"Incidence of some intoxications evolution in Romania in *Apis mellifera carpathica* bees monitored in a bee disease prevention program in the active beekeeping season of 2019"

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Abstract
The aim of this paper consisted in evaluating the intoxication cases and their dynamics during the active beekeeping season of 2019 for Apis mellifera carpathica bees monitored in a program for the prevention of infectious and non-infectious diseases. Following the corroboration of the anamnestic data with the morphoclinical data, suspicion of intoxication with toxic feed (pollen), chemicals (pesticides) and medicinal products (antiparasitic products) was established, excluding other causes of illness. During the period of the study, 113 apiaries from different geographic areas of Romania were monitored; counting a number of 7007 bee families, and was identified a number of 18 apiaries (16%) with susceptibility of intoxication, including a number of 1582 bee families (22.57%). The percentage dynamics of the intoxication cases in the studied bee families was the following: 34.07% intoxication with toxic food, 59.6% intoxication with chemical substances and 6.33% intoxication with drugs. We mention that this proportion of the intoxication was on the background of an active beekeeping season in 2019 with many rainfall and extreme weather phenomena.
Key words*: bees, Apis mellifera carpathica, suspicions of intoxication*

Introduction

Pollinators are a key component of the global biodiversity, providing vital ecosystem services to cultivated and wild plants. There are clear evidence regarding the massive decline of both wild and domesticated pollinators (honey bees), but also the parallel decline of the plants that rely on them. Lowering the pollinators number may lead to the loss of pollination services, with considerable ecological and economical negative impacts that could significantly affect the maintenance of wildlife diversity, the stability of larger ecosystems, plant production, food security and human well-being (Potts G. Simon et al ., 2010).

Bees' intoxications are pathological conditions caused by certain organic or inorganic substances which by direct contact or ingestion cause serious disturbances of cellular metabolism and endanger their vital functions. From the etiological point of view, bees' intoxication is classified as: toxic food intoxication (pollen, honey), chemical substances intoxication (pesticides, paints, artificial combs) and drug intoxication (antiparasitic veterinary products).

Drugs intoxication in bees is quite common, causing behavioural changes (abandonment of the hive, stretched wings, agglomeration of bees on the beehive wall, fall of bees in the grass in front of the hive, trembling of the abdomen) and their death on the bottom of the hive (1, 2, 5, 7).

Intoxication with allelochemicals from the harvesting plants used by bees (alkaloids, coumarins, saponins, cyanogenic and cardiac glycosides, and terpenes) has an important share in bees' morbidity, causing depopulation and economic losses for the beekeepers (3, 12).

Chemical toxicosis is the most dangerous non-contagious bee disease, being produced by phytopharmaceuticals used in agricultural and forestry plant protection (12). The most important of the chemical intoxications is the pesticide intoxication, which affects on one hand the digestive

system (as a result of the ingestion of pesticides with food) and, on the other hand, the bee's nervous and respiratory system, together with the environmental pollution, with effects on all the creatures on our planet (4, 6, 8, 10, 11, 12, 13, 14, 15). Pesticides cause a multitude of sublethal effects on bees, affecting their productive performance, the development of the juvenile, lesioning the nervous system (impairment of learning, mobility and memory), increasing the susceptibility to diseases and affecting the hygiene behaviour in the hive (8).

Recent research has shown the interaction between pesticides, especially neonicotinoid pesticides and pathogenic bee viruses, fact that can lead to significant losses in the bee families (4), but also to the worsening of *Nosema ceranae* infections, which together lead to the collapse of the bees colony (8, 13).

Compared with other insects, bees are highly susceptible to pesticides due to deficiencies in genetically encoded detoxification enzymes (15).

Materials and methods

During the study period, the active beehive season of 2019 (February-July), 113 apiaries from the North, Central, East, South and West of the country were monitored, totalling a number of 7007 bee families.

Laboratory tests for the diagnosis of bees' diseases were carried out in the *Beekeeping* Research and Development Institute (ICDA) Laboratory, and the methodology of the laboratory investigations for the diagnosis of bees' diseases was carried out in accordance with the O.I.E. protocols (*World Organization for Animal Health*, 2008).

Results and discussion

Performing a detailed anamnesis, corroborated with the morphoclinical exam of the bee samples (alive, death), completed with the symptomatological picture (conducted by the veterinarians of the ICDA Beekeeping Pathology Laboratory, helped by the beekeepers from the field) after eliminating other causes (infectious, parasitic or technological) followed by laboratory examinations (direct microscopy of the intestinal content, bacterioscopic, bacteriological, mycological and parasitological exams) the suspicion of intoxication in the studied bees was established.

From a total number of 113 monitored apiaries, summing 7007 bee families, 18 apiaries had intoxication problems (15.92%), representing a total of 1582 bee families (22.57%) (Table 1).

