Losses of Honey Bee Colonies and Risk Factors for their Mortality in Bulgaria During 2020

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Abstract. The study is based on a comparative analysis of the established losses of bee colonies by regions in Bulgaria and on the characterization of the risk factors for available mortality in 2020. Information for different types of forage sources with potential risk for *Apis mellifera* due to pesticide treatment, was 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, were surveyed. Beekeepers were asked to answer questions about the number of wintering honey bee colonies and how many of them after the winter: were alive but had unsolvable queen problems; were lost through natural disaster and were dead or reduced to a few hundred bees. The survey data show that the highest mortality was 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 is 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 were reported in the North Central and Southeast (7%) regions. The presented and analyzed data should be considered when developing activities to protect the honey bee health status in Bulgaria.

Key words: honey bee, *Apis mellifera*, reasons for mortality, forage sources, pesticides.

Introduction

Honey bees are the main pollinators of entomophily vegetation and an important factor for preserving plant diversity in nature. The treatment of agricultural crops with various pesticides is a risk factor for the health and overall vitality of bee colonies. The phenomenon, known today as the Colony Collapse Disorder (CCD), poses a serious challenge for scientists around the world, to analyze the causes of increasing mortality among honey bee populations and to propose measures to protect *Apis mellifera* species. Studies on the problem indicate that the main factors leading to bee losses are *Varroa destructor*, *Nosema* spp., various viruses (mainly *Acute Bee Paralysis Virus* and *Deformed Wing Virus*), pesticides used in

agricultural practice and the nutrition of bee colonies (Potts et al., 2010; van Engelsdorp & Meixner, 2010; Gajger et al., 2017; Steinhauer et al., 2018). Among the reasons for the loss of bee colonies are starvation, theft, crude weather conditions, new pathogens and more. Honey bees are often exposed to the simultaneous effects of multiple stressors, leading to a synergistic result that goes beyond the negative effects of each of them individually (Alaux et al., 2010; Johnson et al., 2013; Tosi et al., 2017; van Dooremalen et al., 2018; Straub et al., 2019; Tomljanović et al., 2020). The study by Steinhauer et al. (2021) indicates small but meaningful growth of successful wintering bee colonies through management practices of a higher level. These results show the positive impact of the designed and used programs, as well as the guidelines provided by scientific experts for work in the target groups of beekeepers. Ullah et al. (2021) presented the viral effects on honey bee populations and indicated that areas of the world responsible for supplying frequently consumed food had a high number of bee losses per year. Various environmental factors that negatively affect the viability of honey bees are: intensive farming with long-term use of pesticides, food shortages, habitat loss, new pathogens and pests (Goulson et al., 2015; McMenamin & Genersch, 2015 ; Tantillo et al., 2015; Brutscher et al., 2016). Ullah et al. (2021) express their vision for a unified health approach worldwide in support of beekeeping and its management in the future.

According to the official data of Agrostatistics (February 2020) in Bulgaria there are 13,771 apiaries with 867,561 bee colonies as of October 1, 2019. Of them total, 58,377 bee colonies were destroyed. Statistics show poisoning as the leading cause with the highest percentage of losses - 24,317 bee colonies.

Since 2008, through the European COST action FA0803 (Prevention of honey bee COlony LOSSes), the international organization COLOSS has been established,

uniting *Apis mellifera* researchers from all over the world. COLOSS (http://coloss.org) is currently an international non-profit association based in Bern, Switzerland. Its main goal is to improve the welfare of bees globally. COLOSS conducts annual monitoring of the condition of honey bee colonies around the world through its network of researchers. According to a study published in 2020 (Gray et al., 2020) on the situation with honey bees in 35 countries, the losses of bee colonies in Bulgaria are 5.8%.

