HONEY BEE DISEASES AND THEIR CONTROL IN BULGARIA AND ESTONIA: A REVIEW

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ABSTRACT

The Western honey bee (*Apis mellifera*) is a species of crucial economic, agricultural and environmental importance. Honey bee colonies suffer from numerous pathogens. These include various bacteria, viruses, fungi and parasites. The aim of the present work is to review and compare information on the most important bee diseases and their control in Bulgaria and Estonia. Based on the data about honeybee diseases from available research and those supplied by the National Reference Laboratories of Bulgaria and Estonia it could be said that in both countries the main problem for beekeeping are the diseases varroosis, nosemosis, American foulbrood, and European foulbrood. Estonia seems to be one of the few countries in the world where *N. apis* (43%) is still individually prevalent, while in Bulgaria nosemosis caused by *N. ceranae* (98%) predominates. Principles of prevention and treatment of bee diseases are similar in both countries and comply with European recommendations.

Key words: *Apis mellifera*, bee diseases, Bulgaria, Estonia, control.

Introduction

Honey bees are species of crucial economic, agricultural and environmental importance worldwide. Besides the source of bee products (honey, pollen, wax, propolis and etc.), they are one of the most effective natural pollinators of a wide variety of crops and wild flora. Bee activity helps the biodiversity preservation and improving the balance of ecosystems.

Western honey bee (*Apis mellifera* L.) is the species used in beekeeping of Bulgaria and Estonia. Both countries are located in the temperate climate zone, Bulgaria - in the south, Estonia - in the north. Bulgaria has long-standing traditions in the beekeeping, a precondition for which is the varied and rich vegetation of the Balkan Peninsula suitable for the production of honey and also the favorable natural, climatic (Forister et al., 2010) and ecological conditions (Shumkova et al., 2018b). The climate in Estonia is characterized by the cold winters and summers that tend to have brief warm period. Beekeeping here is important and widespread but operated rather in small-scale establishments. In temperate climates, weather conditions during winter can put substantial pressure on honey bee colony survival (Switanek et al., 2017; Brodschneider et al., 2019). Also, weather conditions during summer can influence the winter survival of honey bees, as shown in a recent study conducted in the northeastern United States (Calovi et al., 2021). Consequently, high colony losses can also occur during the summer following noticeable losses in the winter (Jacques et al., 2017).

In light of the global increase in honey bee colony losses, risk factors regarding beekeeping management practices and honey bee diseases have been studied intensively during the last decade (Rosenkranz et al., 2010; Forsgren et al., 2018; Gray et al., 2019; Vilarem et al., 2021). Very often bee losses are associated with various stressors such as: pathogens, viruses, bacteria, fungi and parasites which under certain conditions cause the appearance of diseases (Chauzat et al., 2013; Flores et al., 2021). Poor health of honey bees became an increasingly urgent problem which received considerable media and public attention in recent decades (Maxim et al., 2007; Maxim et al., 2010). Many European and North American countries reported high rates of disorders (mortality, dwindling and disappearance) affecting honeybee colonies (Haubruge et al., 2006; Vanengelsdorp et al., 2012). Searching the causes of these phenomena and their control is becoming increasingly urgent (Fries et al., 1996; Fries et al.,2010; Generisch, 2008; Generisch, 2010; Traynor et al., 2020).

In connection with the above, we set a goal to review and compare studies on the most important bee diseases and their control in Bulgaria and Estonia. The data presented chronologically are from scientific developments over the last 20 years and such, sourced by the National Bee Disease Reference Laboratories of both countries.

