Bulgarian Journal of Agricultural Science, 28 (Suppl. 1) 2022
Agricultural Academy

First data on the pesticide residues in samples of honey bees and food stocks in the *Apis mellifera* colonies from Bulgaria

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Abstract

Petrov, P., Ivanova, E., Petkov, N. & Arakelyan I. (2022). First data on the pesticide residues in samples of honey bees and food stocks in the *Apis mellifera* colonies from Bulgaria. *Bulg. J. Agri. Sci., 28, (Supplement 1)*, 84–90

Honey bees are insects of great biological, ecological and economic importance. They are characterized by high sensitivity to environmental pollution and are therefore successfully used for biomonitoring studies. This study presents primary data on the presence of pesticide residues in honey bee samples and food stocks in the honey bee colony – honey, wax and pollen, collected from territories in Bulgaria on signals of high mortality and severe loss of bee colonies. The collected samples have been subjected to chemical chromatographic analysis (via RESID 19/04 LC-MS / MS methods) to determine pesticide residues in the Central Laboratory for Chemical Testing and Control at the BFSA (Sofia, Bulgaria) and in the international laboratory PRIMORIS, Belgium). The performed chemical analyzes provide information on the presence in the tested samples of residues of a total of 27 pesticides, including insecticides, fungicides, acaricides, herbicides and growth regulators, which is a signal for objective danger of high mortality and severe weakening of the honey bee colonies in some areas in Bulgaria. The presented results reveal the need for future detailed studies of risk factors for the health and viability of honey bees, among which are the various chemical nature and mechanism of action of agrochemicals.

Keywords: honey bee colonies; pesticides; honey bee colony losses

Introduction

Honey bees are species of insects with enormous biological, ecological, agricultural and economic significance. They are producers of various products – honey, wax, pollen, propolis, royal jelly and bee venom, used for food and human treatment. *Apis mellifera* species is characterized by high environmental pollution sensitivity and is therefore used for biomonitoring studies in many regions of the world. Studies have shown that the genetic richness of honeybee populations in Europe is threatened by the uncontrolled introduction of genes from other subspecies into adapted local populations, stress from the changing environment and agrochemical pollution, the emergence of new pathogenic species and global climate changes (Meixner et al., 2014; Büchler et al., 2014; Ivanova, 2018). It was reported that the lack of united efforts and targeted activities to prevent these processes would have a detrimental effect on honey bees and wildlife, with a staggering effect on the pollination of wild flora and agricultural plants.

Various environmental factors that have a negative impact on the viability on honey bees are: intensive agriculture with long-term use of pesticides, the shortage of suitable food, the loss of habitats, new pathogens and pests. Ullah et al. (2021) express their vision for a unified health approach worldwide in support of beekeeping and its management in the future.

The harmful effects of pesticides are generally based on their accumulation as residual quantities in the ecological food chain and their toxic effect (including cytotoxic and genotoxic) on living organisms. In recent years, agricultural practice has taken into account the trend of widespread use of neonicotinoids as a new generation of pesticides, which are applied in modern agricultural production to protect crop plants from various pests. Although this class of insecticides is currently being applied in many countries around the world, there is increasing evidence of their toxic effects on honey bees and other pollinators in nature. This is also the reason for restricting or totally banning their use in some European countries. These agrochemicals are characterized by high water solubility, easy penetration in the plant organism, environmental resistance and strong neurotoxic effect on insects that feed or come into contact with the treated plants. In addition to direct toxic effects, they also affect the normal functioning of the honey bee colony. A number of studies (Bonmatin et al., 2003; 2005; Straub et al., 2019) are aimed at clarifying the relationship between the mortality of bee colonies, decreasing numerosity of bee populations and increased use of pesticides, including neonicotinoids, preferred by agricultural producers because of their high efficiency, in particular when treating seeds and areas with corn, sunflower and rape.

To date, studies on the negative impact of pesticides on the health and vitality of honey bees in Bulgaria have not been done, although for the period 2018-2020 reported a total of up to 19% losses of bee colonies in different parts of the country, and some beekeepers report mass poisonings of bees in the regions of their apiaries (Ilieva et al., 2020; 2021).