Among the leading causes of bee losses in Europe and Bulgaria, the negative impact of the use of various pesticides is increasingly mentioned (Tomizawa & Casida, 2003; Elbert et al., 2008; Harper et al., 2009; Gervais et al., 2010; Migdal et al., 2018; Ivanova, 2018; Hayasaka et al., 2019). Nenicotinoids are neurotoxic insecticides that have been reported to have a strong negative effect on the health of honey bees at the population level (Henry et al., 2012; Rundlöf et al., 2015; Tsvetkov et al., 2017). Thiamethoxam and clothianidin because of their chemicals composition have been identified as potential risk agrochemicals to the vitality of the bee colonies (Lundin et al., 2015; Schmuck & Lewis, 2016; Gajger et al., 2017). In Bulgaria, the losses of bee colonies and the complex of reasons for them have been partially studied (Ivanova & Petrov, 2010; Ilieva et al., 2020).

The aim of the present study is to identify, characterize and comparatively analyze the regional differences in the losses of bee colonies and the reasons for them in Bulgaria during the period 2019-2020.

Material and Methods

A national survey among beekeepers in Bulgaria, including a standardized questionnaire for comparability of responses (Van Der Zee et al., 2013), was conducted between April and June 2020 as part of the COLOSS international monitoring survey (Brodschneider et al., 2016, 2018, Gray et al., 2019, 2020). The data were collected by filling in a paper questionnaire and by e-

mail. Beekeepers were asked about the number of wintering bee colonies and how many of these colonies were alive after the winter: a) but with unsolvable problems affecting the queen bee; b) were lost due to a natural disaster (fire, storm, flood, etc.); c) have been lost due to mortality or have been reduced to a few hundred bees. The survey was conducted among professional (members of the National Bee Breeding Association) and independent beekeepers, a total of 64 apiary owners with 6897 bee colonies located throughout the country. The questionnaire also gathered information on the presence in the areas of and around the apiaries of orchards and plantations of rapeseed, corn, sunflower, heather and autumn fodder crops, which could be forage sources and a potential risk factor for pesticide poisoning of the honey bees.

Differences between groups compared were analyzed by the Pearson Chi-Square test.

Results

The data from the present study showed that the total percentage of losses of bee colonies in Bulgaria for the period 2019 - 2020 was found to be 12.11%. The largest losses were reported in the North Central region - 18.93%, and the smallest - in the Northwest region - 1.05%. Regarding the three compared criteria - a) unsolvable problems with the queen bee, b) losses due to a natural disaster and c) mortality or reduction of the bee number in the colonies to a few hundred (Gray et al., 2020), the survey data were as follows: 4.57% - by criterion "a"; 1.97% - by criterion "b" - and 5.57% - by criterion "c". The distribution of bee losses by criteria for different regions is presented in Table 1.

Table 1. Distribution of the honey bee losses in Bulgaria by regions according to the studied criteria: a) unsolvable queen problems; b); natural disaster c) death or reduction to a few hundred bees (in %): $p < 0.05^*$; $p < 0.01^{**}$; $p < 0.001^{***}$.

Regions	a	b	c	Total losses
2019/2020	4.57%	1.97%	5.57%	12.11%
Northwest	0.0	1.05	0.0	1.05 *
North Central	8.62	3.61	6.70	18.93
Northeast	0.13 *	0.26 *	3.59	3.98 *
Southwest	0.0	0.0	2.36 ***	2.36 *
South central	1.39 **	0.70	2.09 ***	4.17 *
Southeast	0.35 *	0.0	7.35	7.70

It is evident that according to criterion "a", the most significant were the losses in the North Central region - 8.62%. In Northwestern and Southwestern Bulgaria there were no reported losses under this criterion, and in Northeastern, Southeastern and South-Central Bulgaria they were 0.13%, 0.35% and 1.39%, respectively - values statistically significantly lower than the average established by this indicator. According to criterion "b", the most losses were reported again in the North Central region - 3.61%. The losses in the South West

and South East regions were zero, and in North West, North East and South-Central Bulgaria the reported bee losses were respectively: 1.05%, 0.26%; and 0.7%. There was a statistically significant difference compared to the average values of this criterion only in terms of losses in the Northeast region. According to criterion "c", the most significant losses of bee colonies were reported for the South East and North Central regions - 7.35% and 6.7%. These values were close to the averages established for our country. In the North East, South

West and South-Central regions the losses according to this criterion were: 3.59%; 2.36% and 2.09%, respectively, the latter two values being statistically significantly lower than the averages established in the course of the study under criterion "c". No losses were reported under this criterion in the North West region (Table 1).