Results and Discussion

The acaricidal effect of ethereal oils of salvia (*Salvia sclarea* L.), basil (*Ocimum basilicum* L.), white marjoram (*Origanum heracleoticum* L.) and dill (*Anethum graveolens* L.) added to food of bees (honey-sugar-candy) has been investigated by Nenchev et al. (2005). Experiment has been carried out in the autumn of 2003. Two control and four experimental groups have been formed from equal bee colonies. Bee colonies have been fed three times at an interval of seven days with honey – sugar candy (100 g single dose), including 1 % ethereal oil for experimental groups. The extensinvasion among bee colonies at the beginning and the end of the experiment and the number of mites (*Varroa destructor*) fallen at the bottom of the hive at an interval of seven days have been detected. Colonies from first control group (K1) have been treated with Rodovar® (active substance amitraz) and those from second control group (K2) have not been treated with acaricidal preparation aiming detection of the number of naturally fallen mites. Control treating with Apiprotect® (active substance coumaphos) has been done at the end of experimental period. The effectiveness of the tested ethereal oils as acaricidal means has been calculated based on the results. It has been found that ethereal oils of salvia, basil, white marjoram and dill, added to honey – sugar candy in a dose 1%, have an acaricidal effect on the mites *V.destructor* . The lower effectiveness (37.38–43.21%) of ethereal oils than this of Rodovar® (99.07%) is as a result of the attachment of the tested ethereal oils to the food of bees.

Zhelyazkova et al. (2005) investigated the influence of Nozestat® (containing iodine and formic acid) on bees invaded with spores of *Nosema apis*. They established that the preparation is well tolerated by bees in the treatment doses recommended by the manufacturer (5 ml/l sugar syrup) when applied three times at intervals of 3 days. The rate of invasion of bees by nosema spores has been reduced up to 4.2 times.

Gurgulova et al. (2006) investigated the antibacterial and antimycotic activity of the ethereal oils of savory (*Saturea montana* L.), thyme (*Thymus vulgaris* L.), white marjoram (*Origanum heracleoticum* L), salvia (*Salvia sclarea* L.), dill (*Anethum graveolens* L.), chamomile (*Matricaria chamomilla* L.), hyssop (*Hyssopus officinalis* L.), basil (*Ocimum basilicum* L), peppermint (*Mentha piperita* L.), lavender (*Lavandula angustifolia* Ch.) and thymol (crystals) to 25 strains of various microorganisms isolated from sick bees and brood: *Paenibacillus larvae var. larvae*, *Paenibacillus alvei*, *Paenibacillus paraalvei*, *Ascosphera apis*. High antibacterial activity with minimum inhibiting concentrations (MIC) = 0.012 – 0.025% has been established in ethereal oils from savory, thyme, white marjoram and thymol (crystals). Salvia, dill, lavender, basil and peppermint oils have had MIC = 0.1%, but chamomile and *H. officinalis* oils have had MIC ≥ 0.2 to all agents causing disease in bees and brood. With regard to the antimycotic activity ethereal oils from savory, thyme, white marjoram and thymol (crystals) have shown MIC = 0.025%, basil and peppermint oils – MIC = 0.1% and lavender, dill, salvia, chamomile, and *H. officinalis* oils – MIC ≥ 0.2 to the *A. apis* strains.

Gurgulova et al. (2008) studied in vitro antibacterial and antifungal activity of two plant products (Ecophil-P® and Green TM®) against some causative agents of brood diseases – *P. larvae*, *Melissococcos pluton*, *P. alvei*, *Brevibacillus laterosporus*, *P. paraalvei*, *A. apis*, as well as the field prophylactic effect of Ecophil–P® against American foulbrood. Their results showed that the investigated products are suitable for use in biological beekeeping.

The toxicity of various concentrations of oxalic acid dihydrate (OA) in aqueous and sucrose solution to *V. destructor* and to honey bees has been assessed using submersion tests of caged bees and by spraying bees in colonies with and without brood (Toomemaa et al., 2010). An aqueous solution of 0.5% OA has given effective control of the mite and has been non-toxic to bees whereas higher concentrations (1.0–2.0%) have been highly toxic to bees. Submersion tests into solutions with 0.1% OA have been acaricidal both in aqueous (59.9 ± 3.7 %) and in 50% sucrose solution (71.1 ± 4.2%) whereas concentrations of 0.2–0.5% OA have been highly effective; OA in sucrose solution has been more toxic to bees than OA in the aqueous solution. Spraying with 0.5% OA solution at a dose of 25 mL per comb in May 2003 and in April 2004 has been 99.01–99.42% effective in mite control in Estonian standard one box long beehives with 22 frames (each 414 × 277 mm, area 1000 cm^2 per comb side). Most mites have fallen after the first spraying. In autumn, spraying test colonies that have had little capped brood once or twice with a 0.5% OA solution has given effective mite control (92.94 ± 0.01% and 91.84 ± 0.02%, respectively) with no noticeable toxicity to bees.