Apiaries with suspicion of intoxication throughout the entire period of the study (active beekeeping year 2019)

Table 1

Active beekeeping year	**2019**
Number of monitored apiaries	113
Number of bee families monitored	7007
Number of apiaries affected by intoxication	18 (15.92 %)
Number of bee families suspected of intoxication	1582 (22.57%)

From a number of 7007 monitored bee families, 1582 bee families (22.57%) were identified with the suspicion of intoxication, of which mortality was found in 30 bee families

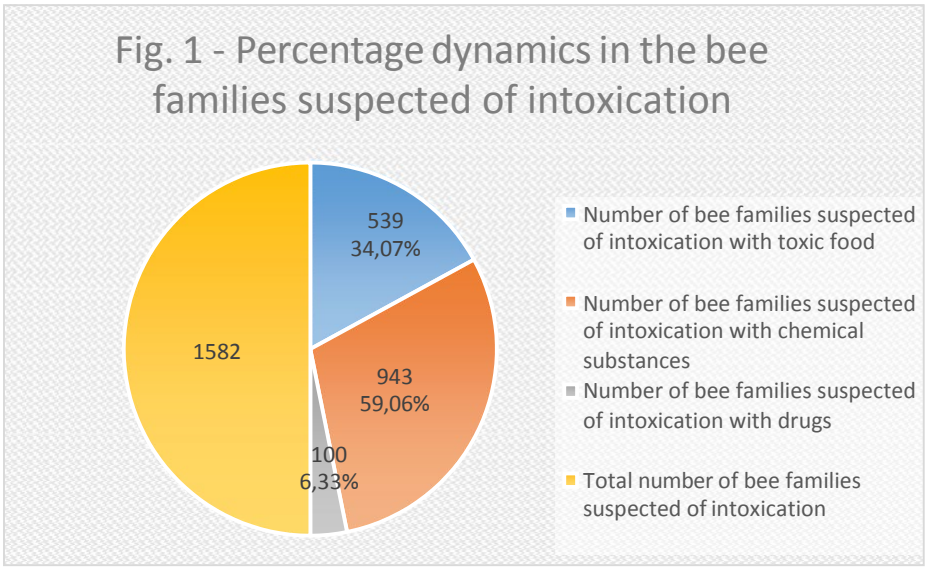
(1.88%), the rest showing depopulation. The percentage dynamics of intoxication cases in the studied bee families was the following: 34.07% toxic food intoxication, 59.6% chemical substances intoxication and 6.33% drugs intoxication (Table 2). We mention that this proportion of intoxication was on the background of an active beekeeping season 2019 with abundant rain and rainwater puddles.

Types of suspected intoxication in the bee families monitored during the period of the active season 2019

Table 2

Type of intoxication	Number of bee families with intoxication suspicion/ active season of 2019	Number of dead bees/ active season of 2019
Number of bee families intoxicated with *toxic food (pollen)*	539 (34.07%)	30 (1.88%)
Number of bee families intoxicated with *chemical substances*	943 (59.6 %)	0
Number of bee families intoxicated with *drugs*	100 (6.33 %)	0
Total number of intoxicated bee families	1582	30 (1.88%)

From Table 2 it is found that during the active season of 2019, the intoxications with sublethal doses of toxic chemical substances had the largest share, being found in 943 bee families (59.6%), followed by the intoxications with toxic food in 539 bee families (34.07%) and drugs intoxications in 100 bee families (6.33%) (Fig.1).



The explanation for this percentage, with the predominance of intoxications with toxic chemical substances, can be attributed to the fact that the beekeeping season of 2019 was a rainy season that allowed rainwater to form puddles and to increase therefore its concentration in chemicals that are toxic to bee. These toxic substances originated either from spraying the

agricultural crops with various pesticides, or by treating the seeds from different crops with toxic chemicals, substances that were emitted into the soil and then concentrated in puddles' water, or from treatments planned by the town halls in order to combat mosquitoes. The low proportion of drug intoxications is explained by the fact that during the main harvesting periods no hive deworming treatments are being carried out and as such, this type of intrusions accidentally occur, either at the beginning of the active season or between large harvesting periods. The distribution of the intoxication suspected cases by counties and months is presented in Table 3.