The losses under criterion "c" were significantly related to the alarming trend of mass poisoning of honey bees in some regions of the country due to the widespread use of various pesticides in agricultural practice. In this regard, information was collected on plant resources, which have been also forage sources for honey bees, but at the same time pose a risk to their health due to treatment with pesticides - orchards, oil seed rape, maize, sunflower, autumn forage crops (Table 2).

Table 2. Availability reported by regions for different types of forage sources as potential risk factor for pesticide poisoning of the honey bees.

Forage sources Regions	Orchards	Oil seed rape	Maize	Sunflower	Autumn Forage Crops
Northwest	+			+	+
North Central	+	+	+	+	+
Northeast	+	+	+	+	+
Southwest		+			
South central	+			+	
Southeast	+	+	+	+	

The data from the present study showed that in the North Central and the Northeast regions all of these crops were grown, and in Southeastern Bulgaria - all, except for autumn forage crops. These results, compared with the information on the reported losses of bee colonies by regions, showed that in the South East and the North Central regions most honey bee colonies have died according to criterion "c" (7.35% and 6.7%, respectively). These significant losses in areas with orchards, sunflower, oil seed rape and maize are indicative of the high danger that these crops pose to honey bees.

Discussion

The results obtained in our study could be compared with the official data from the last published monitoring study of COLOSS (Gray et al., 2020). It notes that for all countries in Europe (31 countries out of a total of 35 participants in the survey) the identified losses by criteria were as follows: 4.1% (95% CI 4.0-4.1%) of bee colonies were lost due to problems with the queen bee; 10.7% (95% CI 10.5-10.9%) - due to death after hibernation and 1.9% (95% CI 1.8-2.0%) - due to natural disaster. The average reported loss rate for the countries included in the study was 16.7% (95% CI 16.4-16.9%). For the period 2018 - 2019, the highest percentage of bee losses - 32% - was established for the territory of Slovenia, and the lowest - for Bulgaria - 5.8%. The authors of the study connect the low reported mortality in Bulgaria with the professionalism of the beekeepers included in the survey, who work on a national program for the preservation of the local Bulgarian honey bee. According to Ilieva et al. (2020) in Bulgaria for the period 2017-2018 were reported 2.04%, and for the period 2018-2019 - 5.22% honey bee colony losses. The losses due to problems with the queen bee are of the order of 1.13% for both compared periods, those due to death or reduction to a few hundred bees - 0.78% and 2.88%, respectively for 2017-2018 and 2018-2019, and due to natural disasters - 0.2% and 1.22% for the two compared periods. The significantly lower established values for our

country according to the criteria "a", "b" and "c" for the past comparative periods could be explained by the fact that in the surveys at that time mainly professional beekeepers participated. As part of the National Bee Breeding Association (NBBA), they work on a national breeding program aimed at protecting the local Bulgarian honey bee *A. m. rodopica*. In addition to representatives of the NBBA, a number of independent beekeepers took part in the present study, which makes it more objective in characterizing the honey bee colony condition in Bulgaria.

In their study, Gray et al. (2019) notes that intensive feeding with any of the indicated plant sources (orchards, oil seed rape, maize, heather and autumn forage crops) is associated with significantly higher honey bee losses. This is why the authors identify them as potential risk factors for the loss of bee colonies in Europe due to the widespread treatment with pesticides (Gajger et al., 2017; Gray et al., 2019; Tomljanović et al., 2020). In this respect, the situation in Bulgaria is similar - orchards, sunflower plantations and rapeseed areas are available in the habitat areas of the majority of the apiaries included in the study on the territory of the country (Table 2).

Conclusions

Honey bee colony losses in Bulgaria for the period 2019 - 2020 are found to be 12.11%.

The most significant are the losses due to mortality or reduction of honey bees in the colony to a few hundred (5.57%).

