obtain primary food resources such as nectar and pollen [32], which are stored in their colonies as honey and beebread [33]. Their foraging is one of the most well-organized behaviors found in social insects [34]. Honey bee foragers use information gathered from their own experience, such as recall of time and place, sugar concentration to determine whether to continue or begin foraging on certain resources [35]. Until now, research has concentrated on commonly foraged feed elements such as pollen and nectar rather than atypically foraged materials that may be ingested under drought. The objectives of the present study were to evaluate the preference of honey bees for different diets. In addition, we assessed honey bee preferences for various diet supplements placed at various distances from the colonies.

2. Materials and Methods

The study was conducted at the Unit of Bee Research and Honey Production, King Khalid University Abha Saudi Arabia. The current study used local honey bee (A. mellifera jemenitica) colonies housed in the Langstroth hives. The honey bee colonies placed at the apiary did not show any clinical illness signs (see Figure 1). All bee colonies were subjected to regularly suggested colony management procedures [36].

2.1. Preparation of Diets

These pollen substitution diets were high in protein, carbohydrates, minerals, and fats. These items were reasonably priced in the local market. The supplemental diets listed below were created. Each diet was tested with three replications for five pollen substitute diets, including naturally collected pollen as a control on diet preference (see Table 1).

Figure 1. A. mellifera jemenitica apiary set-up





Sr.#	Pollen Supplementary Diet	Ratio	Abbreviations
1	Chickpea flour + Cinnamon powder	50:1	CPCM
2	Chickpea flour + Turmeric powder	50:1	CPTM
3	Chickpea flour only	-	CPOY
4	Chickpea flour + Both powders	50:1	СРВН
5	Maize flour + Cinnamon powder	50:1	MZCM
6	Maize flour + Turmeric powder	50:1	MZTM
7	Maize flour only	-	MZOY
8	Maize flour + Both powders	50:1	MZBH
9	Sorghum flour + Cinnamon powder	50:1	SGCM
10	Sorghum flour + Turmeric powder	50:1	SGTM
11	Sorghum flour only	-	SGOY
12	Sorghum flour + Both powders	50:1	SGBH
13	Wheat flour + Cinnamon powder	50:1	WTCM
14	Wheat flour + Turmeric powder	50:1	WTTM
15	Wheat flour only	-	WTOY
16	Wheat flour + Both powders	50:1	WTBH
17	Pollen only as a control	-	PNOY

Table 1. Different supplement formulations

The various supplemental diets were prepared separately first, measured known quantity (see Figure 2), and carefully blended in a dough



Figure 2. Measuring known quantity of diets before mixing.

machine (Hobart dough mixer, model A200, Offenburg, Germany). The flour was fed externally to provide bees easy access to it. Honey bees must vibrate their body to collect powdered substances, a simple process requiring little time and effort [37].

2.2. Bee Visits and Diet Consumption

Every day in the late afternoon, the consumption rate of each diet replicate was calculated by measuring diet weight before and after



feeding in grams. At the end of the trial, the total amount of food consumed in each replicate was also calculated.

2.3. Choice Powder Feeding

This modified procedure used three distances from colonies: 10, 25, and 50 m. Experimental colonies received all five feeds (i.e., CPCM, CPTM, CPOY, CPBH, and PNOY) in separate plates positioned at various distances [37] as indicated in Figure 3.





Figure 3. Different diet supplements at different distances (10, 25, and 50 m) away from the apiary and measuring the number of honey bees.

2.4. Estimation of Honey Bee Numbers

The numbers of honey bees were counted at different time intervals (7–8 A.M., 11–12 A.M., and 4–5 P.M.). During each time interval, the number of honey bees were counted visually three times as indicated in Figure 3.

2.5. Diet Consumption

The diet weight between before and after feeding in grams per colony were calculated to determine the net weight of pollen-supplemented diets ingested within treatments after feeding 10 times to each colony [38].

2.6. Statistical Analysis

The total amount of preferred food consumed and distance preference was compared between treatments with an analysis of variance. The data were calculated as mean and standard error using the SPSS (version 20). Graphs were created with the GraphPad Prism software (version 7.03). Furthermore, the Tukey post hoc test was used for multiple group comparisons at the 0.05 level.

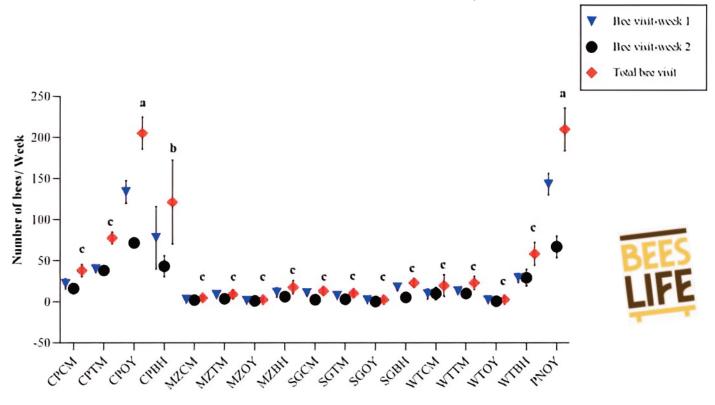
3. Results

3.1. Honey Bee Visitation

Maximum honey bee visitation during the first week was on the PNOY with 143 ± 12.89 /week followed by CPOY (133.67 ± 13.75 /week) and CPBH (78 ± 38.07 /week). The fewest visits were on the MZOY (1.33 ± 0.88 /week) followed by SGOY and WTOY with 2 ± 0.57 /week and $2 \pm$ 1.52/week mean visits, respectively. In the second week, maximum honey bee visits were observed in the CPOY (71.66 ± 6.06 /week) and PNOY (67 ± 13.11 /week). The fewest visits were on the SGOY (0.33 ± 0.33 /week) followed by WTOY (0.66 ± 0.66 /week) and MZOY ($1 \pm$ 0.57/week). However, overall time periods, maximum visits were on the PNOY (210 ± 25.96) fol-



Figure 4. Honey bee visits towards different diet compositions. Whereas, CPCM = Chickpea flour + Cinnamon powder, CPTM = Chickpea flour + Turmeric powder, COPY = Chickpea flour only, CPBH = Chickpea flour + Both powders, MZCM = Maize flour + Cinnamon powder, MZTM = Maize flour + Turmeric powder, MZOY = Maize flour only, MZBH = Maize flour + Both powders, SGCM = Sorghum flour + Cinnamon powder, SGTM = Sorghum flour + Turmeric powder, SGOY = Sorghum flour only, SGBH = Sorghum flour + Both powders, WTCM = Wheat flour + Cinnamon powder, WTTM = Wheat flour + Turmeric powder, WTOY = Wheat flour only, WTBH = Wheat flour + Both powders, PNOY = Pollen only as a control. Different superscript letters indicate significant differences between total diet consumption.



lowed by CPOY (205 \pm 19.32), and the fewest visits were on the MZOY and SGOY with mean visits of 2.33 \pm 1.45 and 2.33 \pm 0.88 followed by WTOY (2.66 \pm 2.18) as indicated in Figure 4.

3.2. Diet Supplement Consumption

The findings showed that honey bees ingested varied amounts of all supplements throughout the research period. Honey bees consumed the highest amount of PNOY diet (404 \pm 28.15 g/week) in week 1, which was followed by CPOY (350 \pm 8.21 g/week) and CPBH (235.67 \pm 7.85/week). The least consumption was for the WTOY diet (5 \pm 4.04 g/week) followed by MZOY (7.66 \pm 4.97 g/week) and SGOY (11 \pm 2 g/week) as indicated in Figure 2. During week 2, the highest diet consumption amount was observed for the CPTM diet (203.33 ± 16.41 g/week) followed by the CPBH diet (192 ± 6.42 g/week), and then PNOY (172 ± 38.15 g/week). However, the overall highest consumption was for the PNOY diet (576 ± 58.85 g) followed by the CPOY diet (463.33 ± 42.84 g), the CPBH diet (427.67 ± 6.35 g), and the CPTM diet (384.67 ± 14.72 g). The least consumption was observed for the WTOY diet (7 ± 6.02 g) followed by the MZCM (16 ± 8.32) and MTTM diets (63 ± 21.37). The remaining diet treatment consumption is indicated in Figure 5.

ANOVA was performed on the honey bee visits and diet consumption, and there were significant differences between the visits to the different treatments during the first week (p < 0.01). Similarly, during the second week, there were significant visits to different diets (p < 0.01). Overall, there were significant variability in some of the treatment visits (p < 0.01) as indicated in Table 2.



Figure 5. Honey bee diet consumption from various pollen substitutes. Whereas, CPCM = Chickpea flour + Cinnamon powder, CPTM = Chickpea flour + Turmeric powder, CPOY = Chickpea flour only, CPBH = Chickpea flour + Both powders, MZCM = Maize flour + Cinnamon powder, MZTM = Maize flour + Turmeric powder, MZOY = Maize flour only, MZBH = Maize flour + Both powders, SGCM = Sorghum flour + Cinnamon powder, SGTM = Sorghum flour + Turmeric powder, SGOY = Sorghum flour only, SGBH = Sorghum flour + Both powders, WTCM = Wheat flour + Cinnamon powder, WTTM = Wheat flour + Turmeric powder, WTOY = Wheat flour only, WTBH = Wheat flour + Both powders, PNOY = Pollen only as a control. Different superscript letters indicate significant differences between total diet consumption.

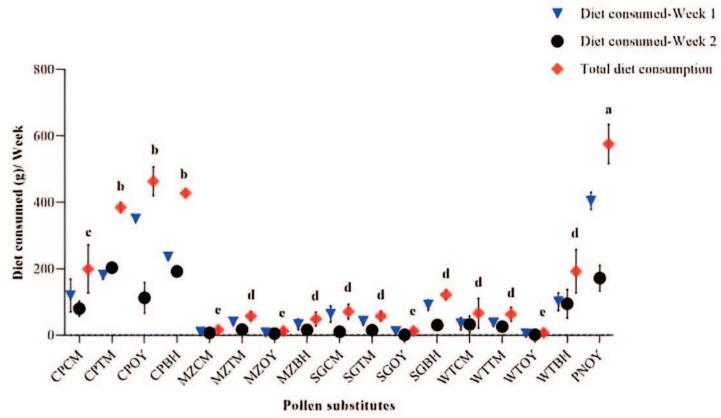


Table 2. Honey bee visit and diet consumption (ANOVA).

Honey Bee Visits	Sum of Squares	Degrees of Freedom	Mean Square	F	<i>p</i> -Value
Week 1	94,945.020	16,34	5934.064	16.883	>0.001
Week 2	26,137.17	16,34	1633.574	14.922	>0.001
Total	218,023.64	16,34	13,626.478	17.913	>0.001
		Diet consumption			
Week 1	705,552.03	16,34	44,097.002	42.080	>0.001
Week 2	238,175.92	16,34	14,885.995	12	>0.001
Total	1,615,024.64	16,34	100,939.020	29.758	>0.001



According to Table 2, the mean diet consumption throughout the entire sample of honey bee colonies showed notable variability during the first week (p < 0.01). The same pattern was observed in consumption for the second week (p < 0.01). Similarly, overall, there were significant differences in diet consumption due to treatment (p < 0.01).

There was a strong positive correlation between the two variables: honey bee visits and diet consumption. During the first week, the Pearson's correlation was 0.957. Similarly, during the second week and in the total observation period, a strong positive correlation was also found between bee visits and consumption: 0.89 and 0.94, respectively.

3.3. Foraging Efforts

The foraging effort was measured by counting the number of bees visiting the best performing diet (chickpea flour mixed with different spices) placed at different distances such as 10 m, 25 m, and 50 m and observed at different time intervals. The maximum mean number of honey bee visits during 7–8 A.M. at a 10 m distance was observed in the PNOY diet (282.5 ± 2.5), which was followed by CPOY (277.5 ± 7.5). The visitation rate was observed in CPCM, which was 75 ± 5, followed by CPBH (79.5 ± 0.5). The maximum mean number of honey bees visiting the PNOY diet (145.5 ± 3) at 25 m was less than the maximum visitation rate to CPOY (252.5 ± 2.5). The least number of visits were observed in the CPTM diet with 50 ± 5 followed by the CPBH diet (51 ± 1). Similarly, at the distance of 50 m, maximum number of honey bees were observed in the PNOY diet (237 ± 7) followed by the CPOY diet (215.5 ± 14.5). The least number of visits (34.5 ± 4.5) were recorded at the CPCM diet followed by the CPBH diet (37.5 ± 2.5) as shown in Figure 6a.

As indicated in Figure 6b, when diets were placed at the distance of 10 m, the maximum number of honey bees during the time of 11-12 A.M. were observed on the PNOY (226 ± 1) followed by CPOY (220.5 \pm 0.5) while the fewest number of honey bees were recorded on the CPBH diet, which was 58.5 ± 0.5 followed by CPCM (65 ± 5). Similarly, when diets were placed at a distance of 25 m, the maximum number of honey bees visited the COPY diet (202.5 \pm 6.5) followed by the PNOY diet (202 \pm 3), while the fewest number of visits were observed on the CPCM diet with 35 ± 5 visits followed by the CPBH diet (46.5 \pm 5.5). In the case of 50 m, maximum visits were observed on the copy diet (180 \pm 10) followed by the PNOY diet (176 \pm 1). The least number of visits were recorded on the CPCM and CPBH diets (27.5 \pm 2.5 and 40.5 \pm 5.5, respectively).