Antibacterial activities of eleven essential oils against *P. larvae* (15 field strains and the reference BCCM / LMG 9820 strain) have been studied by the disk diffusion method and the method of serial dilutions in agar (Roussenova, 2011). The minimal inhibitory concentration (MIC) of essential oils has been determined within 1%–0.015% v/v. Highest activity (MIC ≤ 0.06–0.015% v/v) has been shown by essential oils of cinnamon, thyme, clove, peppermint, lemongrass, sage and oregano. Variable activity has been exhibited by marjoram and tee tree oils. Citrus essential oils have shown the lowest inhibitory effect with MIC ≥ 0.12– 1.0% v/v for mandarin oil and ≥ 0.25–0.5% v/v for grapefruit oil. Roussenova (2011) pointed out that the established antibacterial activity against *P. larvae* encourages further research to include essential oils as an alternative means in the measures for prevention and control of American foulbrood without the use of antibiotics.

A study of 320 bee samples of adult workers from 57 beekeeping farms has been conducted in different regions of Bulgaria during the Spring and Autumn of 2011-2012 (Gurgulova et al., 2013). Adult honey bee samples have been pasteurized, centrifuged, plated on MYPGP agar and then the number of *P. larvae* colonies has been counted. *P. larvae* colonies have been confirmed by PCR. Results have indicated that *P. larvae* spores were found only in 2 samples in autumn of 2012 (from the districts of Sofia and Veliko Tarnovo). Number of *P. larvae* spores has been about 103 counting colony forming units (CFU) /plate of 0.2 ml suspension from 20 adult bees/10 ml distilled water. According to the authors number of CFU from adult bee samples is a good predictor of American foulbrood on a subclinical level. Results supposed that the regular monitoring of bee samples for the presence of *P. larvae* spores and assessment of the epidemiological situation for the disease can reduce the risk of manifestation of American foulbrood before clinical symptoms appearance.

In her thesis, Kaasiku (2014) reported that honey bee colony losses in winter 2012–2013 in Estonia has been 22.55% of the studied 3122 honey bee colonies. The main reasons claimed have been diarrhea and varroosis. In 2013 78.6% of studied 2439 colonies in Estonia have contained *V. destructor* mites. However in spite of the high infestation level of honey bees with varroa mites, the real causes of high mortality according to the author are not clear and need to be studied. The aim of his thesis has been to estimate the effects of the number of pesticides residues found in beehives and the abundance of varroa mites. Also, the relations between the indicators of the colony strength (the amount of brood and honey yield per hive) and the abundance of varroa mites have been studied. In that purpose the samples of comb honey, bee bread and bee brood have been taken from 14 hives in Tartu County, Estonia in June and July 2013. The sampling time has been chosen before and after the flowering of spring oilseed rape. The hives have been placed in landscapes where the proportion of arable land was below 40% (N=7) or above 60% (N=7) in the area of 2 km radius from the hives. The distance between the hives has been at least 4 km. The infestation rate of the studied beehives has been low, being almost zero in June and somewhat higher in July, the average number of varroa mites has been 1.57 per 200 larvae. All the hives have been treated against varroa mites at the beginning of the season with formic acid. The results of the study have shown that the abundance of varroa mites in beehives has been higher in the landscapes where the proportion of arable land has been lower. There has been no correlation between the number of different pesticide residues found in hives and the abundance of varroa mites. The study also has shown that no any difference in the numbers of pesticide residues found in beehives from different landscapes. This could be explained by suggestion that the foraging distances of the honey bees must have been longer than 2 km from the hives so that the foraging territories could have overlapped. The results also have shown no correlation between the honey yield nor the amount of brood in hives. The varroa mites inhabit most of the hives in Estonia and therefore the author suggest that the infestation originates from neighboring hives and apiaries independently from the hive conditions and pesticide residues.