This study provides primary data on the presence of pesticide residues in samples of honey bees and of food stocks in the bee colony, collected from territories in Bulgaria after reports of high mortality and strong reduction in the bee colony strength and number.

Material and Methods

For the period 2018 - 2020, samples of bees and food stocks (honey, pollen, wax) were collected in the bee colonies from apiaries located on the territory of Ruse (Ruse), Burgas (Voden, Zhelyazovo), Sliven (Sliven), Stara Zagora (Dimovtsi) and Plovdiv (Plovdiv – AU) districts – Fig. 1. The honey bee samples were collected from apiaries in the area of Gurkovo and Plovdiv, and the samples from honey, wax and pollen – from apiaries in the areas of Ruse, Burgas and Sliven.

The collected samples were subjected to chemical chromatographic analysis (using RESID 19/04 LC-MS / MS methods) to determine pesticide residues in mg/kg. The test results with values lower than 0.01 mg/kg were reported against a calibration curve with a linear range from 0.01 mg/kg to 0.100 mg/kg in bees and from 0.01 mg/kg to 0.150 mg/kg in honey and are defined as "traces of pesticide residue levels" present.

The analyzes were performed and reported in the Central Laboratory for Chemical Testing and Control (Sofia, Bulgaria) and in the International Laboratory PRIMORIS (Gent, Belgium).

Fig. 1. Sampling locations

Results and Discussion

The performed chemical analyzes provide information on the presence in the tested samples of residues of a total of 27 pesticides: insecticides, including acaricides; fungicides; herbicides and growth regulators (Table 1).

As could be seen from the presented data, residual amounts of 13 insecticides, 6 acaricides, 8 fungicides, 3 herbicides and 2 growth regulators were found in the tested samples.

Tables 2 and 3 present data on the detected amounts of pesticides in the samples of honey bees and of food stocks by region.

The results showed that in the collected samples from the individual settlements the identified pesticides varied in number, composition and quantities (Table 2 and Table 3).

As could be realized from the data obtained, the presence of different types of pesticides was found in all of the tested samples. The higher values of two fungicides – Carbendazim and Tiofanat-metil in the honey bee samples (Table 2), as well as the higher values of Coumaphos and Cyhalothrin in some of the tested samples from food stocks (Table 3) were impressive.

Table 1. Type of pesticides detected in the samples collected from honey bees and of food stocks (honey, wax and pollen).

Insecticides	Fungicides	Acaricides	Herbicides	Growth regulators
Imidacloprid Coumaphos Cyhalothrin Chlorpyrifos (-ethyl) Tau-fluvalinate Fenoxycarb Flonicamid Methoxyfenozid Amitraz Aldicarb Carbaril Clothianidin Dimetoat	Cyprodinil Difenoconazole Thiophanate-methyl Pyrimethanil Tebuconazole Ametocradin Carbendazim Fenamidone	Coumaphos Tau-fluvalinate Amitraz Hexitiazox Dimetoat Fenbutatin oxide	Metholachlor and metholachlor-S Linuron Prosulfocarb	Flumetralin Fenoxycarb

Table 2. Detected amounts of pesticides (mg/kg) in bee samples

Location	Name of the pesticide	Amount of pesticide
Dimovtsi, Gurkovo	Fenoxycarb Flonicamid Pyrimethanil Linuron Methoxyfenozid Tebuconazole Prosulfocarb Amitraz	0.0012 0.0028 0.0020 0.0045 0.0036 0.0035 0.0041 0.0015
Plovdiv-1	Flonicamid Carbendazim Tiofanat-metil Linuron Tebuconazole Prosulfocarb Hexitiazox Amitraz	0.0013 0.0011 0.0019 0.0023 0.0031 0.0038 0.0013 0.0021
Plovdiv -2	Carbendazim Tiofanat-metil Flonicamid Clothianidin Dimetoat Fenamidone Fenoxycarb Tebuconazole Prosulfocarb Hexitiazox Amitraz Fenbutatin oxide	0.035 0.149 0.0088 0.0030 0.0012 0.0013 0.0019 0.0019 0.0032 0.0016 0.0054 0.0034

It should be noted that acaricides used in beekeeping to control *Varroa destructor* together with pesticides for plant protection, were found in our pilot study. The presence of acaricides was found in 66.7% of the collected samples (both in the food stocks of disadvantaged or dead bee colonies and in the carcasses of bees died during the winter-spring period). Three different acaricides (Coumaphos, Amitraz and Tau-fluvalinate) were found in the samples, and the most common were Coumaphos and Amitraz.