When the foraging activity of honey bees at the time of 4–5 P.M. was observed at a distance

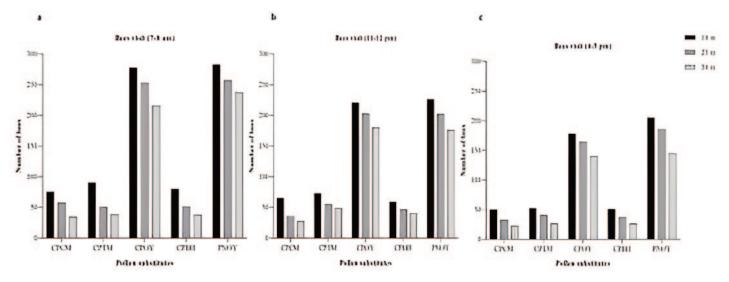


Figure 6. Honey bee visits at different times and pollen substitutes present at different distances: (a) 7–8 A.M. time (b) 11–12 A.M. time (c) 4–5 P.M. time. Whereas, CPCM = Chickpea flour + Cinnamon powder, CPTM = Chickpea flour + Turmeric powder, CPOY = Chickpea flour only, CPBH = Chickpea flour + Both powders, PNOY = Pollen only as a control.



of 10 m, the maximum number of honey bees visiting the diets were found on the PNOY treatment (205 \pm 10) followed by the copy treatment (178 ± 7) . The fewest bees were observed on the CPCM (50 \pm 5) and the CPBH diets (51 \pm 1). At the distance of 25 m, maximum number of bee visits were observed on the PNOY diet (185 ± 10) followed by the copy diet (164 \pm 6). The least number of honey bee visits were recorded on the CPCM (32.5 ± 1.5) and the CPBH diets (37 ± 8). When diets were placed at a distance of 50 m, the maximum number of honey bees visiting the treatments were found on the PNOY diet (145 ± 9), followed by the copy diet (140 ± 10) . The least number of honey bees visiting a diet were found on the CPCM (22.5 \pm 2.5) and the CPBH diets (26 ± 3) as indicated in Figure 6c.

There was a non-statistically significant interaction between time of day, distance from the colonies, and diet types (F (16,45) = 1.017 (p = 0.458). However, a significant interaction was found between time of day and distance (F (16,45) = 3.063 (p = 0.026). Similarly, a significant interaction was found between time of day and diet types (F (16,45) = 33.349 (p = 0.001).

4. Discussion

Like most other invertebrates, honey bees are poikilothermic; they are unable to control their body temperatures and become inactive when the outside temperature becomes intolerable. Due to severely constrained foraging activities during hot weather, their nutritional needs and metabolic activity are reduced [3]. Thus, the present study was designed to offer a substitute for honey bees in the harsh conditions of KSA and to investigate the effects of distance from the bee hive on visitation rate to the diets.

Overall, in our study, honey bees exhibited a significant difference in visitation rates to the different diets. However, maximum visits were observed in PNOY (pollen as a control) (210 \pm 25.96) followed by CPOY (chickpea flour only) (205 \pm 19.32). In terms of diet consumption, PNOY (576 \pm 58.85 g) followed by CPOY (463.33 \pm 42.84 g) were maximum diets consumed which are in agreement with findings of Khan and Ghramh [39]. They concluded that honey bees ingested much more pollen (11.51 \pm 2.22 mg/bee)

and ajeena diet, i.e., commercially available pollen substitute (10.68 \pm 1.29 mg/bee) than any other diet. This attraction towards pollen may be due to the quality of the diet; pollen attracts foragers [40] and is considered a major source of vitamins, minerals, lipids, carbohydrates, sterols, proteins, and amino acids [41]. The nutritional value of protein would be the primary factor in honey bees' selection of pollen for food [42]. In a study conducted in India, four distinct germinated pulse flour diets-germinated horse gram, germinated chickpea, germinated green gram/mungbean, and germinated pea-were used and following feeding, foraging behavior was seen in all diet combinations, including germinated chickpea, germinated green gram, and germinated horse gram [27]. While our findings were in contrast with the study conducted in India, three diet formulas using the four germinated pulses soybean, mungbean, pigeon pea, and chickpea were used, and soybean was the most preferred of the four pulses and three formulations [43]. However, the other substitutes can also be very helpful in drought or harsh conditions when there are few flowers. The fluctuation in floral sources and bee colony population density affect the annual pollen supply for bee colonies in many regions of the world. Since the flora that honey bees need is not consistently present, artificial pollen substitutes and supplements have been utilized to sustain the strength of bee colonies by lengthening the adult lifespan and maintaining brood area [44]. While these supplemented meals and pollen replacements may offer a temporary solution to avoid bee losses in poor foraging settings, it cannot be sustained as a long-term solution in a pollen scarce locale.

In our study, there were reasonable numbers of honey bees that visited and consumed chickpea flour only (CPOY). This can be the best option when there is a pollen shortage. This is because chickpea flour has a good amount of protein (21.70–23.70%), carbohydrates (59.66– 66.42%), fats (4.80–6.36%), ash (2.2–3.46%), total fiber (14.80), and moisture contents (9.35%) [45,46,47]. There are also other flours that have high amounts of proteins and other important elements (Table 3).

In the present study, when honey bee visits were compared at different timings (7–8 A.M.,

Pollen Substitutes	Protein (%)	Carbohydrates (%)	Fat (%)	Ash (%)	Total Dietary Fiber	Moisture (%)	References
	10.55	74.88	0.94	0.94	0.36	12.67	[48]
Wheat flour	11.85	86.04	1.06	0.52	0.54	11.97	[49]
	11.60	78.70	1.70	0.97	-	14.20	[50]
	6.00	-	2.18	0.61	-	10.63	[51]
Maize flour	8.90	-	5.30	1.30	15.60	-	[52]
	8.55	78.77	2.61	0.52	3.68	9.55	[53]
	21.85	66.42	6.36	2.92	-	-	[47]
Chickpea flour	21.70	59.66	5.81	3.46	-	9.35	[45]
	23.70	61.10	4.80	2.2	14.80	-	[46]
Sorghum flour	12.30	73.80	3.60	2.92	-	-	[54]
	12.21	83.45	3.76	0.68	-	-	[55]
	11.50	72.00	2.70	-	-	-	[56]

Table 3. Nutritional content of pollen substitutes reported in different studies.

11-12 A.M., and 4-5 P.M.), and also diets were placed at different distances (10, 25, and 50 m) from the bee hive, maximum activity was recorded in the morning (7-8 A.M.) and at the closest distance of 10 m from the hive. In another study, contrasting results were reported. Honey bees spent the most time foraging during the day, particularly at 12:00 P.M., followed by 14:00 P.M., and finally at 10:00 A.M. every week [57]. Similar to these findings, Pernal and Currie [58] found that honey bees foraged more frequently in the afternoon than in the morning. Forager bees increased their activity and pollen collecting in an onion crop between the hours of 11:00 A.M. and 12:00 P.M. over several days [59]. In all of these studies, maximum bee foraging activity was in the afternoon. This may be due to the effect of rising temperatures and falling relative humidity on anther dehiscence, reaching their highest from 11:00 A.M. to 14:00 P.M. This time period corresponded to the peak pollen-gathering activity of the bees [60]. However, in our case, pollens were used as a control diet detached from flowers and that may be the reason why honey bees showed maximum activity in the morning (7-8 A.M.) instead of the afternoon due to the

free access to pollen early in the morning. In our study, there was a constant increase in the percentage of bees foraging for pollen in the morning, but in the afternoon, that percentage declined. It was discovered that many honey bees flew orientation flights between 12:30 and 14:00 h, especially in sunny conditions, which led to a decreased percentage of pollen foragers in colonies during the middle of the day. This was also documented by a study in China [60]. Additionally, our findings revealed a substantial decrease in foraging activity from 12:00 h to 4-5 P.M., which was likely caused by the high air temperature (exceeding 40 °C). High ambient temperatures make foraging more energy-intensive and can lead to dehydration of foragers. Importantly, foraging distance varies with month differently for the two kinds of forage. In some months, we observe greater distance for one forage type and other months we see the opposite. Overall, this implies that the distance that foraging honey bees must travel is not greatly influenced by one type of forage over another, with summer generally being the season where bees must go farther to gather forage than spring or fall. In the present study, there was a strong positive cor-



relation between the visits and diet consumption (p < 0.01); as the visits increased, the amount of diet consumed also increased. Hence, our results suggest that when food sources are abundant near the bee hive, consumption and foraging activity may increase.

Additionally, more field research is required to ascertain how these supplemental diets affect the health and productivity of honey bee colonies. This research could assist beekeepers in creating more suitable food products that reduce waste and improve the nutritional intake of their bee colonies, especially in harsh conditions when there is a shortage of flora, particularly in the case of the KSA region.

5. Conclusions

In the present study, we examined the preference of honey bees towards different diets and observed the effect of distance on foraging behavior. Overall, honey bees were more attracted to the natural pollens than the other diets. However, this does not mean that honey bees were not attracted to other diets: there were a reasonable number of honey bees that visited the alternative diets such as the chickpea flour only diet. These supplemental diets can be very helpful when there is a scarcity of pollens, and they can play a very important role in the production of honey. In terms of distance, honey bees preferred to visit the food source nearest to the bee hive (10 m), and the preferred time was in the morning (7-8 A.M.).

More research is required to learn how these supplements affect different physiological parameters of honey bee races under diverse climatic situations.

Author Contributions

Conceptualization, K.A.K.; Methodology, K.A.K.; Validation, H.A.G. and K.A.K.; Formal analysis, H.A.G. and K.A.K.; Investigation, H.A.G. and K.A.K.; Data curation, K.A.K.; Writing—original draft, K.A.K.; Writing—review & editing, H.A.G. and K.A.K.; Supervision, H.A.G. and K.A.K.; Project administration, H.A.G. and K.A.K.; Funding acquisition H.A.G. and K.A.K. All authors have read and agreed to the published version of the manuscript.

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Data are contained within the article.

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Conflicts of Interest

The authors declare no conflict of interest.



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References

1. Marcelino, J.; Braese, C.; Christmon, K.; Evans, J.D.; Gilligan, T.; Giray, T.; Nearman, A.; Niño, E.L.; Rose, R.; Sheppard, W.S.; et al. The Movement of Western Honey Bees (Apis mellifera L.) Among US States and Territories: History, Benefits, Risks, and Mitigation Strategies. Front. Ecol. Evol. 2022, 10, 850600. [Google Scholar] [CrossRef]

2. Calderone, N.W. Insect pollinated crops, insect pollinators and US agriculture: Trend analysis of aggregate data for the period 1992–2009. PLoS ONE 2012, 7, e37235. [Google Scholar] [CrossRef] [PubMed]

3. Paray, B.A.; Kumari, I.; Hajam, Y.A.; Sharma, B.; Kumar, R.; Albeshr, M.F.; Farah, M.A.; Khan, J.M. Honeybee nutrition and pollen substitutes: A review. Saudi J. Biol. Sci. 2021, 28, 1167–1176. [Google Scholar] [CrossRef] [PubMed]

4. Awad, A.M.; Owayss, A.A.; Alqarni, A.S. Performance of two honey bee subspecies during harsh weather and Acacia gerrardii nectar-rich flow. Sci. Agric. 2017, 74, 474–480. [Google Scholar] [CrossRef]

5. Alqarni, A.S.; Hannan, M.A.; Owayss, A.A.; Engel, M.S. The indigenous honey bees of Saudi Arabia (Hymenoptera, Apidae, Apis mellifera jemenitica Ruttner): Their natural history and role in beekeeping. Zookeys 2011, 134, 83–98. [Google Scholar] [CrossRef]

6. Alqarni, A.S.; Ali, H.; Iqbal, J.; Owayss, A.A.; Smith, B.H. Expression of heat shock proteins in adult honey bee (Apis mellifera L.) workers under hot-arid subtropical ecosystems. Saudi J. Biol. Sci. 2019, 26, 1372–1376. [Google Scholar] [CrossRef]

7. Taha, E.-K.A.; Al-Kahtani, S.N. Comparison of the activity and productivity of Carniolan (Apis mellifera carnica Pollmann) and Yemeni (Apis mellifera jemenitica Ruttner) subspecies under environmental conditions of the Al-Ahsa oasis of eastern Saudi Arabia. Saudi J. Biol. Sci. 2019, 26, 681–687. [Google Scholar] [CrossRef]

8. Alwadie, H.M. Pollen concentration in the atmosphere of Abha City, Saudi Arabia and its relationship with meteorological parameters. J. Appl. Sci. 2008, 8, 842–847. [Google Scholar] [CrossRef]

9. Taha, E. Seasonal variation of foraging activity, pollen collection and growth of honeybee colonies in Al-Ahsa, Saudi Arabia. Bull. Entomol. Soc. Egypt 2014, 91, 163–175. [Google Scholar]

10. Şahin, M.; Topal, E.; Özsoy, N.; Altunoğlu, E. İklim değişikliğinin meyvecilik ve arıcılık üzerine etkileri. Anadolu Doğa Bilim. Derg. 2015, 6, 147–154. [Google Scholar]

11. Di Pasquale, G.; Alaux, C.; Le Conte, Y.; Odoux, J.F.; Pioz, M.; Vaissière, B.E.; Belzunces, L.P.; Decourtye, A. Variations in the Availability of Pollen Resources Affect Honey Bee Health. PLoS ONE 2016, 11, e0162818. [Google Scholar] [Cross-Ref]