Mõtus et al. (2016) have made the first large scale study demonstrating the status of varroa infestation, its control and related risk factors in Estonian apiaries. Colonies in one hundred and ninety-six apiaries have been sampled during 2012–2013. A questionnaire has been used to record management, as well as treatment procedures applied. The majority of apiaries sampled (95%) have been infested with varroa mites and the mean proportion of affected colonies within apiaries has been high (80.6%). Apiary median colony infestation level has been 2 mites per 300 bees. According to multivariable random-effect logistic regression analysis, the numbers of colonies in an apiary and the presence of a hybrid breed of bees have been associated with higher risk of colony varroa infestation. Apiary management by a professional beekeeper, bees of the Buckfast breed, synchronized treatments between neighboring beekeepers and the presence of farmland around the apiary have been factors protective against colony varroa infestation. Varroa treatment has been applied in 93% of the studied apiaries. Chemical and organic substances have been used by 66 and 61% of beekeepers, respectively in 2012.

Six viruses are considered to cause severe infection in bees which inflicts heavy losses on beekeeping: Deformed wing virus (DWV), Acute bee paralysis virus (ABPV), chronic bee paralysis virus (CBPV), Sacbrood virus (SBV), Kashmir bee virus (KBV), and Back queen cell virus (BQCV) (Shumkova et al. (2018a). That's why the authors have investigated incidence of these viruses in different parts of Bulgaria. A total of 250 adult honey bee samples have been obtained from 50 colonies from eight apiaries situated in three different parts of the country (South, North and West

Bulgaria). The results have shown the highest prevalence of DWV followed by SBV and ABPV, and one case of BQCV.

In connection with the increasing bee mortality in winter of 2016 and 2017 Valtchovski et al. (2017) have made a risk analysis of economically significant diseases in bees in Bulgaria and Macedonia. A total of 205 bee colonies from 36 regions with different landscapes have been examined by RT-PCR diagnostic methods aiming to search pathogens. No clinical signs have been observed in these bee colonies characteristic of the presence of American foulbrood. An exception has been observed in only one case, where *P. larvae* have been proven both in the brood and in the adult bees. Nevertheless, bee colonies have showed 33.33% positive response for American foulbrood in the affected areas, representing 12% of the apiaries studied. With this study authors have proved the vertical transmission of DWV and BQCV in apiaries specialized in queen production. Because of this one finding, they consider, it is imperative to change the rules and requirements for certification of new apiaries.

Shumkova et al. (2018b) have studied the presence of *N. apis* and *N. ceranae* in the area of Bulgaria. A duplex PCR assay has been performed on 108 honey bee samples from three different parts of the country (South, North and West Bulgaria). The results have shown that the samples from the northern part of the country have been with the highest prevalence (77.2%) for *N. ceranae* while those from the mountainous parts (the Rodopa Mountains, South Bulgaria) have been with the lowest rate (13.9%). Infection with *N. apis* alone and co-infection *N. apis / N. ceranae* have not been detected in any samples. These findings suggest that *N. ceranae* is the dominant species in the Bulgarian honey bee. The results have shown that *N. ceranae* is the main *Nosema* species in Bulgaria.

Salkova et al. (2018) have estimated the level of infestation of bee samples with *Varroa destructor*. The investigation has been for a period of two years: 2015–2016. Bee samples have been collected from diseased and dead bee colonies owned by 149 beekeepers. The result have shown that from 220 bee samples tested, 36% have been positive for varroa mite, and negative samples have been 64%. The level of infestation in 39.2% of positive samples has been less than degree of 5%. The authors have concluded that more than a third of the bee samples have been infested with varroa mites. Most of the bee samples have had a low degree of invasion (< 5%) and the average and the high level of invasion of bee samples have been represented by the same values.