The presence of pesticides for plant protection was significantly higher – 24 pesticides from different classes. The most common pesticides found for plant protection in the studied samples were insecticides (N = 10) and fungicides (N = 8). From the other groups, three acaricides, three herbicides and two growth regulators have been identified, one of which was for insects. Only one pesticide was found in one of the samples from food stocks (from the village of Zhelyazovo), while the largest number of pesticides were found in the samples from Plovdiv-1 and 2 (respectively 8 and 12 pesticides) in the carcasses of honey bees from dead bee colonies.

Although the concentration of most pesticides detected was very low (ppb), mixtures of pesticides may have a cumulative and synergistic effect and to lead to an unexpected increase in toxicity compared to that found for individual active substances (Pilling & Jepson, 1993; Mullin et al., 2010).

It is important to note that in some of the studied samples three neonicotinoids were found – Imidacloprid, Clothianidin, Flonicamid.

Adverse effects and cases of death among honey bees have been reported for Europe but without sufficiently detailed analysis of their relationship to neonicotinoids (Hassani et al., 2008). High mortality among bees, impaired orientation and their inability to return to their hive after impact of non-lethal thiamethoxam exposure has been demonstrated in Henry et al. (2012). Treatment with imidacloprid and other neonicotinoids has been shown to significantly reduce the growth rate and production of new queens, reduce the efficiency of natural feeding, increase mortality in worker bees and significantly reduce the number of offspring in colonies of other pollinators (Whitehorn et al., 2012; Gill et al., 2012). The toxic effect and mechanisms of action of such agrochemicals have not yet been fully and thoroughly

Table 3. Detected amounts of pesticides (mg/kg) in food stock samples (honey, wax and pollen).

Location	Tested product	Name of the pesticide	Amount of pesticide
Ruse	Pollen	Cyprodinil Imidacloprid Metholachlor and metholachlor-S	0.047 0.013 0.015
Voden, Burgas	Honey Pollen Wax	Coumaphos Cyhalothrin	0.049 0.39
Zhelyazovo, Burgas	Honey Pollen Wax	Flumetralin	0.26
Sliven -1	Honey Pollen Wax	Cyprodinil Difenoconazole Thiophanate-methyl Chlorpyrifos (-ethyl) Coumaphos	0.050 0.014 0.13 0.010 0.74
Sliven -2	Honey Pollen Wax	Chlorpyrifos (-ethyl) Coumaphos Tau-fluvalinate	0.030 0.93 0.015
Sliven-3	Honey	Aldicarb Ametocradin Carbaril Difenoconazol Pyrimethanil	0.00395 0.00356 0.00361 0.00476 0.00348

studied. Their lethal and sublethal effects on bees' feeding behavior and orientation abilities have been described, but no specific side effects have been studied in field studies (Blacquiere et al., 2012). Possible synergies between widely the used pesticides and the drugs used to improve the health status of bees themselves have not been studied. On a global scale, the use of diverse insecticides has been shown to affect not only honey bee populations, but also the population of birds, butterflies and other insects, which, according to a number of toxicologists, may cause environmental disasters (Tennekes, 2010). According to the author, after treatment of crop plants, these agrochemicals pass through the entire plant, reach nectar and pollen, permeate to the soil, fall into groundwater and act toxically to the living organisms that are in contact with them. They damage not only the insects feeding on the plant but also those that pollinate it. Even low concentrations of pesticides can be highly toxic due to their high persistence in soil and water (Tennekes, 2010; Steinhauer et al., 2018; 2021).