12. Meikle, W.G.; Weiss, M.; Maes, P.W.; Fitz, W.; Snyder, L.A.; Sheehan, T.; Mott, B.M.; Anderson, K.E. Internal hive temperature as a means of monitoring honey bee colony health in a migratory beekeeping operation before and during winter. Apidologie 2017, 48, 666–680. [Google Scholar] [CrossRef]

13. Stoner, K.A.; Nurse, A.; Koethe, R.W.; Hatala, M.S.; Lehmann, D.M. Where Does Honey Bee (Apis mellifera L.) Pollen Come from? A Study of Pollen Collected from Colonies at Ornamental Plant Nurseries. Insects 2022, 13, 744. [Google Scholar] [CrossRef]

14. Topal, E.; Mărgăoan, R.; Bay, V.; Takma, Ç.; Yücel, B.; Oskay, D.; Düz, G.; Acar, S.; Kösoğlu, M. The Effect of Supplementary Feeding with Different Pollens in Autumn on Colony Development under Natural Environment and In Vitro Lifespan of Honey Bees. Insects 2022, 13, 588. [Google Scholar] [CrossRef]

15. Topal, E.; Yücel, B.; Tunca, R.; Kösoğlu, M. Effect of feeding honey bees on colony dynamics. J. Inst. Sci. Technol. 2019, 9, 2398–2408. [Google Scholar] [CrossRef]

16. DeGrandi-Hoffman, G.; Chen, Y.; Rivera, R.; Carroll, M.; Chambers, M.; Hidalgo, G.; de Jong, E.W. Honey bee colonies provided with natural forage have lower pathogen loads and higher overwinter survival than those fed protein supplements. Apidologie 2016, 47, 186–196. [Google Scholar] [CrossRef]

17. Brodschneider, R.; Crailsheim, K. Nutrition and health in honey bees. Apidologie 2010, 41, 278–294. [Google Scholar] [CrossRef]

18. Taha, E.A.; Al-Kahtani, S.; Taha, R. Protein content and amino acids composition of bee-pollens from major floral sources in Al-Ahsa, eastern Saudi Arabia. Saudi J. Biol. Sci. 2019, 26, 232–237. [Google Scholar] [CrossRef]

19. Pereira, K.d.S.; Meeus, I.; Smagghe, G. Honey bee-collected pollen is a potential source of Ascosphaera apis infection in managed bumble bees. Sci. Rep. 2019, 9, 4241. [Google Scholar] [CrossRef]

20. Schittny, D.; Yañez, O.; Neumann, P. Honey bee virus transmission via hive products. Vet. Sci. 2020, 7, 96. [Google Scholar] [CrossRef]

21. Ostiguy, N.; Drummond, F.A.; Aronstein, K.; Eitzer, B.; Ellis, J.D.; Spivak, M.; Sheppard, W.S. Honey bee exposure to pesticides: A four-year nationwide study. Insects 2019, 10, 13. [Google Scholar] [CrossRef] [PubMed]

22. Ricigliano, V.A.; Williams, S.T.; Oliver, R. Effects of different artificial diets on commercial honey bee colony performance, health biomarkers, and gut microbiota. BMC Vet. Res. 2022, 18, 52. [Google Scholar] [CrossRef] [PubMed]

23. Di Pasquale, G.; Salignon, M.; Le Conte, Y.; Belzunces, L.P.; Decourtye, A.; Kretzschmar, A.; Suchail, S.; Brunet, J.L.; Alaux, C. Influence of pollen nutrition on honey bee health: Do pollen quality and diversity matter? PLoS ONE 2013, 8, e72016. [Google Scholar] [CrossRef] [PubMed]

24. Hoover, S.E.; Ovinge, L.P.; Kearns, J.D. Consumption of Supplemental Spring Protein Feeds by Western Honey Bee (Hymenoptera: Apidae) Colonies: Effects on Colony Growth and Pollination Potential. J. Econ. Entomol. 2022, 115, 417–429. [Google Scholar] [CrossRef] [PubMed]

25. Mortensen, A.N.; Jack, C.J.; Bustamante, T.A.; Schmehl, D.R.; Ellis, J.D. Effects of Supplemental Pollen Feeding on Honey Bee (Hymenoptera: Apidae) Colony Strength and Nosema spp. Infection. J. Econ. Entomol. 2019, 112, 60–66. [Google Scholar] [CrossRef]

26. Noordyke, E.R.; Ellis, J.D. Reviewing the efficacy of pollen substitutes as a management tool for improving the health and productivity of western honey bee (Apis mellifera) colonies. Front. Sustain. Food Syst. 2021, 5, 772897. [Google Scholar] [CrossRef]

27. Pande, R.; Karnatak, A. Germinated pulses as a pollen substitute for dearth period management of honey bee colonies. Curr. Biot. 2014, 8, 142–150. [Google Scholar]

28. Kumar, R.; Agrawal, O. Comparative performance of honey bee colonies fed with artificial diets in Gwalior and Panchkula region. J. Entomol. Zool. Stud. 2014, 2, 104–107. [Google Scholar]

29. El-Banby, M.; Gorgui, W. Development of honey bees whose colonies are fed on sugar syrup and different kinds of pollen substitutes. Res. Bull 1970, 610, 1–22. [Google Scholar]

30. Usha, U.; Srivastava, P.; Goswami, V.; Khan, M. Exploration of various flours as pollen substitutes for Apis mellifera L. during Dearth period at Tarai region of Uttarakhand, India. J. Appl. Nat. Sci. 2014, 6, 812–815. [Google Scholar] [Cross-Ref]

31. Younis, M.S. Evaluation of Ten Supplemental Diets to Enhance Some Honey Bee (Apis mellifera L.) Activities during Winter Season in Egypt. Egypt. Acad. J. Biol. Sci. A Entomol. 2019, 12, 101–109. [Google Scholar]

32. Steele, T.N.; Schürch, R.; Ohlinger, B.D.; Couvillon, M.J. Apple orchards feed honey bees during, but even more so after, bloom. Ecosphere 2022, 13, e4228. [Google Scholar] [CrossRef]

33. Wright, G.A.; Nicolson, S.W.; Shafir, S. Nutritional Physiology and Ecology of Honey Bees. Annu. Rev. Entomol. 2018, 63, 327–344. [Google Scholar] [CrossRef]

34. Singh, A.S.; Takhellambam, M.C. A Method to Study Honey Bee Foraging Regulatory Molecules at Different Times During Foraging. Front. Insect Sci. 2021, 1, 723297. [Google Scholar] [CrossRef]

35. Al Toufailia, H.; Grüter, C.; Ratnieks, F.L. Persistence to Unrewarding Feeding Locations by Honeybee Foragers (Apis mellifera): The Effects of Experience, Resource Profitability and Season. Ethology 2013, 119, 1096–1106. [Google Scholar] [CrossRef]

36. Gill, R.J.; Ramos-Rodriguez, O.; Raine, N.E. Combined pesticide exposure severely affects individual-and colony-level traits in bees. Nature 2012, 491, 105–108. [Google Scholar] [CrossRef]

37. Saffari, A.; Kevan, P.G.; Atkinson, J. Consumption of three dry pollen substitutes in commercial apiaries. J. Apic. Sci. 2010, 54, 5–12. [Google Scholar]

38. Amro, A.; Omar, M.; Al-Ghamdi, A. Influence of different proteinaceous diets on consumption, brood rearing, and honey bee quality parameters under isolation conditions. Turk. J. Vet. Anim. Sci. 2016, 40, 468–475. [Google Scholar] [CrossRef]

39. Khan, K.A.; Ghramh, H.A. Nutritional efficacy of different diets supplemented with microalga Arthrospira platensis (spirulina) in honey bees (Apis mellifera). J. King Saud Univ. Sci. 2022, 34, 101819. [Google Scholar] [CrossRef]

40. Cook, S.M.; Awmack, C.S.; Murray, D.A.; Williams, I.H. Are honey bees' foraging preferences affected by pollen amino acid composition? Ecol. Entomol. 2003, 28, 622–627. [Google Scholar] [CrossRef]

41. Roulston, T.; Buchmann, S.L. A phylogenetic reconsideration of the pollen starch-pollination correlation. Evol. Ecol. Res. 2000, 2, 627–643. [Google Scholar]

42. Ghosh, S.; Jeon, H.; Jung, C. Foraging behaviour and preference of pollen sources by honey bee (Apis mellifera) relative to protein contents. J. Ecol. Environ. 2020, 44, 4. [Google Scholar] [CrossRef]

43. Sihag, R.C.; Gupta, M. Development of an artificial pollen substitute/supplement diet to help tide the colonies of honeybee (Apis mellifera L.) over the dearth season. J. Apic. Sci. 2011, 55, 15–29. [Google Scholar]

44. Safari, A.; Kevan, P.G.; Atkinson, J.L. Feed-Bee: A new bee feed is added to the menu. Bee Cult. 2006, 134, 47–48. [Google Scholar]

45. Jagannadham, K.; Parimalavalli, R.; Babu, A.S.; Rao, J.S. A study on comparison between cereal (wheat) and non cereal (chickpea) flour characteristics. Int. J. Curr. Trend Res. 2014, 3, 70–76. [Google Scholar]

46. Sreerama, Y.N.; Sashikala, V.B.; Pratape, V.M.; Singh, V. Nutrients and antinutrients in cowpea and horse gram flours in comparison to chickpea flour: Evaluation of their flour functionality. Food Chem. 2012, 131, 462–468. [Google Scholar] [CrossRef]

47. Ouazib, M.; Dura, A.; Zaidi, F.; Rosell, C.M. Effect of partial substitution of wheat flour by processed (germinated, toasted, cooked) chickpea on bread quality. Int. J. Agric. Sci. Technol. 2016, 4, 8–18. [Google Scholar] [CrossRef]

48. Kulkarni, S.; Desai, A.; Ranveer, R.; Sahoo, A. Development of nutrient rich noodles by supplementation with malted ragi flour. Int. Food Res. J. 2012, 19, 309. [Google Scholar]

49. Sharoba, A.M.; Farrag, M.; El-Salam, A. Utilization of some fruits and vegetables wastes as a source of dietary fibers in cake making. J. Food Dairy Sci. 2013, 4, 433–453. [Google Scholar] [CrossRef]

 Berton, B.; Scher, J.; Villieras, F.; Hardy, J.J.P.T. Measurement of hydration capacity of wheat flour: Influence of composition and physical characteristics.
 Powder Technol. 2002, 128, 326–331. [Google Scholar] [CrossRef] 51. Nasiri, F.D.; Mohebbi, M.; Yazdi, F.T.; Khodaparast, M.H.H. Effects of soy

51. Nasiri, F.D.; Mohebbi, M.; Yazdi, F.T.; Khodaparast, M.H.H. Effects of soy and corn flour addition on batter rheology and quality of deep fat-fried shrimp nuggets. Food Bioprocess Technol. 2012, 5, 1238–1245. [Google Scholar] [CrossRef]

52. Campechano, C.; María, E.; de Dios Figueroa Cárdenas, J.; Arámbula Villa, G.; Martínez Flores, H.E.; Jiménez Sandoval, S.J.; Luna Bárcenas, J.G. New



ecological nixtamalisation process for tortilla production and its impact on the chemical properties of whole corn flour and wastewater effluents. Int. J. Food Sci. Technol. 2012, 47, 564–571. [Google Scholar] [CrossRef]

53. Leoro, M.G.V.; Clerici, M.T.P.S.; Chang, Y.K.; Steel, C.J. Evaluation of the in vitro glycemic index of a fiber-rich extruded breakfast cereal produced with organic passion fruit fiber and corn flour. Food Sci. Technol. 2010, 30, 964–968. [Google Scholar] [CrossRef]

54. Kulamarva, A.G.; Sosle, V.R.; Raghavan, G.V. Nutritional and rheological properties of sorghum. Int. J. Food Prop. 2009, 12, 55–69. [Google Scholar] [Cross-Ref]

55. Palavecino, P.M.; Penci, M.C.; Calderón-Domínguez, G.; Ribotta, P.D. Chemical composition and physical properties of sorghum flour prepared from different sorghum hybrids grown in Argentina. Starch-Stärke 2016, 68, 1055–1064. [Google Scholar] [CrossRef]

56. Zaparrart, M.; Salgado, J. Chemical and nutrition evaluation of whole sorghum flour (Sorghum bicolor, L. Moench), complementation with bean and milk whey, application in baking. Arch. Latinoam. De Nutr. 1994, 44, 151–157. [Google Scholar]

57. Khan, K.A.; Ghramh, H.A. Pollen source preferences and pollination efficacy of honey bee, Apis mellifera (Apidae: Hymenoptera) on Brassica napus crop.

J. King Saud Univ. Sci. 2021, 33, 101487. [Google Scholar] [CrossRef]

58. Pernal, S.F.; Currie, R.W. The influence of pollen quality on foraging behavior in honeybees (Apis mellifera L.). Behav. Ecol. Sociobiol. 2001, 51, 53–68. [Google Scholar] [CrossRef]

59. Yucel, B.; Duman, I. Effects of foraging activity of honeybees (Apis mellifera L.) on onion (Allium cepa) seed production and quality. Pak. J. Biol. Sci. 2005, 8, 123–126. [Google Scholar] [CrossRef]

Zhang, H.; Zhou, Z.; An, J. Pollen release dynamics and daily patterns of pollen-collecting activity of honeybee Apis mellifera and bumblebee Bombus lants-chouensis in solar greenhouse. Insects 2019, 10, 216. [Google Scholar] [CrossRef] 61. Disclaimer/Publisher's Note: The statements, opinions and

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www.andermatt-biovet.de/de-de

Andermatt BioVet





Apitherapy is a way of natural healing with bees and bee products that works in everyday life - prevention, curative and rehabilitation.