Potential risk factors for honey bee vitality could be forage sources such as orchards, sunflowers, oil seed rape, maize and autumn forage crops due to their pesticide treatment.

References

Agrostatistics. (2020). *Ministry of Agriculture, Food and Forestry*. Retrieved from mzh.government.bg

Alaux, C., Ducloz, F., Crauser, D. & Le Conte, Y. (2010). Diet effects on honeybee immunocompetence. *Biological Letters, 6*(4), 562-565.

Brodschneider, R., Gray, A., Adjlane, N., Ballism A., Brusbardism V., Charrièrem J.-D., Chlebo, R., Coffey, M. F., Dahle, B., C de Graaf, D., Dražić, M. M., Evans, G., Fedoriak, M., Forsythe, I., Gregorc, A., Grzęda, U., Hetzroni, A., Kauko, L., Kristiansen, P., Martikkala, M., Martín-Hernández, R., Medina-Flores, C. A., Mutinelli, F., Raudmets, A., Ryzhikov, V. A., Simon-Delso, N., Stevanovic, J., Uzunov, A., Vejsnæs, F., Wöhl, S., Zammit-Mangion, M. & Danihlík, J. (2018). Multi-country loss rates of honey bee colonies during winter 2016/2017 from the COLOSS survey. *Journal of Apicultural Research 57(3)*, 452–457.

Brodschneider, R., Gray, A., van der Zee, R., Adjlane, N., Brusbardis, V., Charrière, J.-D., Chlebo, R., Coffey, M. F., Crailsheim, K., Dahle, B., Danihlík, J., Danneels, E., C de Graaf, D., Dražić, M. M., Fedoriak, M., Forsythe, I., Golubovski, M., Gregorc, A., Grzęda, U., Hubbuck, I., İvgin Tunca, R. I., Kauko, L., Kilpinen, O., Kretavicius, J., Kristiansen, P., Martikkala, M., Martín-Hernández, R., Mutinelli, F., Peterson, M., Otten, C., Ozkirim, A., Raudmets, A., Simon-Delso, N., Soroker, V., Topolska, G., Vallon, J., Vejsnæs, F. & Woehl, S. (2016). Preliminary analysis of loss rates of honey bee colonies during winter 2015/16 from the COLOSS survey. *Journal of Apicultural Research 55(5)*, 375–378.

Brutscher, L. M., McMenamin, A. J. & Flenniken M. L. (2016). The buzz about honey bee viruses. *PLoS Pathog.*, 12 Article e1005757.

Elbert, A., Haas, M., Springer, B., Thielert, W. & Nauen R. (2008). Applied aspects of neonicotinoid uses in crop protection. *Pest Management Sciences 64(11)*: 1099-1105.

Gajger, I.T., Sakač, M., Gregorc, A. (2017). Impact of thiamethoxam on honey bee

queen (*Apis mellifera carnica*) reproductive morphology and physiology. *Bulletin of Environmental Contamination and Toxicology. 99(3),* 297-302. doi: 10.1007/s00128-017-2144-0.

Gervais, J. A., Luukinen, B., Buhl, K. & Stone, D. (2010). *Imidacloprid Technical Fact Sheet. Corvallis: National Pesticide Information Center*, Oregon State University Extension Services.

Goulson, D., Nicholls, E. K., Botias, C. & Rotheray, E. L. (2015). Combined stress from parasites, pesticides and lack of flowers drives bee declines. *Science*, 347.

Gray, A., Adjlane, N., Arab, A., Ballis, A., Brusbardis, V., Charrière, J.-D., Chlebo, R., Coffey, M. F., Cornelissen, B., Amaro da Costa, C., Dahle, B., Danihlík, J., Dražić, M. M., Evans, G., Fedoriak, M., Forsythe, I., Gajda, A., de Graaf, D. C., Gregorc, A., Ilieva, I., Johannesen, J., Kauko, L., Kristiansen, P., Martikkala, M., Martín-Hernández, R., Medina-Flores, C. A., Mutinelli, F., Patalano, S., Raudmets, A., Martin, G. S., Soroker, V., Stevanovic, J., Uzunov, A., Vejsnaes, F., Williams, A., Zammit-Mangion, M. & Brodschneider, R. (2020). Honey bee colony winter loss rates for 35 countries participating in the COLOSS survey for winter 2018–2019, and the effects of a newqueen on the risk of colony winter loss, *Journal of Apicultural Research, 59(5)*, 744-751.