In Bulgaria, the Food Safety Agency (BFSA) with the Risk Assessment Centre publishes periodic views and studies on the issue (including on its official site). In 2013, a partial ban on the use of neonicotinoids clothianidin, imidacloprid, thiamitoxam within the territory of the European Union (EU) has entered into force. At the same time, European legislation allows for derogations to the ban in cases of "danger that could not be contained by other reasonable means" (Article 53 of Regulation (EC) No 1107/2009). In January 2016, the BFSA took advantage of the right of derogation and allowed the imidacloprid and thiamethoxam preparations for plant protection in our country in 24 administrative districts, which is contrary to the regulated restricted use and poses a real danger to the health of the honey bees, other pollinators, many kinds of insects, birds. Since the beginning of 2018, the use of the three most toxic preparations from the neonicotinoid group has been banned for the application of open areas across the EU. Preparations could be used only for growing greenhouse fruits and vegetables. However, the danger of direct and indirect toxic effects for ecosystems exists. There is also the danger of a potential toxin effect on honey bees and in relation to veterinary medicinal products used in beekeeping to combat varroatosis (mainly coumaphos, synthetic pyrethroids and amitraz) and the risk of synergistic interactions between these medicaments and the used in the agricultural practice agrochemicals.

Unfortunately, the practice shows, and in some media, agricultural and beekeeping forums, it is unofficially reported about the possibility banned for the territory of the European Union agrochemicals to be imported from other countries outside the EU and to be used in our country. This assumption is confirmed by some of the results presented in the present study.

Currently, the International COLOSS Association conducts annual monitoring studies on the loss of bee colonies in Europe and the world, and Bulgaria is part of this network of monitoring activities over the past 4 years (Brodschneider et al., 2018; Gray et al., 2019; 2020; Ilieva et al., 2020; 2021). These comparative analysis worldwide show that our country is among the group of countries with the least losses, but also that over the years there has been a negative trend towards increasing these losses (Gray et al., 2019; 2020).

According to Johnson et al. (2010) the harmful effects of pesticides from the group of insecticides are expressed in acute poisoning, leading to the death of bees or an effect on their overall fitness and their cognitive abilities, which impairs the integrity and viability of bee colonies. Our study highlighted the high levels of the pesticide Coumaphos, an organophosphorus insecticide and methicide that were often used to control varroasis. The researches of Williamson et al. (2013 a, b) show that organophosphate pesticides could adversely affect olfactory learning and memory in bees, with acute exposure to sublethal doses of coumaphos enhancing this effect. The same authors found that contamination with organophosphates affects the movement and behavior of bees – they often clean the abdomen and defecate.

As is well known, honey bees are also exposed to plant protection products during spraying by collecting and accumulating contaminated food supplies of honey and pollen in the hive. According to Johnson (2015), surface and guttation drops (dew) are also sources of pesticides. In addition, Oruç et al. (2020) also add the direct exposure to dust particles with pesticides during seed sowing. The authors found that bee losses could reach between 30% and 80% during the sowing of maize in the spring, as dust particles contaminated with pesticides released from seeders and blown away by the wind can infect bee colonies located 15 – 25 km from the areas sown with corn.

In general, the studied samples identified particularly dangerous for bees pesticides from the group of insecticides, especially those of the class of neonicotinoids – Imidacloprid, Clothianidin, Flonicamid, synthetic pyrethroids – Cyhalothrin, Tau-fluvalinate, organophosphates – Chlorpyrifos (-ethyl), Coumaphos, Dimetoat and carbamates (Carbaril, Fenoxycarb).

In addition to the data and analysis of this study, it should be noted that the following changes were found during its implementation period:

- In the contaminated bee colonies, a demographic effect was observed, leading to their weakening and their death during the winter and spring seasons. Mortality of bee colonies in winter and spring (January-April) of 2021 varies from 24.0% (Plovdiv) to 45.6% (Dimovtsi) and 94.1% (Sliven) and in other living honey bee families there was a strong weakening of their strength from 60-70% to 80-90%;
- In the spring and summer there was a strong violation of the age composition of the family – mostly young hives remained, most of the flying bees died outside the hive, and on the site in front of the hive was constantly exported dead bees with obvious signs of poisoning throughout the active period;
- In bee colonies contaminated with pesticides, unusual behavior of live bees was observed on the sanitary site in front of the hive – bees showed paralytic chaotic movements, accompanied by paralysis and death. The perishing bees were not as many as acute poisoning, but these effects were detected every day;
- As a result of violations of temporal polyethism, age and the number of bees in the hive, they failed to meet the needs of the family to maintain 34-35°C, which leaded to the manifestation of ascospherosis (Plovdiv) and European foulbrood – EFB (Sliven);
- In the colonies there was a spotty brood, as well as the death of larvae of different ages;
- There was found increased frequency of loss and replacement of queens;
- In artificial breeding of queen bees (Plovdiv) a decrease in the number of accepted larvae and in the number of newly hatched queens was observed. When the queen cells were opened, it was established that either the larvae have died at different stages of their development, including the pre-pupae and pupae, or the queens in the queen cells were fully formed, but were blackened and dead in them;
- Variations in the worker bee behavior in the bee colony and a tendency for increasing the level of aggressiveness was reported.

The results obtained and the observations on the condition of honey bee colonies give reason to summarize that, unlike acute poisoning, which is easy to diagnose, chronic poisoning (due to exposure of honey bees to sublethal doses of pesticides) could have a delayed and long-lasting effect on the growth and survival of honey bee colonies. There is a loss of adult bees and brood, as a result of which colonies are gradually but severely weakening. Subsequently, they are looted by other bees, or die during the winter-spring period. In our opinion, chronic poisoning with long-term effects is one of the main reasons for the loss of bee colonies during this period.

The findings of the present study are evidence of the need for targeted monitoring, taking into account the relationship between levels of pollution with agrochemicals and mortality of bee colonies by region in Bulgaria.

Conclusion

The established data on the presence of residual pesticides in bee samples and food stocks in bee colonies is a signal of an objective danger of high mortality and severe weakening of bee colonies in some regions of Bulgaria.

The presented results reveal the need to develop measures for conservation of the national genetic resource of *Apis mellifera* and to conduct activities for detailed studies of risk factors for the health and viability of honey bees, including the various agrochemicals and their mechanism of action in nature.

Acknowledgments

This study is supported by the National Research Fund through the contract KP-06-H5112 / 2021 "Complex assessment of genetic and environmental factors related to the losses of honey bees (*Apis mellifera* L.) in Bulgaria".

References

Blacquiere, T., Smagghe, G., van Gestel., C. A. M & Mommaerts, V. (2012). Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment. *Ecotoxicology*, *21*, 973-992.

Bonmatin, J. M., Moineau, I., Charvet, R., Fleche, C., Colin, M. E. & Bengsch, E. R. (2003). A LC/APCI-MS/MS method for analysis of imidacloprid in soils, in plants, and in pollens. *Analytical Chemistry*, *75*, 2027-2033. -

Bonmatin, J. M., Marchand, P. A., Charve, R., Moineau, I., Bengsch, E. R. & Colin M. E. (2005). Quantification of imidacloprid uptake in maize crops. *Journal of Agricultural and Food Chemistry*, *53*, 5336-5341.

Brodschneider, R., Gray, A., Adjlane, N., Ballism, A., Brusbardism, V., Charrièrem, J.-D., Chlebo, R., Coffey, M. F., Dahle, B., de Graaf, D. C., 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, Mutinelli, C. A., Raudmets, F., Ryzhikov, A., Simon-Delso, V. A., Stevanovic, N., Uzunov, J., Vejsnæs, A., Wöhl, F., 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.

Büchler, R., Costa, C., Hatjina, F., Andonov, S., Meixner, M. D., Le Conte, Y., Uzunov, A., Berg, S., Bienkowska, M., Bouga, M., Drazic, M., Dyrba, W., Kryger, P., Panasiuk, B., Pechhacker, H., Petrov, P., Kezić, N., Korpela, S. & Wilde, J. (2014). The influence of genetic origin and its interaction with environmental effects on the survival of *Apis mellifer*a L. colonies in Europe. *J. Apic. Res*., 2014; *53(2),* 205–214.

Gill, R. J., Ramos-Rodriguez, O. & Raine, N. E. (2012). Combined pesticide exposure severely affects individual- and colony-level traits in bees. *Nature,* 491-105-U119.

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.