The origins of modern apitherapy go back at least to Dr. Philip Terč, (born in Czech Republic, worked and died in Maribor, Slovenia) the pioneer of modern apitherapy. Humans has been a user of apitherapy since we first came into contact with bees on this planet, and it can be said that in the past, bee products have indeed been used for therapeutic purposes. On the celebration of the first World Bee Day in Žirovnica, Slovenia, sadly deceased, President of Apimondia, the Irishman Philip McCabe, said: "Honey was medicine before medicine was invented". This was his way of emphasising that bee products are not just food supplements and stimulants, but that we, beekeepers, have in our hands some very power-

ful functional foods that, if we know how to use them properly, can really benefit our health.

For example, every beekeeper comes into contact with bee venom several times, as they are stung by bees while working if they do not wear protective clothes. It was while working with bees that Dr. Philip Terč made an important discovery and became a pioneer of modern apitherapy. As a rheumatic patient, the pain of working with bees (when they stung him) had subsided, and he had already been researching the relationship of bee venom to healthy and sick organisms since 1878/79. Thus, between 1878-87, he examined 173 cases of diseases, injecting a total of 39,000 bee stings, and by 1912 had cured 543 of the 658 cases of his patients. Through his deliberate experiments and critical clinical observation, he built his bee therapy on the apitoxin: rheumatism relationship and, as can be seen from Terč's writings, simultaneously paved the way for allergology and immuindirectly stimulated nology, and the pharmaceutical industry to produce apitoxin preparations.

As pharmaceuticals have moved up the ladder of medical science, we have eventually forgotten about the folk remedies that have been used throughout history to help relieve illness and maintain well-being. Bee products are highly concentrated, biologically active substances that, if used correctly and continuously, can be of great benefit to us. It is important how they are obtained, at what temperatures they are stored, and in what premises and packaging they are stored before they reach the users. Then it is our job to be able to give the right advice on how they should be used, along with adherence to official medicine. Some of apitherapists present apitherapy in the media, organise presentations to inform the general public about the impor-

> tance of bees and their products, thus working in the direction that (I hope) all beekeepers strive for - to give bee products the value and price they deserve on the market.

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Apitherapy is not a touristical offer, but a service provided by a certified apitherapist, either in his or her apiary, which is equipped for apitherapy, or in a room suitable for that service. This involves several iterations of the apitherapy treatment until the desired result is achieved.

As apitherapists, it is our job to give the right advice on how people should use bee products, together with respect for official medicine. It should be strict that when using bee products in apitherapy, we are only talking about sustainable, organically produced bee products. In our work, we must be well aware of our own capabilities and those of bees (bee venom especially) and bee products. We are guided by the phrase: 'Primum non nocere', which means 'first do no harm'. This requires continuous education and cooperation between apitherapists.

Climate conditions all over the world, not only in Europe, limit us and bees with extreme weather events such as too warm winters, too soon and too hot springs with exreme cold and rain, drought in summers with extreme storms and floods, fires, and too hot and long autumns. Beekeepers are faced with reduced honey yields and fewer varieties of honey. From the past, when honey was extracted a few times a year when the bees were moved to different pasture areas, to the fact that some beekeepers feed bees in beeseason so that they do not starve to death, it makes sense to think about the way and focus of beekeeping. Here, beekeepers who also produce other bee products such as wax, propolis, pollen, royal jelly, drone homogenate and bee

venom are at an advantage. All these products, obtained in an organic way, unheated and undried, allow a wide use in apitherapy, which, in these climatic conditions, can save professional beekeeping businesses. Let us think about what we can change, adapt and offer to the people around us.

I am not suggesting that all beekeepers become apitherapists, not at all! But rather that professional beekeepers shift their activities to organic and offer their produce to apitherapists who may be in short supply and need it for their therapeutic work. It is very important for beekeepers to be aware of what we have in our hands - a real natural pharmacy. It is up to the beekeepers' associations to raise awareness among their members, who are certainly free to decide which practice to follow, but it is one possible way. In my opinion, the future of beekeeping is in apitherapy.

> Source: https://www.slovenskabiografija.si/oseba/sbi692294/ (23.3.2022); Slovenskabiografija.si

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MONTHLY REPORTS ON ECONOMIC FRAUD IN HONEY IN THE EU

Economic fraud in the food industry, particularly concerning honey, has become a critical issue that the European Union (EU) is actively addressing through comprehensive monthly reports. These reports highlight various instances of food fraud, outline the EU's response, and emphasize the importance of food integrity.

Overview of Honey Fraud

Honey fraud primarily involves the adulteration and mislabeling of honey products. This can include the addition of cheaper sweeteners like sugar syrup, misrepresentation of the geographical origin, or selling non-pure honey as pure. Such fraudulent activities not only deceive consumers but also harm honest producers and compromise food safety standards.

EU's Monthly Food Fraud Summary Reports

The EU's Joint Research Centre (JRC) publishes monthly food fraud summary reports that detail incidents across various food categories, including honey. The reports from 2023 and 2024 reveal several key trends and actions:

E February 2024:

Brazil, honey and royal jelly – In the operation "Xaropel II" Federal police seized 8000 bottles of adulterated honey and dismantled a criminal organization selling 15 tons per month of sugar syrups as real honey, with a profit margin of 2 000%.

■ *April 2024:*

USA, honey and royal jelly - The U.S Food & Drug Administration (FDA) has released data





from an assignment related to Economically Motivated Adulteration (EMA) on honey, conducted between April 2022 and July 2023. The sampling (107 samples of imported honey) was designed to identify products that contained undeclared sweeteners less expensive than honey, such as syrups from cane and corn. 3% of samples were violative.

■ *May 2024:*

Italy, Honey and royal jelly - The authorities seized 600 kg of wildflower honey mislabelled as acacia honey;

Austria, honey and royal jelly: Several beekeepers had reported the poor health of their bee colonies after using embossed wax sheets manufactured by a specific company. Analyses have notably revealed the presence of molecules (pesticides, hydrocarbons, fatty acids) that do not correspond to the normal contents of authentic beeswax or to those of waxes directly supplied by beekeepers.

November 2023:

Brazil, honey and royal jelly - The Ministry of Agriculture and Livestock (Mapa) found adulteration with C-4 sugars (such as corn or sugar cane syrups) in 7.46% of the 67 honey samples analyzed. For sampling, 67 samples were collected in 42 of the 194 honey-producing establishments registered (21.65% of the total establishments in Brazil).

March, 2023:

Brazil, honey and royal jelly - The Ministry of Agriculture and Livestock (Mapa) carried out a campaign to analyze adulteration in honey samples collected in retail stores. 14 % of 99 samples presented adulteration by C-4 sugars, and 31.5 % of 109 samples were non-compliant for levels of hydroxymethylfurfural and amylase.

Pakistan, honey and royal jelly - The authorities seized 8.1 tons of expired honey ready to be smuggled and sold in the markets.

■ February, 2023:

Brazil, honey and royal jelly – In operation "Dextrin", the authorities closed a company adulterating bee honey with glucose, dextrin and other chemical products.

The summary reported multiple cases of adulterated honey mixed with sugar syrups, which were identified through advanced analytical techniques. These findings underscore the ongoing issue of economic adulteration in the honey sector.

These reports highlight instances of honey mislabeling, particularly concerning the false declaration of geographical origins. The EU's reinvolved sponse stricter scrutinv and enforcement of labeling laws to ensure transparency and consumer trust. The focus shifted to international cooperation. where several coordinated efforts with non-EU countries helped intercept fraudulent honey imports. This collaborative approach is crucial in addressing the global nature of food fraud. The latest report indicated a rise in fraud detection due to improved surveillance and reporting mechanisms. Enhanced analytical methods have been pivotal in detecting sophisticated forms of honey adulteration, leading to more significant regulatory actions.

Global Insights and Regional Discrepancies

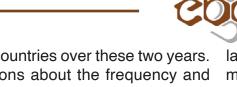
Despite the existence of the website dedicated to reporting food and beverage fraud in the European Union, an analysis of these reports reveals a global perspective. For the years 2023 and 2024, the majority of the reported actions by authorities have come from Brazil and the USA. Apart from two incidents in Italy and Austria, there have been no published reports of honey seiz-



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ures in European countries over these two years. This raises questions about the frequency and thoroughness of inspections within the EU. It is evident that the issue of counterfeit honey in Europe is significant, yet underreported.

Need for Increased Monitoring and Reporting

To better safeguard consumers and maintain market integrity, it is imperative to increase both the frequency of market inspections and the transparency of reporting on these issues. By advocating for more stringent and regular controls, European authorities can help ensure that consumers are better informed and protected against the rising tide of honey fraud.

Conclusion

The persistent issue of honey fraud requires continuous vigilance and robust action from regu-

latory bodies. The EU's monthly food fraud summary reports play a crucial role in highlighting the prevalence of such fraud, informing stakeholders, and guiding policy decisions. As the EU continues to enhance its monitoring and enforcement strategies, these efforts will be vital in ensuring the integrity of honey and protecting both consumers and honest producers.



Director of ANA LAB DOO PANCEVO Republic of Serbia Accredited laboratory in accordance with ISO/IEC 17025:2017. office@ana-lab.rs Member of the EBA Scientific Committee

For more detailed information, you can refer to the monthly food fraud summary reports available at: https://knowledge4policy.ec.europa.eu/food-fraud-quality_en

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Modern society faces a number of challenges when it comes to food safety and the compliance of products on the market. Among these challenges, economic fraud is a serious problem that directly threatens the health and trust of consumers. In this article, we will deal with the analysis of economic frauds related to honey on the market of the Republic of Serbia.

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We are increasingly faced with questions about the authenticity, origin and quality of the food we consume, and such concerns are justified given the growing trend of economic food fraud.

Economic frauds represent a serious challenge for societies around the world, having a significant impact on various social aspects. These scams not only lead to the loss of money and resources, but also create serious consequences for the standard of citizens, gross domestic product (GDP), migration, the demise of villages and agriculture.

One of the direct impacts of economic fraud on society is reflected in the reduction

of the standard of living of citizens. Through fraudulent investments, corruption or market manipulation, people lose their savings, pensions, or even jobs. This directly affects their ability to meet basic needs, such as food, housing, education and health care. Economic frauds can seriously threaten the economic growth and development of а country. Manipulations in business, falsification of financial reports or unfair distribution of resources lead to a violation of investor confidence, a decrease in foreign investment and make lending difficult. All this together has a negative



effect on GDP, limiting the potential for job creation and improving living conditions.

When economic fraud grips a society, it can encourage migration of citizens in search of a better life. People often leave their homes in search of a more stable economic environment. creating demographic changes in the country of origin, while destination countries experience an influx of new labor. Economic fraud often affects rural areas, where agriculture and villages are key segments of society. Through unfair practices, reduction of rural support or false promises to farmers, economic frauds contribute to the demise of villages and decline in agricultural production. This has far-reaching effects on local communities, causing social dispersion and the loss of traditional ways of life. The connection between economic fraud and social aspects in society is complex and requires comprehensive measures to prevent and deal with the consequences. Transparency, strengthening justice systems, and educating citizens about financial literacy are key steps toward building a more resilient society that can deal with the challenges of economic injustice.

It is estimated that TENS OF BILLIONS OF EUROS are lost in the national budgets of the countries of the Western Balkans due to economic fraud in the region, due to the uncompetitiveness of national producers and entire economic sectors. Economic frauds directly affect all holders of economic and state systems, and represent an invisible factor that is very difficult to detect and sanction. Economic fraud is directly related to the hyper-production of cheaper products whose origin is difficult to determine, and with its growth and connection with import lobbies, interest groups, the criminal milieu and corrupt elements in public institutions, it affects the weakening of the state apparatus, and therefore the life standard of citizens.

Corruption, which allows the weakening of controls on the market, as well as on the borders, allows such cheap goods to freely overflow national markets and creates a dumping effect on national producers whose competitiveness should be in the first place and in the national interest of each country. Instead, internal factors, which base their business model on trade, i.e. sales, increase the pressure for even more such goods to reach the end consumers, and thus national producers become uncompetitive. With the decrease in sales of domestic products, the number of new jobs also decreases, which then directly affects the number of unemployed people who have to either retrain or look for work abroad. This triggers an outflow of population, and parts of the territory begin to empty. As a large percentage of settlements in the territory of the Western Balkans are directly or indirectly connected to agriculture (in Serbia, over 80 % of the population is directly or indirectly connected to agriculture), so economic fraud directly or indirectly affects all layers of society. Regardless of whether it is a winemaker or a viticulturist, beekeeper, or perhaps a construction company, or a company engaged in the production of process technology, economic fraud in food affects them all.

It is wrong to look at final products such as a jar of honey, or a bottle of wine, but also a tetrapack of milk or a dairy product, as if it is the product of only that producer, because this is not the case. Even though these products enter the market as final products of designated producers, they still represent the products of the entire society, because they all participate together to make these products and put them on the market.

