

## EVALUATING SUSPICIONS OF INTOXICATION IN BEES IN 2018-2020

Vasilică Savu<sup>1</sup>, Agripina Șapcaliu<sup>1</sup>, Tache Bogdan<sup>1</sup>, Roxana Zaharia<sup>2</sup>, Viorica Lagunovschi-Luchian<sup>3</sup>, Luiza Bădic<sup>4</sup>, Ion Rădoi<sup>3</sup>

<sup>1</sup>Beekeeping Research and Development Institute, 013975 Bucharest, Romania

<sup>2</sup>Research and Development Institute for Plant Protection Bucharest, Romania

<sup>3</sup>University of Agronomic Sciences and Veterinary Medicine Bucharest, Romania

<sup>4</sup>Spiru Haret University, 030045 Bucharest, Romania

\* Correspondence address:

Research-Development Institute for Plant Protection

8 Ion Ionescu de la Brad Blvd., 013813, Bucharest, Romania

Phone: 004-021-2693231

Email: roxyanna\_21@yahoo.com

**Abstract:** The purpose of this work was to evaluate suspicions of intoxication in bees in 2018-2020. Bee health was monitored and samples were collected (live bees, dead bees, honey, pollen, hive fragments) from bee colonies in apiaries located in various geographical areas of Romania. Corroborating the anamnestic data, morphoclinical examinations and laboratory tests, other causes of disease were excluded and the diagnosis was suspicion of intoxication. During this period, 317 apiaries were monitored out of which 32 apiaries were identified to display suspicion of intoxication, out of which 4 apiaries in 2018, 18 apiaries in 2019 and 10 apiaries in 2020. The suspicion of intoxication diagnosis included 4079 bee colonies, out of which 1896 colonies were diagnosed in 2018, 1582 in 2019 and 601 in 2020. The bee colonies under suspicion of intoxication were evaluated and categorized as follows: toxic food intoxication 49.5% (2018), 34.07% (2019) and 14.31% (2020); chemical substance intoxication 40% (2018), 59.6% (2019), 35.10% (2020) and medicine intoxication 10.5% (2018), 6.33% (2019) and 50.59% (2020).

**Keywords:** bees, *Apis mellifera carpathica*, suspicion of intoxication

### INTRODUCTION

Evaluating suspicions of intoxication in bees in 2018-2020 in Romania is of special importance for prophylaxis and human health. By harmonizing the sanitary-veterinary legislation and the agricultural policies in Romania with the European legislation, we aim for the protection of bees as main pollinators in agriculture (Asiminei et al., 2016). The etiopathogenesis of intoxications in bees targets the mechanism of cell metabolism alteration by organic or inorganic substances that by being ingested or by direct contact affect the vital functions (European Food Safety Authority, 2019). The most frequent suspicions of intoxications in bees are the ones caused by toxic food, medications and chemical substances (Asiminei et al., 2016; Savu & Șapcaliu, 2013; Gregorc et al., 2018).

The intoxication by toxic food has been described by various authors, who outlaid aspects of the toxicity of allelochemical substances in foraging plants that caused morbid states, mortality and depopulation in bee colonies (Asiminei et al., 2016; Gray et al., 2019; Mustard & Wright, 2004; Coulon et al., 2019).

Medical intoxications are especially significant due to the high percentage, serious manifestations and depopulations of hives (Asiminei et al., 2016; Rădoi et al., 2019; Kamler et al., 2003; Tomasz et al., 2016).

Intoxications by chemical substances and especially by pesticides were presented in numerous congresses, symposia and in various international publications, being monitored

through research programs (Riedl et al., 2016; Asiminei et al., 2016; Böhme et al., 2018; Savu et al., 2019; Scott et al., 2018)

## MATERIALS AND METHODS

Researches took place during the beekeeping seasons of 2018-2020 and were based on the monitoring of apiaries in various geographical areas in the south-east and south of Romania. Monitoring was founded on a protocol focused on anamnesis and morphoclinical examination of bee colonies, with the purpose to establish the suspicion of intoxication, after eliminating other causes of depopulation and mortality in bees (infectious diseases, parasitoses, technological errors) (Meikle & Holst, 2015). The laboratory tests carried out in the pathology laboratory of the Beekeeping Research and Development Institute Bucharest, on samples of live and dead bees, based on O.I.E. (World Organisation for Animal Health, 2008) methodology and protocols, established the suspicion of intoxication. The number of monitored apiaries during these beekeeping seasons was 317, out of which 136 apiaries in 2018, 113 apiaries in 2019 and 68 apiaries in 2020. The monitored apiaries had a number of 16,910 bee colonies, of which 6004 colonies were monitored in 2018, 7007 in 2019 and 3899 colonies in 2020.