Distribution of the cases with intoxication suspicion between January and July 2019

Table 3

Month /2019	**Number of affected apiaries**	**County**	**CLINICAL SIGNS**	**Type of intoxication suspicion**	**Observations**
FEB.	**1**	SB	Small, blackened bees with exteriorised proboscis, wings stretched at 90 degrees, high mortality	**Drugs intoxication**	The irrational use of various external antiparasitic substances
APR.	**5**	GR, GR, PH, IF, VS	Depopulation, diarrhea, constipation, bloating, bees with the proboscis pulled out, exteriorised needle	**Toxic food intoxication** (pollen)	Without Nosema (after rain)
MAY	**8**	SB, GR, IF, DB, DB, SB, SM, AG	Blackened bees pulled out at the entrance of the hive, exteriorised needle, blackened head in the cell, uncoordinated movements, paralysis, depopulation, dead bees with crowded legs, wings stretched at 90 degrees, exteriorised proboscis, reduced activity in the hive and outside, paresis, mortality	**Chemical intoxication** (pesticides, mosquito sprays, etc.)	(after rain)
JUL.	**4**	CV, TL, IF. GR	Depopulation, small and blackened bees, abdominal bloating, diarrhea	**Toxic food intoxication** (linden pollen)	Without Nosema (after rain)

The suspicion of intoxication with toxic food (pollen) was identified in the active beekeeping season during harvesting, after rain, and was characterized symptomatically by: bloating, diarrhea, constipation, exteriorisation of the genital apparatus in drones, pollen glued to the bees' feet, their presence on the flying board, paresis, inability to fly (Figure 2, 3, 4, 5, 6, 7).

Fig. 2. Morphoclinical aspects: bloating of the adult bee's abdomen in the pollen intoxication suspicion

Fig. 3. Intoxication with toxic food – pollen, buckwheat honey (stains of diarrhea on the frames from the hive)

Fig. 4. Pollen intoxication in working bees (wet pollen attached to the bees' feet)

Fig. 5. Pollen intoxication in drones (exteriorization of the genital apparatus)

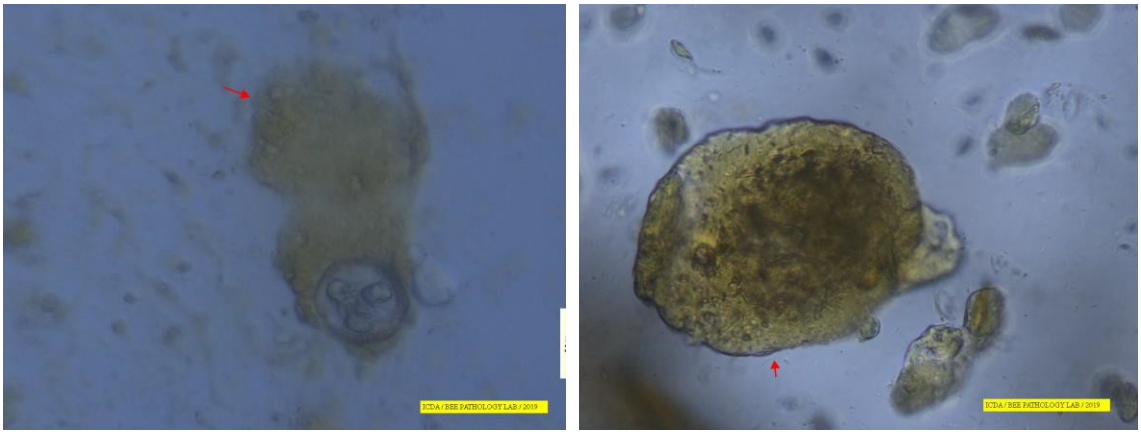


Fig. 6. Diarrheal feces (left) or constipation (right) in the intoxication with toxic food

The suspicion of intoxication with *chemical substances* has been identified in the beekeeping season during harvesting (fruit trees, colza, sunflower, linden), after pesticide and / or mosquito spraying, followed by heavy rains with rain water puddles, but also following the use of some toxic paints for the protection and individualization of the hives, or the beekeepers using artificial combs containing toxic paraffin solvents. (Figures 7, 8)

Fig. 7. Morphoclinical aspects in the intoxication with *chemical substances* (pesticides) after spraying the plant cultures (fruit trees, colza, etc.)

Fig. 8. Morphoclinical aspects in the suspicion of intoxication with chemical substances (blackened bees with the wings stretched at 90 degrees)

The usage of *drugs* without following the indications in the package leaflet and also the use of some medicinal products intended for other animal species without knowing the exact dose of administration in bees, have led to the emergence of brutal drug intoxication cases, shortly after treatments, with a mortality rate of 100% (one apiary in February).

Conclusions

1. Suspicions of intoxication in bees represented 22.57% of the bee families, and mortality rate was 1.88%, accompanied by significant depopulation.

2. The largest share of the suspected intoxications in the active season of 2019 was attributed to the chemical substances intoxication (59.6%), followed by toxic food intoxication (34.07%) and drugs intoxication (6.33%).
3. By educating the beekeepers and following the legislation regarding the prescription of veterinary medicines by specialists, the impact of intoxications on bees can be limited.
4. The intoxication with chemical substances has decreased by applying some administrative and training measures for the beekeepers.
5. Applying some intoxication prevention and *environment protection* measures during the bees' harvesting period represents a good measure to limit the economic losses.

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.

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