Also, it is wrong to reason that only those producers are affected by economic fraud in food, because if they do not have a job, then no one in





the chain will have a job and that then affects all of us as a society. With the decline in living standards, people cannot afford new things, new cars, clothes, apartments, houses, trips, and so on. Consequently, the construction industry, the auto industry, the textile industry and all other sectors of production feel the consequences. And the consequences are also felt in the state apparatus, because there is not enough money for the salaries of the employees, inspectors receive less pay, so they are forced to engage in corrupt activities in order to satisfy their living demands. By reducing the criteria, reducing controls on the market, the door is opened to unfair competition, the gray zone of traffic, black production, fake products of original brands, and all this directly affects society and its dis-

Economic frauds directly affect the filling of the state budget due to the impossibility of collecting multiple income taxes, value added taxes, taxes and contributions for wages, taxes on excise goods. The state then resorts to increasing imports in order to compensate for budget deficits and collect import VAT and customs rates for certain categories of products, but in this way it further increases the influence of unfair competition, which creates an even greater dumping effect on domestic producers. The reduction of budget funds reduces the possibilities of new infrastructure projects, construction of highways, school institutions, hospitals, as well as strengthening the quality of learning in higher education institutions. Salaries in education and healthcare stagnate, and inflation raises the cost of living, and people decide to seek happiness in other destinations in the world. Highly skilled personnel are leaving the country and emigration is increasing.

placement and dying out.

Based on the information of the Republic Institute for Statistics of the Republic of Serbia, in the past 11 years, the number of inhabitants in the Republic of Serbia has decreased from 7.22 to 6.64 million inhabitants. Even 580,000 inhabitants less in 11 years! This is twice the population of Novi Sad. This is a direct indicator of the white plague, a large number of people are leaving the country, there is an elderly population that is dying, we are becoming an old nation, and fewer babies are being born and this trend must change. Our interest is for this trend to turn around and for people to start returning to their country, and for our birth rate to in-Therefore, crease. the fiaht against economic fraud IS a national interest of the **GREATEST** state importance and we have to deal with this problem in a serious way, because economic fraud is a form of economic war.

By strengthening the

fight against economic food fraud, the state gains strength, problems with corruption are solved, state institutions are strengthened, salaries of employees in the state sector

are increased, so there are no more reasons for corrupt actions, the number of inspectors in the field increases, and therefore strengthens the fight against the gray zone of traffic, illegal flows of goods, regulates and strengthens regulations in terms of criminal policy against those who engage in, support or are involved in economic fraud on the market. By strengthening state services, new regulations are introduced that protect national markets from the overflow of cheap goods with a dumping effect.

The situation with honey in Serbia: The fight against counterfeiting and challenges on the market

Beekeeping in Serbia is going through a difficult period, faced with various challenges that



SUMER MOVEMENT FOR FOOD QUALITY" Association.

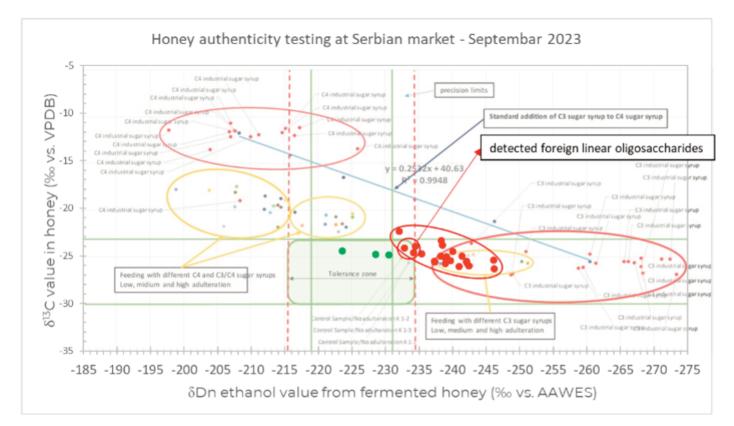
Honey samples were tested for authenticity (botanical origin of sugar), and tests were done in the accredited laboratory ANA LAB DOO Pančevo, which specializes in isotopic tests in food.



The action determined that as many as 22 honey samples did NOT pass the authenticity test, which represents as much as 88 % of the

total number of tested honey samples (25) (Graphical preview of isotopic results for tested honey samples from Serbian market - Red cluster with red spots). All laboratory reports were forwarded to the competent authorities - the Veterinary Administration and the Veterinary Inspection of the Ministry of Agriculture of the Republic of Serbia for further steps in accordance with the regulations.

The Serbian Federation of Beekeeping Organizations (SPOS), as the umbrella beekeeping organization in Serbia, has a direct interest in starting a fierce fight against honey counterfeiting in Serbia, because the state of Serbia co-financed the construction of the NAS MED facility with 20 % of its total value, which is owned by SPOS, and for which is currently repaying a loan in the amount of 450,000.00 euros. The entire factory has cost around 1,800,000 euros so far. The interest of beekeepers is that they can make a living from their work and that they can market their honey from the NAS MED Plant on the Serbian market. Since the market is left to itself, i.e. there are no controls on economic fraud and malfeasance by unfair competition, this practically means that it is impossible to sell real honey on the market, given that beekeepers are initially not competitive in price and no one is interested in their honey, while counterfeits are sold on the





market and commits direct tax evasion. The situation is alarming, and if these problems are not solved, the Serbian beekeepers will find themselves in an even more difficult situation.

ANA LAB, specialized laboratory that deals with testing the authenticity of food, was founded on the foundations of many years of scientific and research work by Mr. Ivan Smajlović and his professional team. Ivan Smajlović is recognized as one of the leading experts in the field of isotopic testing and determining the authenticity of food on a global level, and his work focuses on the detection of economic fraud in food products, such as wine, fruit brandies, fruit juices, honey, milk and dairy products (www.ana -lab.rs).

ANA LAB stands out for its accreditation by the Accreditation Body of Serbia (ATS) according to the international standard ISO/IEC 17025:2017 for product conformity testing. This accreditation enables the laboratory to operate in the food control system in the Republic of Serbia and internationally.

This action of testing the authenticity of honey and other products such as wine and fruit brandies of the Republic of Serbia opened up new issues in the functioning of official inspection bodies and the entire food control system in the Republic of Serbia. After the announcements in the media, at the end of last year, more precisely on 26.12.2023. the report of the State Auditor's Institution ON THE AUDIT OF BUSINESS FEASIBILITY "FOOD SAFETY IN THE REPUB-LIC OF SERBIA" was published (https:// www.dri.rs/izvestaj/12588), which pointed out numerous non-conformities and exposed all the problems that exist in the relevant Ministry of Agriculture of the Republic of Serbia.

In the summary conclusions, it was established that:

1. The planning and legislative framework in the area of food safety has not been fully established, because operational goals have not been elaborated in detail, significant areas have not been fully regulated, and a planned approach to crisis management has not been established.

3.2 Public policy documents, which detail the objectives of the strategy in the area of food safety, have not been adopted.

3.2 The areas of general conditions for food safety and food hygiene conditions in the

food business are not fully regulated by secondary legislation.

3.2 The field of food fraud is not fully regulated by law, which affects the negative perception of consumers about the food safety system.

3.2 A planned approach to managing crisis situations in the area of food safety has not been established.

2. The institutional framework provided by the Law on Food Safety was not effectively established, because the activities of the Expert Council did not sufficiently contribute to the assessment of risks in the field of food safety and food fraud at the national level, the capacities of the National Reference Laboratory were not fully utilized, and the coordination of inspection supervision and food monitoring has not been effectively established.

2.1. The activities of the Expert Council did not sufficiently contribute to the risk assessment in the field of food safety and food fraud at the national level.

2.2. The capacities of the Directorate for National Reference Laboratories are not fully utilized.

2.3. The laboratories, which performed laboratory tests as part of official controls within the scope of competence of the official inspection bodies of the Ministry of Health, were not selected through a competition.

2.4. Official control plans have not been adopted, which is why control priorities have not been determined and coordination between competent authorities in the food control system has not been established.

2.5. Coordination of inspection supervision has not been fully established due to insufficient activity of the Working Group for Food.

2.6. Monitoring of food is not carried out continuously.

3. While the Institute for Public Health of Serbia "Dr. Milan Jovanović Batut" annually reported to the public about the healthiness of food and bottled water, the rapid notification and alarm system did not sufficiently contribute to the effective notification of the public about the existence of risks to human health caused by food.

3.1. The rapid notification and warning system did not sufficiently contribute to the effec-



threaten the survival of this traditional activity. Counterfeits of honey, problems with purchase, costs associated with beekeeping, difficulties in product placement and the ubiquitous crisis in the beekeeping sector have become a serious burden for beekeepers. In addition, increasing costs for fuel, beekeeping equipment, bee health protection products, and control of various viruses that threaten bees further complicate the situation. Increased bee mortality due to the use of pesticides and insecticides, changing climatic conditions and the reduction of honey pastures are additional burdening factors.

The Serbian Federation of Beekeeping Organizations (SPOS / SFBO) has recognized these challenges and is committed to protecting the interests of beekeepers. In order to fight against counterfeiting of honey, SPOS has been fighting on the domestic market for years. Detecting fake honey, cheating buyers, and price manipulation were the main tasks of the organization. Achieving stability in the purchase prices of honey became possible only after SPOS, with the support of the state, established a facility for marketing honey called "NAŠ MED"

(OUR HONEY). This initiative enabled beekeepers to compete in the market and achieve fair prices for their pure and authentic honey.

The reduced control of honey on the market recently has led to the fact that it is literally difficult for real beekeepers to sell their honey, because they are not competitive in price with counterfeiters. The regulation on honey quality is written so that even complete counterfeits can pass honey quality control. Determining the

quality of honey based on only a few basic physico-chemical quality parameters is enough to prove that it is really authentic honey. Today, all those parameters can be technologically adjusted so that fake honey can meet the minimum standards for honey and be released on the market as such, creating a dumping effect on real honey. The problem is increasing, because honey is sold not only in retail chains, but also in public markets and other open public spaces. More rigorous and constant market control with adequate laboratory analysis and confirmation of authenticity from a specialized laboratory is the only way to bring order to the market and solve the dumping problem we currently have in the field.

However, with the beginning of the war in Ukraine, there was a destabilization of the honey market in the European Union. Increased import of cheap honey from Ukraine and China resulted in a drop in honey prices on the world market, making Serbian honey uncompetitive. Counterfeiters took advantage of this situation, placing fake honey on the domestic market, often cooperating with retail chains to increase their profits.

Due to unfavorable market conditions, many beekeepers are forced to give up beekeeping. Unfair competition, high costs and climate change have led to serious problems in the sector. In order to cope with the challenges, this year (2024) at the XV State Beekeeping Fair, SPOS hosted delegates from over 18 European countries, and on that occasion the European Beekeeping Association - EBA (European Beekeeping Association) was formed. This alliance brings together national beekeeping or-

> ganizations from 26 European countries with the aim of a joint fight against honey counterfeiting in Europe. The motto "we choose European honey" emphasizes the importance of supporting local beekeepers and preserving the authenticity of beekeeping products.

The European Beekeeping Association has an ambitious goal lobbying state institutions, including Brussels, in order to improve the situation in beekeeping and

reduce the amount of fraud in the honey trade. This initiative aims to preserve the tradition of beekeeping, support beekeepers and promote high-quality, authentic honey produced by European beekeepers. This aims to create a fair market environment in which beekeepers will cope with challenges and continue their important role in preserving the bee ecosystem.

The fact is that in Serbia there are around 20,000 beekeepers who live from beekeeping and collecting honey. This represents a serious number of citizens, and since beekeeping is mostly a family activity, we can conclude with a





simple calculation that 20,000 families live from beekeeping, which amounts to about 80,000 souls. Looking from that social perspective, it is very important to preserve beekeeping in Serbia and help it recover and stand on its feet again. With the fight against counterfeit honey and against dumping, which kills the competitiveness of Serbian beekeepers, the honey market will once again be strengthened and, therefore, the price of honey will stabilize, which will directly mean more income for beekeepers in Serbia, and therefore a better standard of living for everyone those engaged in beekeeping.

SPOS - Serbian Federation of Beekeeping Organizations (SFBO)

Among other things, the Executive Board of SPOS gave a report on the opportunities in the world honey market with reference to the export and import of honey to the People's Republic of China, as the largest honey trader in the world.

In 2022, China exported a record amount of honey for a record amount and ranked first in the world in both positions. In 2020 and 2021, China was the second country after New Zealand in terms of honey export value. The average export price in 2022 was \$1.78 per kilogram. It is not the price of honey that the beekeeper receives, but the total export price with all shipping costs to the customer anywhere in the world. Honey was exported to 53 countries of the world. The first five largest buyers were (in thousands of tons): Great Britain - 34.3; Japan – 29.8; Belgium – 25; Poland – 15.5; Spain – 7.1. The volume of honey imports to China in 2022 decreased by 20% compared to 2021 and was the smallest in the last five years. The price of honey imports has also dropped significantly.

The average import price of honey in 2022 was 19.1 dollars per kilogram. There is, therefore, a HUGE difference in the export and import price of honey, because China buys the highest quality honey for its sophisticated market of more than 5 million enormously wealthy people, mostly manuka honey. China, by all accounts, will retain the leading position in terms of the value of honey exports in 2023 (waiting for data) as well as in 2022. The biggest contender for the leadership position is New Zealand, as was the case in 2020 and 2021.

China is also a big importer of honey. Honey was imported from 22 countries. Its five largest suppliers are (in tons): New Zealand - 1,612; Russia – 839; Thailand – 361; Australia – 223; France - 151. China is the third largest buyer of New Zealand honey (14%), after the USA (28%) and Great Britain (16%), followed by Australia (14%) and Japan (9%). The value of manuka honey from New Zealand, imported to China, accounts for 80% of the total value of honey.