**Table 1.** Monitored apiaries in 2018-2020

Beekeeping year	2018	2019	2020	Total
Monitored apiaries (total number)	136	113	68	317
Monitored bee colonies (total number)	6004	7007	3899	16,910

## RESULTS AND DISCUSSIONS

Corroborating the results of morphoclinical examinations of live and dead bees samples with anamnestic data collected from beekeepers, and eliminating infectious, parasitic, mycological and technological causes through laboratory tests (direct microscopy, bacterioscopic and parasitologic examination), the suspicion of intoxication was established. The suspicion of intoxication was based on an anamnestic examination that indicated the time of bee depopulation and mortality after phytosanitary and medication treatments without observing dosages and recommendations, nectar and pollen foraging after rains or from potentially toxic plants, as well as based on the morphoclinical table.

Subsequent to establishing the suspicion of intoxication, 32 apiaries were identified as being under this suspicion, out of which 4 apiaries in 2018, 18 apiaries in 2019 and 10 apiaries in 2020 (table 2).

**Table 2.** Suspicions of intoxication in 2018-2020

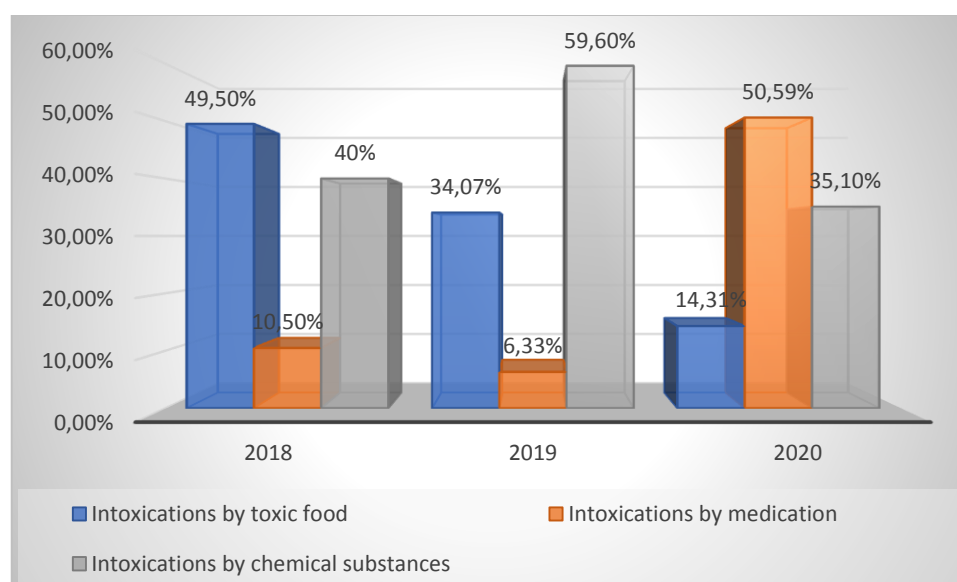
Beekeeping year	2018	2019	2020	Total
Apiaries under suspicion of intoxication (%)	4 (2.9 %)	18 (15.92 %)	10 (14.7%)	32 (10.09%)
Bee colonies under suspicion of intoxication (%)	1896 (11.21%)	1582 (22.57%)	601 (15.41%)	4079 (24.12%)

The morphoclinical examination on samples of live and dead bees as result of intoxications showed constipation, alternating diarrhea, bloating, paresis, externalized proboscis. At hive level, one noticed depopulation, high mortality, dead bees at the entrance and in front of the hive, reduced bee activity in the hive and outside it.

The suspicion of intoxication was established according to the laboratory diagnosis in 32 apiaries of the 317 monitored during the study period. The total number of bee colonies under suspicion of intoxication was 4079, out of which 1896 colonies in 2018, 1582 colonies in 2019 and 601 colonies in 2020. The intoxication categories suspicious in bee colonies during the study period of 2018-2020 are presented in table 3 and figure 1.