Gray, A., Brodschneider, R., Adjlane, N., Ballis, A., Brusbardis, V., Charrière, J.-D., Chlebo, R., Coffey, M. F., Cornelissen, B., da Costa, C. A., Csáki, T., Dahle, B., Danihlík, J., Dražić, M. M., Evans, G., Fedoriak, M., Forsythe, I., de Graaf, D., Gregorc, A., Johannesen, J., Kauko, L., Kristiansen, P., Martikkala, M., Martín-Hernández, R., Medina-Flores, C. A., Mutinelli, F., Patalano, S., Petrov, P., Raudmets, A., Ryzhikov, V. A., Simon-Delso, N., Stevanovic, J., Topolska, G., Uzunov, A., Vejsnaes, F., Williams, A., Zammit-Mangion, M. & Soroker, V. (2019). Loss rates of honey bee colonies during winter 2017/18 in 36 countries participating in the COLOSS survey, including effects of forage sources. *Journal of Apicultural Research, 58(4)*, 479-485.

Harper, B., Luukinen, B., Gervais, J. A., Buhl, K. & Stone, D. (2009). Diazinon Technical Fact Sheet. *Corvallis: National Pesticide Information Center*, Oregon State University Extension Services.

Hayasaka, D., Kobashib, K. & Hashimotoa, K. (2019). Community responses of aquatic insects in paddy mesocosms to repeated exposures of the neonicotinoids imidacloprid and dinotefuran. *Ecotoxicology and Environmental Safety, 175*, 272–281.

Henry, M., Béguin, M., Requier, F., Rollin, O., Odoux, J.-F., Aupinel, P., Aptel, J., Tchamitchian, S. & Decourtye, A. (2012). A common pesticide decreases foraging success and survival in honey bees. *Science, 336*, 348-350.

Ilieva, I. I., Ivanova, E. N., Petrov, P. P. & Petkov, N. G. (2020). Honey-bee Colony Losses in Bulgaria: A Case Study during the Period 2017-2019. *Acta zoologica bulgarica, 15*, 117-122.

Ivanova E. N. & Petrov P. P. (2010). Regional differences in honey bee winter losses in Bulgaria during the period 2006-9. *Journal of Apicultural Research 49*(1), 102-103.

Ivanova, E. N. (2018). *Population-genetic richness of the Bulgarian honey bee and the history of a 25-year scientific journey, scientific monograph.* University Publishing House "Paisii Hilendarski". 314 p.

Johnson, R. M., Dahlgren, L., Siegfried, B. D. & Ellis, M. D. (2013). Acaricide, fungicide and drug interactions in honey bees (*Apis mellifera*). *PLoS One 8(1)*, p. e54092.

Lundin, O., Rundlöf, M., Smith, H. G., Fries, I. & Bommarco, R. (2015). Neonicotinoid insecticides and their impacts on bees: a systematic review of

research approaches and identification of knowledge gaps. *PloS One, 10,* Article e0136928.

McMenamin A. J. & Genersch E. (2015). Honey bee colony losses and associated viruses. *Current Opinion in Insect Science, 8*, 121-129.

Migdal, P., Roman, A., Popiela-Pleban, E., Kowalska-Góralska, M. & Opalinski, S. (2018). The Impact of Selected Pesticides on Honey Bees. *Polish Journal of Environmental Studies, 27*(2), 787-792.

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O. & Kunin, W. E. (2010). Global pollinator declines: trends, impacts and drivers. *Trends in Ecological Evolution*, *25*(6), 345-353.