According to research conducted by the "CONSUMER MOVEMENT FOR FOOD QUALITY" Association from Pančevo, 88 % of honey on the Serbian market is counterfeit

On 11.09.2023. Association "CONSUMER MOVEMENT FOR FOOD QUALITY" carried out the first action of testing the market for food quality and on that occasion 25 samples of honey were sampled from 7 retailers shops in the territory of the city of Pančevo:

- 1. AMAN,
- 2. DIS,
- 3. GOMEX,
- 4. IDEA,
- 5. LIDL,
- 6. MAXI,
- 7. MARKET MERE

Sampling was carried out for the Consumer Protection Association by the accredited laboratory Food Testing Center (CIN DOO), from Belgrade. 2 units of each product (with the same LOT numbers, same filling dates and times) were sampled, with one unit sampled for analysis and the other unit left for potential super-analysis. For each sample, a sampling record was made by the sampler from CIN DOO Belgrade. The sampling was also attended by members of the "CON-



tive notification of the public about the existence of risks to human health caused by food.

3.2. Reporting to the public about the healthiness of food and bottled water is carried out by the Institute for Public Health of Serbia "Dr. Milan Jovanović Batut" at annual level.

All this together opened up additional questions and, in conjunction with the problems of agriculturists (farmers, beekeepers, fruit growers and winegrowers), opened up questions about the negative trend in the agriculture of the Republic of Serbia and raising questions related to corruption within the Ministry of Agriculture of the Republic of Serbia.

After the devastating statistical data that the import of agricultural products (meat and meat products, as well as milk and diary products) increased to 660 million euros in the first 6 months of 2024, and that at the same time the total export of meat from the Republic of Serbia was only 72.5 tons, farmers rose up and demanded serious reforms and changes in the leading positions in the Ministry of Agriculture of the Republic of Serbia.

In this connection, information has reached the public that 4 years ago, the Republic of Serbia had an operational laboratory as part of the Directorate for National Reference Laboratories of the Republic of Serbia, the highest arbitration and control body for food quality control, and that at the moment when that laboratory was supposed to go for accreditation, without explanation the entire project was stopped and the equipment was ordered to be taken out of the premises of the Directorate for National Reference Laboratories of the Republic of Serbia. The subsequent report of the state auditor only confirmed that corruption in the departmental Ministry of Agriculture has caught so much that it is doing everything to suppress control and put operational systems on their feet, which are prescribed by the Laws of the Republic of Serbia.

Without operational DNRL as the highest arbitration body for food control, there is no final and ending word on the quality of food, that is, the samples that are analyzed in the official sample flow. This entails the impossibility of epilogue in the courts, because therefore in the processes of expertise in the court processes they cannot be carried out in an appropriate manner and appropriate verdicts cannot be delivered. Therefore, unfair competition can continue unhindered with further operations and economic frauds can continue to undermine the entire society of the Republic of Serbia. Persons in significant government positions are subsidized by lobbyists whose aim is to maintain the status quo as long as possible, because this in itself ensures extra profit for certain parties.

In the future, consumer associations will continue with their activities and we expect more feedbacks from the market about the quality and authenticity of the food that is marketed to the citizens of the Republic of Serbia.



Ivan Smajlović Director of ANA LAB DOO PANČEVO Republic of Serbia Accredited laboratory in accordance with ISO/IEC 17025:2017. office@ana-lab.rs Member of the EBA Scientific Committee

<u>BEES</u>

HARNESSING HONEY BEE BIODIVERSITY IN THE FIGHT AGAINST HONEY FRAUD

The estimated cost of global food fraud ranges from €25 to €37 billion annually. Honey, celebrated for its taste, nutritional value, and health benefits, is unfortunately one of the prime targets. Considering that in 2022, the European Union (EU) produced 236,019 tonnes of honey, alarmingly a recent EU-wide coordinated action revealed that 46% of the sampled honey was suspected of being subject to fraud. At the EU level, the EU Honey Directive (Directive (EC) 110/2001) consolidates the EU-wide definition of honey, specifying common rules on composition, and determining the main labelling information. Despite these regulations, globalisation and complex supply chains have contributed greatly to a rise in honey fraud. These trends undermine market prices and consumer trust, inflicting substantial harm on the beekeeping sector, where honey prices are crucial for economic sustainability.

Honey fraud occurs through mislabelling and adulteration. Mislabelling involves false claims about the botanical, geographical (including regional, territorial, and topographic), or entomological origin of honey. Noteworthy is that the EU Honey Directive remains vague on honey bee subspecies entomological derivation. Conversely, adulteration involves mixing honey with cheaper, low-quality honeys or adding sweeteners such as sugar syrups. These practices artificially inflate the price of low-value honey to that of a higher quality which fetches a better price on the market.

Traditionally, fraudulent honey is detected using the presence of pollens which indirectly indicates the geographical origin of the honey. Other analytical methods target physical-chemical characteristics of honey, such as sugars, proteins, amino acids, minerals, and trace elements. However, these methods lack universality and are adopted arbitrarily in different countries. An exciting and novel approach is the use of DNA markers, which has great potential for identifying both botanical and entomological origins of honey. As living things continuously shed genetic material, honey bees leave traces of both the flowers they visited and their genetic material, in the honey they produce. Scientists have developed tools to detect this genetic material and use these biomarkers to identify honey fraud. However, only if the local honey bee subspecies is reared this novel parameter can be utilised to assess entomological origin. This provides a strong incentive for beekeepers to rear their regional autochthonous bees, offering an added tool to combat honey fraud.

Malta, an EU member state in the Mediterranean, has a long tradition of beekeeping and hosts an endemic subspecies of honey bee, Apis mellifera ruttneri. As is the case with other regional subspecies of honey bees in their own native habitats, A. m. ruttneri is key for sustainable beekeeping in Malta due to its adaptations and increased production. It also offer an added opportunity in the fight against honey fraud,



The Maltese Honey Bee (Apis mellifera ruttneri) [© Niki Alexander Mallia 2024]

should environmental DNA in honey be used to determine authenticity.

In addition to testing, increasing consumer awareness is another crucial way to address honey fraud. To that end, the Foundation for the Conservation of the Maltese Honey Bee has since May 2023, advocated for the declaration of the Maltese Honey Bee as Malta's National Insect. This initiative aims not only to safeguard local biodiversity, but also boost public interest in beekeeping and bee-derived products. This elevated status would also translate to the enhanced value and prestige of local honey. Promoting honey as a product derived from both local flora and an endemic subspecies declared as the National Insect, holds great potential for the economic sustainability of Malta's fragile beekeeping sector.

Hive frame full of Maltese honey, made by the Maltese Honey Bee (Apis mellifera ruttneri) [© Abner Joe Buttigieg 2022]





At the European level, the principle of honey authenticity, based not only on local flora but also on endemic honey bee ecotypes, should be given serious consideration. This approach offers a dual safeguard in promoting sustainable honey production amidst globalisation, climate change, and other challenges: it ensures authenticity, quality, and increased valorisation by protecting against honey fraud; while also conserving the increasingly threatened biodiversity of honey bee ecotypes.

> **Dylan Farrugia & Abner Joe Buttigieg** Administrators at the Foundation for the Conservation of the Maltese Honey Bee

Interested in finding out more about the NGO and our initiatives? can the QR code now!



We extend our gratitude to the editorial board for allowing us to publish this article.

Congratulations to the EBA's executive and associated partners on the launch this project. This initiative serves as an ideal platform to internationally promote coordinated efforts aimed at safeguarding honey bee diversity and the beekeeping sector, upon which it depends!



EBA HEADQUARTERS

THE BEEKEEPING CENTER OF SLOVENIA INTRODUCES ITSELF

The Slovenian Beekeepers' Association unites more than 210 beekeeping societies and nearly 8,000 beekeepers. The average age of beekeepers in Slovenia is 59 years. We work with Carniolan grey bee and we use the AŽ hive in 90% of beekeeping practices. In total, Slovenian beekeepers have around 200,000 bee colonies. Our common home is the Beekeeping Center of Slovenia, located in Brdo pri Lukovica.

Starting in 2024 it also serves as the headquarters for the European Beekeeping Association. We are excited to introduce you to our Beekeeping Center, which is a great source of pride for Slovenian beekeepers!

SLOVENIAN BEEKEEPING CENTER

Slovenian beekeepers first came to the idea to build an educational beekeeping center in the 1960s, and the decision to follow up on this idea was made in 1967. In 1969, they bought land in this area, in 1972, they obtained a building permit, laid the foundation stone and constructed the building all the way to the roof. The construction was then stopped, and it was not resumed until 2000. The Slovenian Beekeeping Center was fully built and opened on 26 May 2002.



The Slovenian Beekeeping Center includes:

- the seat of the Slovenian Beekeepers' Association.

 – editorial office of the Slovenski čebelar (Slovenian beekeeper) magazine,

- the headquarters of the Honeydew Flow Observation and Forecasting Department,

 the headquarters of the Beekeeping Public Advisory Service,

- the headquarters of a recognized breeding organization,

- the headquarters of the European Beekeeping Association (EBA),

- the Janez Goličnik Beekeeping Library,

 – a laboratory for internal quality control of bee products,

- a bee product tasting center,

- a beekeeping academy,

- conference rooms (3) for various events,

- the Čebelarna shop,

- the Pri Čebelici restaurant.

In addition, the Slovenian Beekeeping Center is also a venue for:

- research;

beekeeping promotion and beekeeping technology development;

 presentations of honey plants and medicinal herbs;

 supplying beekeepers with equipment and necessary tools, along with equipment presentations;

 activities for the preservation of natural and cultural heritage in the field of beekeeping;

 activities related to professional field trips, business tourism and beekeeping tourism;

 various camps and guided tours according to pre-arranged programs.

3D-KRANJICA





3D-Kranjica is a unique educational and promotional tool created using the latest 3D printing technology based on micro-CT and a mechatronic assembly with computer control and audiovideo support on the screen with speakers. 3D-Kranjica – a physical model of a bee on a 1:100 scale (size of 1.5 meters) with a morphological similarity of over 90% to a real-life Apis mellifera carnica worker bee. The 3D-Kranjica model is mounted on a rotating platform built into a replica of an apiary and contains moving parts and nine educational animations. The 3D-Kranjica will be able to "fly" and take part in various events around the world. The model includes a



3D printed model of a bee for the blind and visually impaired in the size of 20 cm.

HONEY STORY PAVILION

The pavilion is a transportable apiary for education, presentation and promotion of Slovenian beekeeping and honey products.

It is equipped with the latest video and audio technology.

It takes us on a journey to the world of bees with the help of sight, smell, touch and finally taste, with a very special honey station. Half of the interior of the pavilion is dedicated to the presentation of beehives and honey production technology. The hives portray the life of bees with special video content installed inside honeycombs. Under the hive, a beehive scale is installed, along with a weather station and video surveillance of the exterior of the pavilion. Further on, honey extraction, storage and packaging are





presented, according to all the standards of safe and healthy food production. Key information on bees and beekeeping is also highlighted.

The second part of the interior of the pavilion is dedicated to the presentation of all bee products. Next to the sales counter, there is a space for tasting different types of honey. For this purpose, a special honey tasting station was developed, which has never been seen before.

The exterior of the pavilion is interesting as well. Next to the honey station, signs are installed which present the types of honey, all bee products, our indigenous Carniolan honey bee and an important segment of our beekeeping – apither-





apy. On the other side of the pavilion, highlights in the history of beekeeping are presented on a timeline. The tradition of Slovenian beekeeping is well-represented by painted beehive panels and facades of AŽ beehives. Meanwhile, children will get to know the key characteristics of bees with the help of didactic games.

The Honey Story pavilion will not leave any visitor indifferent, neither during the day nor at

night, as the specially illuminated ambience emphasizes the honey drops in the honeycomb, which are placed on the outside of the pavilion, as well as the motifs engraved in glass inside the pavilion.

When the pavilion is not travelling, it is located at the headquarters of the Slovenian Beekeepers' Association and can be admired by visitors.

VR - BEEKEEPING

VIRTUAL BEEKEEPING is a unique project for people to learn more about beekeeping, and a fun experience for everyone to boot.

You will be able to try it for free on your first visit to the beekeeping center.

Using the virtual reality application called "Beekeeping VR", you will embark on a virtual journey inside an apiary and a beehive and learn more about the typical working day of a bee family. You will take a walk around the apiary and see the space in a 360-degree technique.

You will be able to move around the tools that are needed to work with bees, open the beehive and hold the honeycombs full of bees while accompanied by the soothing sound of their buzzing.

And do not forget: bees can normally sting, but that is not the case in this apiary.



"JANEZ GOLIČNIK" BEEKEEPING LIBRARY



In our library collection, we keep books on beekeeping, manuals, publications of beekeeping societies, theses, international beekeeping magazines, videos about the life of bees and other pamphlets. In the beekeeping library, we also keep all the volumes of the Slovenski čebelar ('Slovenian beekeeper') magazine and its predecessors – Slovenski čebelar in sadjerejec ('Slovenian beekeeper and fruit grower') and Slovenska čebela ('Slovenian bee').



There are ten reading spots available. A librarian can give you advice and help you find material.

The material can be viewed in the reading room, while newer literature can be borrowed out. Membership is free.

LEARNING PATH FOR THE BLIND AND VISUALLY IMPAIRED

Cooperation of partners in the Čebelja pravljica pripoveduje ('The Story of a Bee') project enabled the conceptual design and development of an innovative tourist product that connects the Beekeeping Center in Brdo pri Lukovici and Rajh's homestead in Dol pri Ljubljani, two important tourist spots in the framework of the Heart of Slovenian Local Action Group initiative.