**Table 3.** Intoxication categories suspicious in bee colonies in 2018-2020

Intoxication categories	2018	2019	2020	Total
Intoxications by <i>toxic food (pollen)</i>	938 (49.5%)	539 (34.07%)	86 (14.31%)	1563 (38.31%)
Intoxications from <i>medication</i>	200 (10.5%)	100 (6.33%)	304 (50.59%)	604 (15.04%)
Intoxications by <i>chemical substances</i>	758 (40%)	934 (59.6%)	211 (35.10%)	1903 (46.65%)
Affected bee colonies	1896	1582	601	4079



**Figure 1.** The intoxication categories suspicious in bee colonies in 2018-2020 period

The results presented in table 3 show a rise in the percentage of intoxications from chemical substances from 40% in 2018 to 59.6% in 2019, followed by a drop to 35.1% in 2020. The suspicion of intoxication by chemical substances (pesticides), shows during foraging (especially rape and sunflower), after pesticide sprinkling, followed by rain periods. Failure to observe foraging interdiction after chemical substance sprinkle on cultivated plants, namely failure to close honeybees in the hive during sprinkle periods (after local authorities notify the beekeeper on the sprinkles schedule in the foraging area), could be the cause of the high percentage of honeybee colonies suspicious of intoxication with chemical substances.

The analysis of data in the table also indicate a decrease in percentages of intoxications with toxic food (toxic pollen, high fructose corn syrup - HFCS etc), from 49.5% in 2018 to 34.07% in 2019 and then to 14.31% in 2020. Typical for this type of intoxications is their occurrence in the active beekeeping season, after rains in the foraging period and less in the inactive season, primarily with symptoms like: diarrhea, bloating, humid pollen stuck to bees'

legs on the flight board of the hive, bees' death. Rădoi et al. (2019) presented the symptom of drone's genitalia inflammation in the suspicions of intoxication from toxic food.

In the 2018-2020 period, according to the table 3, we notice an increased percentage of intoxications by medication from 10.5% in 2018 to 6.33% in 2019, to 50.59% in 2020. Intoxications from medication occurred mostly as result of beekeepers' failure to observe recommendations in prospects of anti-parasitosis medication for honeybees, as to the mode of administration and dosage, as well as to storage conditions for these products, and to the use of anti-parasitosis drugs of other animals on honeybees. Manifestations in such cases were brutal and led to mortality up to 100% or massive depopulations, honeybees having externalized proboscis, blackened worker bee chitin (Rădoi et al., 2019).

Further analysis of the data in the table also indicates a decrease in the number of bee colonies affected by intoxications from 1896 affected bee colonies in 2018, to 1582 colonies in 2019 and then to 601 colonies in 2020. This decrease may be due to beekeepers' increased awareness of the foraging interdiction during melliferous plants sprinkling, and to their understanding of the need for prophylactic measures regarding intoxications in honeybees and to traffick restrictions generated by the Covid-19 pandemic.

## CONCLUSIONS

The evaluation of suspicions of honeybees' intoxications in Romania was done based on monitoring bee health in apiaries with economic loss from depopulation and mortality in various areas of the country in the 2018-2020 period.

A number of 317 apiaries were monitored, out of which 136 in 2018, 113 in 2019 and 68 in 2020, and 4 apiaries were diagnosed with suspicions of intoxications in 2018 (1896 honeybee colonies), 18 apiaries in 2019 (1582 honeybee colonies) and 10 apiaries in 2020 (601 honeybee colonies).

The suspicion of intoxication by toxic food was predominant in 2018 (49.5%) and dropped in 2020 (14.31%). The suspicion of intoxication from medication increased from 10.5% in 2018 to 50.59% in 2020. The suspicion of intoxication by chemical substances increased from 40% in 2018 to 59,6% in 2019, and then decreased to 35.10% in 2020.

The decrease in the number of honeybee colonies under suspicion of intoxication in the 2018-2020 period from 1896 colonies in 2018 to 601 colonies in 2020 may be justified as a result of prophylactic measures and traffick restrictions due to the Covid-19 pandemic.

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Compliance with ethical standards: The research does not involve human and/or animal experimentation.

Conflict of interest: The authors declare that they have no conflict of interest. We mention that the research conducted has no connection with the activity of official territorial or central laboratories nominated for the monitoring and control of bee diseases.

## REFERENCES

ASIMINEI, S., SOLCAN, G., SECASIU, V., MITROIU, M.D., PUCHIANU, G., ISAN, E., ANDERCO, S., DOBRE, G. (2016). Patologia albinei melifere. Ed. "Ion Ionescu de la Brad" Iasi, ISBN 978-973-146-224-9, 325-256.