Rundlöf, M., Andersson, G. K. S., Bommarco, R., Friesm I., Hederström, V., Herbertsson, L., Jonsson, O., Klatt, B. K., Pedersen, T. R., Yourstone, J. & Smith, H. G. (2015). Seed coating with a neonicotinoid insecticide negatively affects wild bees. *Nature, 521*, 77-80.

Schmuck, R. & Lewis, G. (2016). Review offield and monitoring studies investigating the role of nitro-substituted neonicotinoid insecticides in the reported losses ofhoney bee colonies (*Apis mellifera*). *Ecotoxicology, 25*, 1617-1629.

Steinhauer, N., Kulhanek, K., Antúnez, K., Human H., Chantawannakul, P., Chauzat, M.-P. & van Engelsdorp, D. (2018). Drivers of colony losses. *Current Opinion in Insect Science, 26*, 142-148.

Steinhauer, N., van Engelsdorp, D. & Saegerman, C. (2021). Prioritizing changes in management practices associated with reduced winter honey bee colony losses for US beekeepers. *Science of the Total Environment, 753*, 141629.

Straub, L., Williams, G. R., Vidondo, B., Khongphinitbunjong, K., Retschnig, G., Schneeberger, A., Chantawannakul,

P., Dietemann, V. & Neumann, P. (2019). Neonicotinoids and ectoparasitic mites synergistically impact honeybees. *Sciientific Reports, 9,* 8159.

Tantillo, G., Bottaro, M., Pinto, A. Di, Martella, V., Pinto, P. Di & Terio, V. (2015). Virus infections of honeybees *Apis mellifera*. *Italian journal of food safety, 4*(3), 5364.

Tomizawa, M. & Casida, J. E. (2003). Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors. *Annual Review of Entomology, 48,* 339-364.

Tomljanović, Z., Cvitković, D., Pašić, S., Volarević, B., Tlak Gajger, I. (2020). Production, practices and attitudes of beekeepers in Croatia. *Veterinary Arhives, 90*(4), 413-427.

Tosi, S., Nieh, J. C., Sgolastra, F., Cabbri, R. & Medrzycki, P. (2017). Neonicotinoid pesticides and nutritional stress synergistically reduce survival in honey bees. *Proceedings of the Royal Society B: Biological Sciences, 284*(1869) ar. num. 20171711.

Tsvetkov, N., Samson-Robert, O., Sood, K., Patel, H. S., Malena, D. A., Gajiwala, P. H., Maciukiewicz, P., Fournier, V. & Zayed A. (2017). Chronic exposure to neonicotinoids reduces honey bee health near corn crops. *Science, 356*, 1395-1397.

Ullah, A., Tlak Gajger, I., Majoros, A., Dar, S. A., Khan, S., Kalimullah, Haleem Shah, A., Nasir Khabir, M., Hussain, R., Khan, H. U., Hameed, M. & Anjum, S. I. (2021). Viral impacts on honey bee populations: A review. *Saudi Journal of Biological Sciences, 28*, 523–530.

van der Zee, R., Gray, A., Holzmann, C., Pisa, L., Brodschneider, R., Chlebo, R., Coffey, M. F., Kence, A., Kristiansen, P., Mutinelli, F., Nguyen, B. K., Noureddine, A., Peterson, M., Soroker, V., Topolska, G., Vejsnæs, F. & Wilkins, S. (2013). Standard survey

methods for estimating colony losses and explanatory risk factors in *Apis mellifera*. In Dietemann V., Ellis J.D. & Neumann P. (Eds.), *The COLOSS BEEBOOK, Volume I*: Standard Methods for *Apis mellifera Research. Journal of Apicultural Research, 52(4)*.

van Dooremalen, C., Cornelissen, B., Poleij-Hok-Ahin, C. & Blacquière, T. (2018). Single and interactive effects of Varroa destructor, Nosema spp., and imidacloprid on honey bee colonies (*Apis mellifera*). *Ecosphere, 9(8)*, ar. num. 02378.

van Engelsdorp, D. & Meixner, M. D. (2010). A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them *Journal of Invertebrates Pathology, 103*, S80-S95.