The project enabled the adjustment of the beekeeping learning path of the Slovenian Beekeepers' Association in the vicinity of the Slovenian Beekeeping Center to the needs of the blind and visually impaired, which will enable them, by means of an electronic tablet, to order food and drinks by themselves and to walk the learning path near the Slovenian Beekeeping Center of Slovenia without the need for further assistance.



CARNIOLAN HONEY BEE GALLERY

The Carniolan Honey Bee gallery is part of the newly built winter-summer garden and is available as additional premises for catering as well.

The Carniolan Honey Bee gallery has permanent and occasional exhibitions. His also used as a multi-purpose venue of the Slovenian Beekeepers' Association for various events held at the Slovenian Beekeeping Center.





BEEKEEPING CAMPS

Summer camps are a great opportunity for children to get valuable and unforgettable experience. They are supervised by dedicated mentors who prepare a variety of activities in order to teach children how to work together as well as other important life skills. At the camp, children make new acquaintances and friendships, create unforgettable memories and get used to a certain degree of independence, which prepares them better for adulthood.

"PRI ČEBELICI" RESTAURANT

The "Pri Čebelici" restaurant is located in a beautiful spot in the heart of nature – in 'Čebelarski dom' in Brdo pri Lukovici.

The restaurant serves delicious food and beverages, offers accommodation, and also ac-

cepts reservations for events such as: business meetings, weddings, birthdays, baptisms, confirmations and communions; there is also a congress hall with all the necessary equipment for different business meetings.







ČEBELARNA SHOP

When visiting the Slovenian Beekeeping Center, you will have the opportunity to purchase honey products, souvenirs, beekeeping literature, beekeeping equipment ...





CONFERENCE ROOMS AT THE SLOVENIAN BEEKEEPERS' ASSOCIATION



It is possible to rent conference halls with all the necessary equipment for seminars, consultations, business meetings and conferences:

* Anton Janša Hall: for 100 to 130 people,

* Peter Pavel Glavar Hall: for 25 to 30 people,

* Anton Žnidaršič Hall: for 20 to 25 people.



TEAM BUILDINGS

In the beautiful environment of the Beekeeping Center and on the premises of the Slovenian Beekeepers' Association, it is possible to organize a team building, which includes:

a guided tour on the beekeeping learning path,

- a visit to the apitherapy apiary,

– a visit to the observation hive (in spring and summer),

- tasting of honey and other bee products,

- honey drink tasting,

- a short presentation on Slovenian beekeeping, - team building is concluded by a picnic or a normal meal.

We adapt all programs to the wishes of our visitors.

Contact: Slovenian Beekeeping Center Brdo 8 1225 Lukovica, Slovenia +386 1 72 96 100 info@czs.si www.czs.si



European Beekeeping Association



T: +386 4 537 85 10 • M: + 386 41 618 775 • info@medikoel.com • www.medikoel.com



INTRODUCING FACTORY FOR PLACEMENT HONEY OF THE SERBIAN FEDERATION OF BEEKEEPING ORGANIZATIONS (SPOS) "NAŠ MED" LLC.

The Serbian Federation of Beekeeping Organizations (SFBO - in Serbian SPOS) was founded 127 years ago.

In 2020, the Federation built and opened the factory for honey marketing of its about 8,000 member beekeepers. Honey is sold without intermediaries, which practically means that you buy honey directly from SPOS beekeepers.

Factory mode of operation

When beekeepers extract honey from hives, they report the quantities to the factory

for sale. Samplers go out in the field and sample honey, and then they seal the barrels. In addition to individual samples, aggregate samples are made for one lot of honey of 20-24 tons, and sent to international accredited laboratories at the buyer request. After the good results of the analysis arrive, the honey is taken from the beekeepers and transported to the factory, where it is homogenized, once again analysed, packaged and delivered to buyer. If a buyer becomes a strategic partner by signing a contract with the secure marketing of larger quantities of honey, the buyer can get honey under the most favourable conditions possible.

NO BEES, NO LIFE · EBA MAGAZINE

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How to buy honey

The buyer needs to be assured of the quality of the honey while the honey is in the "Naš med" factory. Subsequent complaints are not possible. After the final sampling is performed by the buyer, or a person designated by the buyer or an institution at the buyer's request, the buyer or an institution at the buyer's request, the buyer sends the sample to the laboratory of the buyer's choice. The packaging of the sampled honey is sealed with the buyer's seals. If the analysis is good, the buyer pays for the honey before the truck sets off.

Available quantities

In good years, we have much more than 2,000 tons of honey for export, but the availability should always be checked in advance, because we work only with the honey of our members, we do not import honey, and it sells out quickly.



Honey selection

Serbia has the following types of honey for export: acacia, sunflower, mountain meadow, honeydew, linden, rapeseed...





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MAXIMUM QUALITY STANDARDS

1) The Plant implemented HACCP, BRC FOOD 9 and HALAL.

2) SFBO (SPOS) owns the **private quality standard** "Good Beekeeping Practice" which implies a series of measures and procedures a beekeeper needs to implement in order to create the preconditions for obtaining top quality honey:

www.spos.info/wp-content/uploads/2016/06/Good_Beekeeping_Practice_Guide-SERBIA.pdf

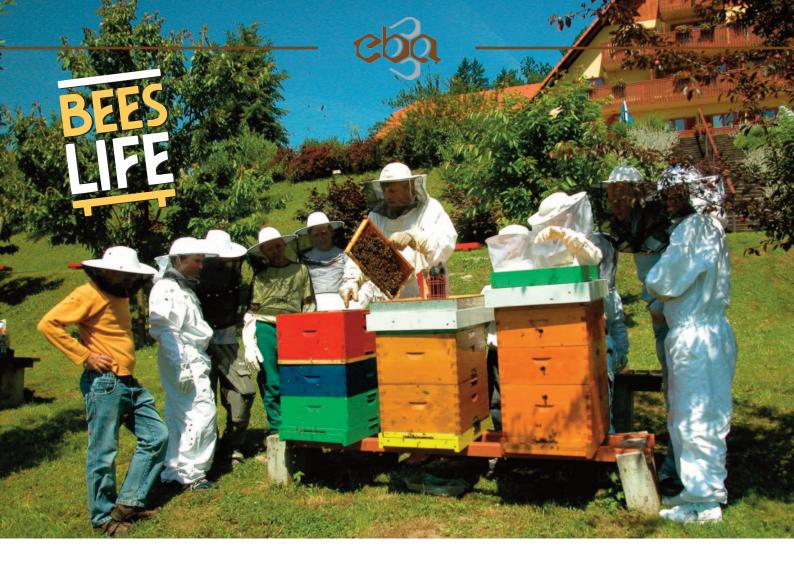


Honey is exported in metal barrels of 290 kg (with bisphenol-free coating), smaller barrels or buckets, as well as in any other smaller packaging from 30 g to 1,000 g, according to the customer's choice. Honey is also packed in 12 g teaspoons, as well as in small bags.

Nenad Portić, director +381 60 444 07 07 Factory for collecting and placement honey from SPOS` beekeepers "NAŠ MED" LLC. 2 Solunskih ratnika St. Rača, Republic of Serbia www.nasmed.rs, nasmed.rs@gmail.com



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PRESENTATION OF THE PUBLIC ADVISORY SERVICE ON BEEKEEPING IN SLOVENIA

The Ministry of Agriculture, Forestry and Food of the Republic of Slovenia awarded a first concession to the Slovenian Beekeepers' Association for performing the public advisory service on beekeeping in 2008. In 2021, Ministry awarded the third concession to the Slovenian Beekeepers' Association for the period 2022 to 2028. The Service operates pursuant to annual Public Advisory Service on Beekeeping Programme. The primary tasks of the Public Advisory Service for Beekeeping are advice to beekeepers and education of beekeepers, publication of professional and promotional materials, we participate in the preparation of development beekeeping programs in Slovenia, advise on the preparation of regulations in the field of beekeeping and we are raising awareness among the youth and the general public about beekeeping which include training of children in beekeeping clubs, organization of promotional campaigns for the general public.

The Advisory Service is particularly focused on the qualifications of beekeepers so that they



will be better managers of their apiaries and produce flawless and healthy products for human consumption. The tasks of specialist advisers of the Public Advisory Service in beekeeping in the fields of technology, economy and safe food are performed in 2024 by 11 people in the total scope of at least 7 full-time consultants (PDM) annually and the administrator and accountant in the total scope of one employee. Besides the regularly employed, there are 18 field advisors working for the Advisory Service.

These consultants are additionally trained beekeepers who advise beekeepers on the implementation of general preventive measures and in examining bee colonies, in evaluating the correctness of the colony development and in determining any possible changes in bee behaviour and broods, in preparing bees for transfer and in internal control of honey. They also assist beekeepers who are just starting their beekeeping career. In the field, they carry out workshops that are intended to transfer knowledge between beekeepers.

Beekeeper training is also taken care of in the Programme in the form of specific training courses at which specialist can transfer their knowledge to beekeepers. This knowledge covers beekeeping technologies and economy as well as safe food. We have prepared the Guidelines for Good Hygienic Practices in Beekeeping based on the principles of the HACCP system. Most Slovenian beekeepers have already been qualified to implement these guidelines. We place a great deal of stress on internal control of honey; we have our own laboratory for analysing the physical-chemical parameters of honey. The laboratory performs internal control of honey and provides expert consultancy to beekeepers based on the findings. We also help beekeepers in honey labelling.

The Janez Goličnik specialist library operates within the framework of the Public Advisory Service on Beekeeping and is housed in the Beekeeping Centre at Brdo pri Lukovici. The library offers over 3,000 units of specialist beekeeping literature that we use to train beekeepers and in-





form the general public. The library includes domestic literature and many materials from other countries.

With the intention of increasing the number of young beekeepers, beekeeping clubs are held at primary and secondary schools with over 2.000 children. They are led by beekeepers – mentors and teachers. Mentors benefite from a 40-hour syllabus for beekeeping clubs for the lower and higher-level groups, numerous educational materials, various practical beekeeping materials and beekeeping equipment.

The Advisory Service introduced notification and awareness-raising of the general public on the importance and role of beekeeping, on the role of bees in the pollination of plants and on the

EBELICA

significance of healthy and high-quality honey. It publishes a great deal of educational and promotional materials, publishes articles and participates in radio and television shows, etc. We carry out numerous promotional campaigns such as attending fairs, organising "Honey Days", the "open house" day of slovenien beekeepers, we participate to honey breakfast in kindergartens and schools etc.

Lidija Senič Head of Services of SBA

MOJA PRIJATELJICA

🛚 matica,

🔹 troti.

o čebele delavke,

Življenje v panju:



OBFES, NO LIFE · EBA MAGAZINE





EBAAT

THE SEVENTH BEEKEEPING FAIR OF THE SOUTHEAST BALKANS IN VRANJE (SERBIA)



The 7th Southeast Balkan Bee Fair will be held in Vranje on September 21, 2024.

EBA and SFBO (Serbian Federation of Beekeeping Organizations) have agreed on cooperation, and the President of EBA, General Secretary of EBA and President of the Scientific Commission of EBA will hold a Round Table with the presidents of the national beekeeping associations of the region, as well as all interested beekeepers.

SERBIAN FEDERATION OF BEEKEEPING ORGANIZATIONS CITY OF VRANJE in partnership with EUROPEAN BEEKEEPING ASSOCIATION (EBA) UNION OF BEEKEEPING ASSOCIATIONS OF NORTH MACEDONIA ASSOCIATION OF PROFESSIONAL BEEKEEPERS OF GREECE UNION OF BEEKEEPERS ASSOCIATIONS OF THE REPUBLIC OF SRPSKA NATIONAL FEDERATION OF BEEKEEPERS OF ALBANIA with support ASSOCIATION OF BEEKEEPERS "MATICA" VRANJE organize





SEVENTH BEEKEEPING FAIR OF THE SOUTHEAST BALKANS "OPEN BALKANS"

(North Macedonia, Greece, Republika Srpska, Albania, Bulgaria, Turkey, Montenegro) Sports Hall of the City of Vranje Date: September 21, 2024 Working hours: 7-19 hours

FREE ADMISSION FOR ALL FREE PARKING FOR EXHIBITORS AND VISITORS IN THE COURTYARD OF THE SPORTS HALL COMPLETELY FREE STANDS FOR EXHIBITORS UP TO 20% PROMOTIONAL DISCOUNT FOR EXHIBITOR FEES FOR PAYMENTS BY APRIL 5, 2024 AND 10% FOR PAYMENTS BY JULY 5, 2024.

THE PROGRAM

9.00 Start of sales exhibition

10.30-11.00 CEREMONIAL OPENING OF THE FAIR

- Vranje Mayor Slobodan Milenković
- President of SFBO, MD. Rodoljub Živadinović
- Representative of the Ministry of Agriculture

11.00-11.15 CULTURAL AND ART PROGRAM

11.15-12.15 EXPERT LECTURE ON BEEKEEPING

Lecturer: Dr. Vet. honey ZLATKO TOMLJANOVIĆ, Zagreb, Croatia, is a professional beekeeper with 150 hives and head of the Beekeeping Advisory Department of the Ministry of Agriculture of the Republic of Croatia, national coordinator of the Climate Farm Demo project, as well as head of the Working Group of the Ministry of Agriculture for climate-smart agricultural practices

Topic: CLIMATE SMART BEEKEEPING

12.15-12.45 Discussion



16.00-17.00 ROUND TABLE FOR PRESIDENTS OF BEEKEEPING ASSOCIATIONS AND BEEKEEPING ALLIED REGIONS: Topic: HOW WILL THE EUROPEAN BEEKEEPING ASSOCIATION (EBA) SAVE BEES, BEE-

KEEPERS AND CONSUMERS?