- BÖHME, F., BISCHOFF, G., ZEBITZ, C.P.W., ROSENKRANZ, P., WALLNER, K. (2018). Pesticide residue survey of pollen loads collected by honeybees (*Apis mellifera*) in daily intervals at three agricultural sites in South Germany. *PLoS ONE*, 13, e0199995. <https://doi.org/10.1371/journal.pone.0199995>.
- COULON, M., SCHURR, F., MARTEL, A.C., COUGOULE, N., BÉGAUD, A., MANGONI, P., DALMON, A., ALAUX, C., CONTE, Y. Le, THIÉRY, R., RIBIÈRE-CHABERT, M., DUBOIS, E. (2017). Metabolisation of thiamethoxam (a neonicotinoid pesticide) and interaction with the Chronic bee paralysis virus in honeybees. *Pesticide Biochemistry and Physiology*, 144, 10-18.
- EUROPEAN FOOD SAFETY AUTHORITY. (2019). Conclusion on the peer review of the pesticide risk assessment of the active substance thiacloprid. *EFSA Journal*, 17, 5595.
- GRAY, A., BRODSCHNEIDER, R., ADJLANE, N., BALLIS, A., BRUSBARDIS, V., CHARRIÈRE, J.D., CHLEBO, R., COFFEY M.F., CORNELISSEN B., AMARO DA COSTA C. (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 Science*, Journal of Apicultural Research, 58:4, 479-485, DOI:10.1080/00218839.2019.1615661
- GREGORC, A., MOHAMED, A., BLAIR, S., PATRICIA, R.K., ADAMCZYK, J. (2018). Toxicity of Selected Acaricides to Honey Bees (*Apis mellifera*) and Varroa (*Varroa destructor* Anderson and Trueman) and Their Use in Controlling Varroa within Honey Bee Colonies. *Insects*, 9, 2,55, doi: 10.3390/insects9020055 <https://doi.org/10.1016/j.pestbp.2017.10.009>.
- KAMLER, F., DALIBOR, T., JIŘINA, P., HAJŠLOVÁ, J., MAŠTOVSKÁ, K. (2003). Intoxication of honeybees on chemical treated winter rape: problem of its verification. *Bulletin of Insectology*, 56, 1, 125-127.
- MEIKLE, W.G., HOLST N. (2015). Application of continuous monitoring of honeybee colonies. *Apidologie*, 46, 10-22.
- MUSTARD, J., WRIGHT G. (2004). Intoxicated Honey Bees May Clue Scientists Into Drunken Human Behavior. Ohio State University. ScienceDaily, <https://www.sciencedaily.com/releases/2004/10/041025123121.htm>
- OIE (WORLD ORGANISATION FOR ANIMAL HEALTH) (2009). Manual of *Diagnostic Tests and Vaccines for Terrestrial Animals (mammals, birds and bees)*, Section 3.2. Apinae, vol.1 (NB: Version adopted in May 2016)
- RADOI, I., MILEA, F.G., CODREANU, I., SAVU, V., SAPCALIU, A., BĂDIC, L. (2019). Aspects regarding the progress of some poisoning processes in bees monitored in a prophylaxis program of infectious and non-infectious diseases in this species. *Journal of Environmental Protection and Ecology*, 20, 4, 1690-1697.
- RIEDL, H., JOHANSEN, E., BREWER, L., BARBOUR, J. (2006). How to Reduce Bee Poisoning from Pesticides. *PNW 591*, A Pacific Northwest Extension publication. <https://catalog.extension.oregonstate.edu/sites/catalog/files/project/pdf/pnw591.pdf>
- SAVU, V., SAPCALIU, A. (2013). *Patologia albinelor*. Editura Fundației România de Măine. București, 31-38.
- SAVU, V., SAPCALIU, A., RĂDOI, I., MILEA, F.G., CODREANU, I., RAITA, S., BĂDIC, L. (2019). Evaluating of some intoxications cases, their types and dynamics in Romania in *Apis mellifera carpathica* bee monitored in active beekeeping season of 2019. *Lucrări Științifice-Medicină Veterinară, Universitatea de Științe Agricole și Medicină Veterinară "Ion Ionescu de la Brad" Iași*, 61, 9-16, 1454-7406.
- SCOTT, T.O., TROY, DA., Wu-SMART, J. (2018). Interactions between pesticides and pathogen susceptibility in honey bees. *Current Opinion in Insect Science*, 26, 57-62. <https://doi.org/10.1016/j.cois.2018.01.006>
- TOMASZ, K., NIEWIADOWSKA, A., POSYNIK, A. (2016). Pesticide Poisoning of Honeybees: A Review of Symptoms, Incident Classification, and Causes of Poisoning. *Journal of Apicultural Science*, 60, 2, 5-24.