Rusenova et al. (2019) attempted to detect *P. larvae* spores in naturally contaminated honeys by conventional PCR and to determine the sensitivity of the reaction with different primer pairs in order to assess its potential for American foulbrood control. The results from the study showed 70% sensitivity of the PCR in honey (14 out of 20 samples) with spore content $\geq 10^2$ cfu/g honey. The authors have concluded that the false negative results in conventional PCR tests of bee honeys with low contamination levels could have a negative impact on measures for disease control and could permit spread of spores out of the affected bee family. For adequate control of American foulbrood they recommend firstly to isolatate *P. larvae* spores on suitable agar, which to be following by PCR identification in bacterial colonies.

Gray et al. (2019) have presented loss rates of honey bee colonies over winter 2017/18 from 36 countries, including 33 in Europe, from data collected using the standardized COLOSS questionnaire. The 25,363 beekeepers supplying data passing consistency checks in total wintered 544,879 colonies, and reported 26,379 colonies with unsolvable queen problems, 54,525 dead colonies after winter and another 8,220 colonies lost through natural disaster. The overall loss rate has been 16.4% of honey bee colonies during winter 2017/18, but this has varied greatly from 2.0 to 32.8% between countries. The overall loss rate in winter 2017/18 has been highest in Portugal (32.8%). Bulgaria, new country to this monitoring study, has had the lowest loss rate of just 2.0%, though based on data from only 27 professional beekeepers. Loss rate for Estonia has been about 16.4%, through based on data from 169 professional beekeepers. Winter losses related to queen problems have varied between 1.1% in Bulgaria to 4.3% in Estonia.

Ilieva et al. (2021) have made a comparative analysis of the established losses of bee colonies by regions in Bulgaria and characterization of the risk factors for available mortality in 2020. Information for different types of forage sources with potential risk for *A. mellifera* due to pesticide treatment has presented and analyzed. By using the international standardized COLOSS questionnaire for 2020, members of the National Bee Breeding Association and independent beekeepers, owners of a total of 64 apiaries (over 6,800 bee colonies), located in all regions in Bulgaria, have been surveyed. Beekeepers have been asked to answer questions about the number of wintering honey bee colonies and how many of them after winter have been alive but had unsolvable queen problems, lost through natural disaster, and dead or reduced to a few hundred bees. The survey data have shown that the highest mortality has been found for the North Central region (19%), and the lowest – for the Northwest (1%) and Southwest (2%) regions. Among the reasons for the loss of bee colonies, the leading one has been the mortality of honey bees or their significant reduction in the colonies, which is also related to the negative impact of the applied pesticides in the studied areas. In this aspect, the most serious problems have been reported in the North Central and Southeast (7%) regions.

Shumkova et al. (2021) have investigated the effects from the application of the herbal supplements NOZEMAT HERB® (NH) and NOZEMAT HERB PLUS® (NHP) on overwintering honey bee colony survival and on total protein and lysozyme content. To achieve this, in early autumn 2019, 45 colonies have been selected and treated with these herbal supplements. The obtained results have shown that both supplements have a positive effect on overwintering colony survival. Considerable enhancement in longevity of "winter bees" has been observed after the application of NHP, possibly due to the increased functionality of the immune system and antioxidant detoxification capacity.

In Estonia, the DWV has been found in 98.5% of the studied bee colonies, while the ABPV has been found in only one bee colony. A few cases of *A. apis* were also found (Karise, 2021).

Naudi et al. (2021) have studied prevalence of *Nosema* spp. in bee colonies in Estonia and Latvia. For this aim they have selected 30 apiaries in Estonia and 60 in Latvia that have been positive for *Nosema* spp. Obtained results have shown that both species have presented in Estonia and Latvia, but *N. apis* has been dominant in Estonia (43%), and *N. ceranae* in Latvia (47%). According to the authors pathogens are very persistent, since 5 years after the previous investigation, only 33% of infected apiaries in Estonia and 20% of infected apiaries in Latvia, have been free of any pathogens.

Tummeleht et al. (2022) have performed a study using the two-year data collected in frames of the European Commission EPILOBEE project. Previously, the data from Estonian apiaries have been analyzed together with the data from all 17 participating European countries in the consortium. In the project 196 apiaries containing 2,439 colonies all over Estonia have been included. The study has aimed to clarify the risk factors that would predict colony losses in Estonia. According to data collected the main factors increasing colony mortality after winter have been the size of the apiary, *V. destructor* mite count, infestation with *P.* larvae and lack of farmlands around the apiary. No significant risk factors in relation to honey bee summer mortality have been detected (Tummeleht et al., 2022).