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, Mutinelli, C. A., Patalano, F., Raudmets, S., Martin, A., Soroker, G. S., Stevanovic, V., Uzunov, J., Vejsnaes, A., Williams, F., 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.

Hassani, E., Dacher, A. M., Gary, V., Lambin, M., Gauthier, M. & Armengaud, C. (2008). Effects of sublethal doses of acetamiprid and thiamethoxam on the behavior of the honeybee (*Apis mellifera*). *Arch. Environ. Contam. Toxicol*., *54(4),* 653-661.

Henry, M., Beguin, 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 Zool. Bulg*., *Supplement 15*, 117 – 122.

Ilieva, I. I., Ivanova, E. N., Petrov, P. P.& Petkov, N. G. (2021). Losses of honey bee colonies and risk factors for their mortality in Bulgaria during 2020. *Ecologia Balkanica*, 173-180.

Ivanova, E. (2018). The population-genetic wealth of the Bulgarian honey bee and the history of a 25-year scientific journey. Scientific monograph. Paisii Hilendarski University Publishing House. Plovdiv, 314. ISBN 978-619-202-393-5 (BG).

Johnson, R. M., Ellis, M. D., Mullin, C. A.& Frazier, M. (2010). Pesticides and honey bee toxicity – U.S.A. *Apidologie*, *41(3),* 312-331. https://doi.org/10.1051/apido/2010018.

Johnson, R. M. (2015). Honey bee toxicology. *Annual Review of Entomology, 60*, 415-434. https://doi.org/10.1146/annurev-ento-011613-162005.

Meixner, M. D., Francis, R. M., Gajda, A., Kryger, P., Andonov, S., Uzunov, A., Topolska, G., Costa, C., Amiri, E., Berg, S., Bienkowska, M., Bouga, M., Büchler, R., Dyrba, W., Gurgulova, K., Hatjina, F., Ivanova, E., Janes, M., Kezic, N., Korpela, S., Le Conte, Y., Panasiuk, B., Pechhacker, H., Tsoktouridis, G., Vaccari, G. & Wilde, J. (2014). Occurrence of parasites and pathogens in honey bee colonies used in a European genotype - environment - interactions experiment. *Journal of Apicultural Research 53 (2),* 215-229. http://dx.doi.org/10.3896/IBRA.1.53.2.04.

Mullin, C. A., Frazier, M., Frazier, J. L., Ashcraft, S., Si-monds, R. & Pettis, J. S. (2010). High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health. *PLOS ONE*, *5(3),* e9754. doi:10.1371/journal.pone.0009754.

Oruç, H. H., Çayci, M.& Sariyev, R. (2020). Sudden and prevalent deaths of foraging honey bees in early spring during sowing of clothianidin coated maize seeds between 2013 and 2018 in Turkey. *J. apic. sci.*, *64 (1)*, 67-76. DOI: 10.2478/JAS-2020-0007.

Pilling, E. D. & Jepson, P. C. (1993). Synergism between EBI fungicides and a pyrethroid insecticide in the honeybee (*Apis mellifera*). *Pesticide Science*, *39*, 293–297, doi:10.1002/ ps. 2780390407.

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. *Sci. Rep., 9,* 8159.

Tennekes, H. (2010). The significance of the Druckrey–Küpfmüller equation for risk assessment-The toxicity of neonicotinoid insecticides to arthropods is reinforced by exposure time. *Toxicology, 276 (1),* 1–4.

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.

Whitehorn, P. R., O'Connor, S., Wackers, F. L. & Goulson, D. (2012). Neonicotinoid pesticide reduces bumble bee colony growth and queen production. *Science*, *336*, 351-352.

Williamson, S. M., Baker, D. D. & Wright, G. A. (2013a). Acute exposure to a sublethal dose of imidacloprid and coumaphos enhances olfactory learning and memory in the honeybee, Apis mellifera. Invertebrate *Neuroscience*, *13(1),* 63-70. DOI: 10.1007/s10158-012-0144-7.

Williamson, S. M., & Wright, G. A. (2013b). Exposure to multiple cholinergic pesticides impairs olfactory learning and memory in honeybees. *The Journal of Experimental Biology*, *216*, 1799-1807. DOI: 10.1242/jeb.083931.