Participants:



Boštjan Noč (Slovenia) President of the European Beekeeping Association



Biljana Tomić (Serbia) Secretary General of the European Beekeeping Association



Dr. Urška Ratajc (Slovenia) President of the Scientific Commission of the European Beekeeping Association

Participation in the Round Table is free for all interested beekeepers.

BASIC INFORMATION ABOUT THE FAIR

The Seventh Beekeeping Fair of the Southeast Balkans will be held in Vranje with international participation, with complex sales, exhibition, educational, promotional, economic and tourist content.

SFBO, in cooperation with the City of Vranje, has ensured that the entrance to the fair will be free for all interested beekeepers and citizens this year.

We expect a large attendance of the fair by beekeepers from Serbia, Macedonia, Albania,

Greece, Bulgaria, Turkey and Montenegro, because this part of the Balkans simply does not have a similar manifestation of such a large scale, and our beekeeping equipment is of exceptional quality and fair prices. The incredibly large visit to the Fair so far has proven that.

CONDITIONS OF PARTICIPATION IN THE FAIR

SFBO, with its significant participation in the organization of the fair, secured significantly more favorable prices for the exhibition space than the



regular collective prices valid at the State Beekeeping Fair in Belgrade, since the fairs proved to be an exceptional opportunity for the placement of larger quantities of honey from our beekeepers, placement of equipment at favorable prices for beekeepers and especially for contracting future business - which is an increasingly frequent phenomenon that brings the biggest profit to the exhibitors, due to the visit of a large number of foreign buyers of beekeeping equipment wholesale.

Each stand will have an inscription with the name of the company or the owner, which is included in the price.

PRICE LIST FOR EXPOSITION AT THE FAIR

REGISTRATION PRICES in EUR (without 20% VAT) for payments from July 6, 2024 until the beginning of the Fair:

Beekeeping equipment and other (for stalls and stands up to 9 square meters)* 320 EUR

Beekeeping equipment and other (for stands from 10 to 18 square meters)* 400 EUR

Beekeeping equipment and other (for stands from 19 to 27 square meters)* 720 EUR

Bee products (for companies and nonmembers of SFBO* 304 EUR

Bee products (for SFBO members)* 101 EUR

Literature (in written and electronic form)* 18 EUR

*- the price is increased by 10% in case the exhibitor wants to sell bee products (honey, pollen, propolis, raw wax, perga, royal jelly... or preparations based on bee products) at the counter or stand intended for the sale of equipment. At the Seventh Fair in Vranje, it will not be possible to choose the location of the stand.

The rental of several stands by one exhibitor is allowed, but the stands cannot be connected to each other, but must be separated.

Stands for bee products (for non-SFBO members) cannot be larger than 9 square meters. For SPOS members, stands for bee products are up to a maximum of 1.5 meters wide.

This year, the organizer will provide each exhibitor with at least 1 stand of 9 square meters, which means a space of 3x3 meters, partially covered by a roof and marked with a board with the name of the exhibitor or his company, with one table 1-2 m long. If the exhibitor rents more than 9 square meters, he arranges all the space that exceeds 9 square meters.

Occupying space outside of the exhibition stand is not allowed. If any of the exhibitors does that, they will be immediately removed from the fair.

The sale of any other products than bee products (including mixtures and products based on bee products) is not allowed at the stands for which a preferential registration fee has been paid for SFBO members for the display of bee products and products based on them. In the event that this happens, the exhibitor will be immediately removed from the stand, and he is also obliged to pay double the registration fee at the first subsequent SPOS fair in which he participates (regardless of whether it is a fair in Vranje or Belgrade). Exhibiting beekeepers - members of SFBO, who pay a preferential registration fee this year, must be members of SFBO in 2023 and 2024 in order to exhibit under preferential conditions.

All exhibitors are obliged to market their goods at the fair in accordance with legal regulations, otherwise they bear full legal responsibility.

APPLICATION FOR PARTICIPATION

Application for participation in the fair is carried out according to the following procedure. Make the payment to the SFBO account at Poštanska štedionica: 200-2361360102033-24, and the payment slip with the information written on the prescribed form (name and surname, full address, telephone numbers, email, list of everything you are exhibiting, name of the beekeeping society through which you are a member of SFBO...) sent to the address of SFBO with the indication "For the fair in Vranje".

Exhibitors from abroad pay exactly the same registration fee (in euros), but for payment instructions they must contact SFBO accountant Jagoda Milenkovic on phones +381 11 61-28-



071, +381 60 444-01-24, working days from 9 until 1 p.m., or by e-mail: sposbg@sezampro.rs and spos.rs@gmail.com

OTHER

Provided: free parking for exhibitors and visitors located right next to the hall.

OBTAINING MARKETING SPACE OUT-SIDE THE STAND: Obtaining marketing space, outside the exhibitor's stand, is paid to SFBO in addition to the registration fee, 23,9 EUR (+20% VAT), per meter of the longer side of the advertisement, or 19,9 EUR (+20% VAT) for advertisements whose longer side is shorter than one meter. The exhibitor creates the advertisement himself, chooses the place for it and places it himself (in accordance with the safety regulations). In the case of placing an advertisement outside the paid advertising space, the exhibitor at the first subsequent SFBO fair pays twice the registration fee.







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NO BEES, NO LIFE · EBA MAGAZINE

Issue 2, August 2024 · www.ebaeurope.eu

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EUROPEAN MEAD MAKERS CONFERENCE A GATHERING OF MEAD ENTHUSIASTS AND HONEY PRODUCERS

Kings of Mead, in collaboration with the European Mead Makers Association (EMMA) and pitnemiody.pl, are delighted to announce the European Mead Makers Conference. This event is set to become a cornerstone for both professional and home mead makers across Europe and the globe.



Event Details:

- Date: February 27, 2024 March 1, 2024
- · Location: Krakow, Poland

Event Overview

The European Mead Makers Conference offers a unique platform for mead enthusiasts to enhance their knowledge, network with fellow aficionados, and partake in enriching activities. The conference will feature a series of lectures and presentations delivered by industry experts, designed to provide valuable insights and foster the exchange of ideas and experiences.

Key Highlights

1. Educational Sessions: Attendees will have the opportunity to attend a variety of lectures and presentations that cover a broad range of topics related to mead making. These sessions are tailored to benefit both novices and seasoned mead makers, ensuring a comprehensive learning experience for all.

2. **Networking Opportunities**: The conference is an excellent occasion to meet fellow



mead makers, exchange contacts, and forge new partnerships. The informal meetings in local restaurants will include mead tasting sessions, where participants can share their creations and receive feedback from peers.

3. **Mead Tasting Sessions**: These sessions offer a relaxed environment for attendees to showcase their own mead. Participants are encouraged to bring their mead, allowing others to sample and discuss the nuances of each brew.

4. **Honey Madness Cup**: having its premiere in 2024, a competition exclusively for beekeepers and apiary owners who produce their own honey. This competition is an extension of the renowned Mead Madness Cup, organized in collaboration with bee honey sommelier Olga Gavrylik. Unlike traditional competitions, honeys in the Honey Madness Cup will be judged on organoleptic qualities by honey sommeliers from the Italian organization Alba del Miel.



European Beekeeping Association (EBA) as Patron

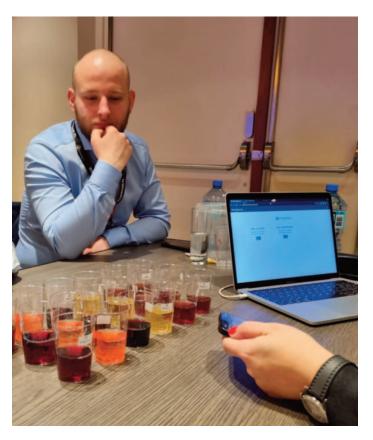
This year, we are honoured to have the European Beekeeping Association (EBA) as a patron of the European Mead Makers Conference. The EBA is committed to three primary goals:

• Combating Counterfeit Honey: Addressing the influx of counterfeit honey in the European market to protect the integrity of genuine honey



products.

• Advocating for Special Subsidies: Promoting the introduction of subsidies per beehive, acknowledging the crucial role bees play in pollination and ecological improvement.



Fighting Against Toxic Pesticides: Working

to prevent the improper use of pesticides that are harmful to bee populations.

The EBA's involvement underscores our shared commitment to quality, authenticity, and the well-being of the bee-keeping community. Their support enhances the conference's focus



on sustainable practices and the promotion of genuine honey and mead products.

Testimonials

"We are thrilled to expand our horizons with the Honey Madness Cup, offering a new dimension to our beloved Mead Madness Cup. This initiative underscores our commitment to promoting quality and excellence in both mead and honey production." - Olga Gavrylik, Bee Honey Sommelier

Conclusion

The European Mead Makers Conference promises to be an event filled with learning, net-

working, and celebration of mead and honey craftsmanship. Whether you are a professional mead maker, a hobbyist, or a honey producer, this conference provides an unparalleled opportunity to connect, learn, and grow within the community.

For more information and to register, please visit https://emmconference.com/en/home/.

We look forward to welcoming you to an unforgettable experience at the European Mead Makers Conference.











We are looking for the EUROPEAN CHAMPION

Which will be the best European honey 2024?

Participate in the European honey contest, which will be held for the first time in 2024 in Koper, Slovenia! An internationally recognized expert committee will declare the three best honeys in each category, which will be announced on the 7th of December 2024 at a professional and culinary event in sunny Koper, Slovenia. The best honeys will receive:

• The title of EUROPEAN CHAMPION 2024,

• unique plaques and awards,

• medal logos to mark the award-winning series of honey,

• special exposure in the European Parliament,

• analysis reports on high quality and safety,

• the right to use the flattering title for the next two years!

Rules and conditions of participation:



organizer	assessment category	sample collection	address to collect samples	registration fee/sample	information	day of evaluation	closing ceremony
Coastal beekeeping society Koper, Slovenian beekeeper's Association, Municipality Koper	European	up to and including 16.9.2024 In person or by mail.	Čebelarska zveza Slovenije, Brdo pri Lukovici 8, 1225 Lukovica, Slovenia	70 € Transfer: OBALNO ČEBELARSKO DRUŠTVO KOPER <u>Bošamarin</u> 30 6000 Koper TRR, Banka Koper: 10100035841107	Aljaž Debelak, <u>aljaz.debelak@czs.si</u> 01/729 61 29 <u>Klavdijo</u> Babič, <u>klavdijo.babic@siol.net</u> 041 825 680	26.9.2024	7.12.2024 in Koper, Slovenia
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SPONSORSHIP REQUEST AND METHOD OF ADVERTISING IN THE MAGAZINE

On behalf of the European Beekeeping Association (EBA),I am writing to seek your support in the form of sponsorship to help ensure the smooth and effective operation of our Association.

The EBA is dedicated to promoting and supporting beekeeping across Europe. The Association was founded out of necessity, as bees and beekeepers are essential for our ecosystem and society. Without beekeepers there are no bees, and whithout bees there is no pollination, leading to a lack of food on planet Earth.

EBA works for bees, beekeepers and consumers.

Our mission is to:

- 1. Fight against counterfeit honey that flooded the European market;
- 2. Introduction of incentives per beehive as agro-ecological programme;
- 3. Fight against the improper use of chemicals that are harmful to bees;

In return for your generous support, we offer various sponsorship benefits. We believe that this partnership would be mutually beneficial and would significantly contribute to the advancement of the european beekeeping sector.

ADVERTISING IN THE MAGAZINE:

1. Through sponsorship packages;

2. It is possible to pay for an ad only for 1/4 page (100 euros), for a larger area by agreement. The entire page cannot be obtained, it belongs only to the General Sponsor.

IT CONTINUES



EBA

sponsorship packages

GOLD sponsor - 5.000 euros:

Advertisement on the EBA website Presentation at all EBA events, logo on all EBA correspondence 12 advertisements in the EBA monthly e-magazine in A4 page size

SILVER sponsor - 3.000 euros:

Advertisement on the EBA website Presentation at all EBA events, logo on all EBA correspondence 12 advertisements in the EBA monthly e-magazine in half A4 page size

BRONZE sponsor - 2.000 euros:

Advertisement on the EBA website 12 advertisements in the EBA monthly e-magazine in the size of 1/4 A4 page

EBA SUPPORTER - 1.000 euros:

Advertisement on the EBA website 12 advertisements in the EBA monthly e-magazine in the size of 1/8 A4 page

These are basic packages, but we are open to different forms of cooperation, which we agree on individually. We would be delighted to discuss this opportunity further and explore how we can align our goals with your organization's values.

Thank you for considering our request. We look forward to the possibility of working together.

Yours sincerely,

Boštjan Noč President of the European Beekeeping Association

ebga

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The responsibility for the correctness of the English language in the magazine lies with the authors of the texts.

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Advertising in the magazine: 1. Through sponsorship packages; 2. It is possible to pay for an ad only for 1/4 page (100 euros), for a larger area by agreement. The entire page cannot be obtained, it belongs only to the General Sponsor.

The total number of pages in the magazine is not fixed.

There are no fees for published texts and photos.

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