In a two-year period (2020-2021) Salkova and Gurgulova (2022) have investigated honey bee samples from Bulgaria for presence of the two most common and widely distributed honey bee parasites. They have tested 185 bee samples by morphological and light microscopic methods. The obtained results have shown that 32.43% of bee samples have been infested with *V. destructor*. Degree of the infection in the bees has ranged from 0.5% to 60%. Spores of *Nosema* spp. have been established in 25.40% of samples with a degree of infection in the range $3x10^5$–$26x10^6$ per bee. Mixed infections of both parasites have been observed in 32.43% of the samples. Negative samples have been with the lowest value of 9.74%.

Brodschneider et al. (2023) have surveyed 28,409 beekeepers maintaining 507,641 colonies in 30 European countries concerning varroa control methods. The set of 19 different *Varroa* diagnosis and control measures has been taken from the annual COLOSS questionnaire on honey bee colony losses. The most frequent activities have been monitoring of *Varroa* infestations, drone brood removal, various oxalic and formic acid applications. Correspondence analysis and hierarchical clustering on principal components have shown that six varroa control options (not necessarily the most used ones) significantly contribute to defining three distinctive clusters of countries in terms of *Varroa* control in Europe. The six most important *Varroa* control methods to differentiate these clusters have been based on the following active ingredients of veterinary medicinal products (and their means of administration): amitraz (strips), amitraz (fumigation), formic acid (long-term evaporation), fluvalinate, oxalic acid (trickling) and coumaphos. Cluster I (eight Western European countries) has been characterized by use of amitraz strips. Amitraz strips have been applied most during August, September and October. Fifteen countries from Scandinavia, the Baltics, and Central-Southern Europe, among which Bulgaria and Estonia, have been included in Cluster II. Most beekeepers in this cluster apply oxalic acid and formic acid. This cluster has been characterized by long-term formic acid treatments. Formic acid has typically applied after honey harvest, in July, August and September. Cluster III has been formed by seven Eastern European countries characterized by dominant usage of amitraz-based products applied mainly via fumigation, with two seasonal peaks (late summer and a smaller one at the end of winter). All countries in this cluster have had registered national amitraz fumigation products shortly after the arrival of *Varroa* on their territory, around the mid-1960s. The median number of different treatments applied per beekeeper has been lowest in cluster III. Based on estimation of colony numbers in included countries, Brodschneider et al. (2022) have extrapolated the proportions of colonies treated with different methods in Europe. The authors suggest that about 62% of colonies in Europe are treated with amitraz, followed by oxalic acid for the next largest percentage of colonies. Data regarding the strategy in Bulgaria and Estonia have been obtained by 51 professional beekeepers (6897 bee colonies) and 178 beekeepers (6746 bee colonies) respectively. The indicated results have shown a similar percentage of colonies treated with amitraz, but in Bulgaria they prefer to use it by fumigation, and in Estonia by strips. Acccording to Brodschneider et al. (2022) main methods of mite control in both countries have been varroa monitoring, biotechnical methods (drone brood removal), essential oils, most often based on thymol, as well as synthetic pyrethroids based on flumethrin and taufluvalinate. The results of this survey show that oxalic and formic acid are widely applied both in Bulgaria and Estonia. The differences observed are as follows: in the exposure of formic acid - short treatment are preferred in Bulgaria, and long treatments in Estonia; a small percentage of beekeepers in Estonia use the hyperthermia method, which is not applied in Bulgaria; coumaphos strips are used by some beekeepers in Bulgaria, but not in Estonia. The data published show also that Bulgarian honey bees have good survival without varroa treatment.

Toomemaa and Kaart (2022) have investigated the application of queen caging to prevent autumn brood rearing. In comparative experiments, cages equipped with queen excluders on both sides have been used. Only cages of the experimental group have been supplied with comb sections. Survival of queens and influence of various factors on brood rearing in cages and in the nest after the queen had been released have been compared. The factors included have been the position of caged queens in the nest (in the middle or in the periphery), comb sections with empty or honey-filled cells, or with drone cells. The queens have not perished in cages supplied with a worker comb section, but 8% of them have perished in cages without it. One queen has perished in the cage with drone comb section. The number of colonies rearing brood and the brood area in the nest has been higher than those in the cage. There has been no correlation between the position of the caged queen in the nest and brood production in the cage and later in the nest. Comb section fullness has had no impact on brood rearing in the cage. In cages with a drone comb section, no brood has been observed besides eggs, but the total brood area (including eggs) has not been differ from the worker comb section group in cages and later in the nest. According to the authors caging of the queens on an empty worker comb section for 21 days supports their survival and enables the beekeeper to carry out successful Varroa control earlier in autumn.

Prevalence of *Nosema* spp. in Bulgaria has been investigated by Salkova and Naudi (2022). One hundred and fourteen samples of bees from 82 apiaries located in different regions of the country have been tested. The results have showed that 74.6% of the samples have been positive and 25.4% - negative for nosemosis. Of the positive samples, 47.4% have had an invasion rate of 2 to 10 million spores / bee, followed by those with up to 1 million spores / bee (17.5%) and the smallest number of samples has shown an invasion rate of over 10 million / bee (9.6%).

Naudi et al. (2022) have infected the royal jelly in queen cells with *Nosema* spores to see whether and how it affects the development of honey bee queens. Seven groups of grafted honey bee larvae have been established and treated as follows: high and low concentrations of *N. ceranae* and *N. apis*, mixes of both species in both concentrations, and untreated control. After allowing nurse bees to fill the queen cells with royal jelly, an injection of 50 000 spores or 10 000 spores has been added into the royal jelly. It has been found that only *N. apis* decreased the hatching rate of honey bee queens both in single and mixed treatment at high dosages, but morphological deviations in unhatched pupae has not been detected.

Salkova et al. (2024) evaluated and compared the varroacidal efficacy and mite mortality dynamic during autumn treatment of honey bee colonies in two experimental areas (Boychinovtsi-Northwestern Bulgaria and Zlatiya – Northeastern Bulgaria), treated with three available veterinary medicinal substances. They conducted clinical studies on the efficiency of three acaricidal combinations (AC) - one, based on 3.6 mg flumethrin/strip (AC-1) and two containing essential oils - first one with composition: 5 g thymol plus 2 g peppermint oil/lamellae (AC-2), and the second one with composition: 4 g thymol plus 2 g peppermint oil/ lamellae (AC-3). Product containing coumaphos and an additive with oxalic acid for the control treatment was used. After 35 days of AC-1 exposure, 94.5% and 87.82% efficiency were achieved in the apiaries in Boychinovtsi and Zlatiya, respectively. Efficiencies of the combinations tested (AC-2 and AC-3) for 45 days were 97% and 95% in the Zlatiya apiary, and 91% and 80% in the Boychinovtsi apiary respectively. The results of the experiments showed the absence of resistance to the tested substances.

Conclusion

The analysis of the presented data showed that honey bees in Bulgaria and Estonia are affected by various diseases: with parasitic (varroosis), fungal (nosemosis, Stonebrood), bacterial (American foulbrood, European foulbrood), and viral nature (DWV, SBV, CBPV, Israeli acute paralysis virus (IAPV), KBV, BQCV). The most significant among them are varroosis, nosemosis and American foulbrood. Winter and summer bee mortality and pesticide poisoning are also among the main challenges for beekeepers in both countries.

The following differences between the two countries are observed: nosemosis in Bulgaria is more severe and with greater losses compared to Estonia, due to the predominant spread of the more pathogenic causative agent – *N. cerane*; European foulbrood is common in Bulgaria, while no cases of this infection have been reported in Estonia in the last 10 years; method of hyperthermia treating varroosis is applied in Estonia, which is not accepted in Bulgaria; treatment of varroosis with formic acid in Bulgaria is with short term, and in Estonia - with long term; some beekeepers in Bulgaria, unlike in Estonia, still use Coumaphos strips.

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