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Effects of pesticides on bee populations and safety of bee honey in Ukraine

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ABSTRACT

To prevent pest contamination of crops, they are treated with plant defense agents, the action of which is aimed at the destruction or development and reproduction control of hazardous organisms. But also these chemical agents cause pollution of environmental ecosystems. Furthermore, the use of pesticides on honey bees often leads to mass mortality of the bees and contamination of nectar and pollen. Honey, made by the bees of such nectar, may contain pesticide residues that are toxic to a bee brood and harm the viability and productivity of bee colonies. One hundred seventy-two samples of bee honey and 40 samples of dead bees were studied from different regions of Ukraine. Eight hundred thirty-seven bee colonies died from pesticide poisoning of the honey bees in 2021. The bees most died due to thiamethoxam (523 bee colonies), clothianidin 400 (bee colonies), and lambda-cyhalothrin (342 bee colonies). In 2022, the poisoning of the honey bees, from which 1,130 bee colonies died, was caused by seven insecticides. Lambda-cyhalothrin (653 bee colonies), thiamethoxam (352 bee colonies), imidacloprid (342 bee colonies), clothianidin (325 bee colonies), and acetamiprid (320 bee colonies) were most frequently detected. 11 insecticides, 11 fungicides and 2 each of acaricides and herbicides were found in the honey. There were 425 detection cases of insecticides, 285 fungicides, 8 acaricides, and 3 herbicides. In 2021-2022, 16 insecticides of the 3rd toxicity class were found in the dead bees.

Keywords: pesticide, honey bees, bee honey, safety, food product

INTRODUCTION

The increasing demand for food products stimulates the widespread use of pesticides when growing crops. Crops are treated with plant defense agents for the destruction of the development and reproduction control of hazardous organisms. But also these chemical agents cause pollution of environmental ecosystems. Furthermore, the use of pesticides on honey bees often leads to mass mortality of the bees and contamination of nectar and pollen **[1]**, **[2]**.

Honey bees, as the main pollinators of crops, play an important role in supporting an ecological balance and are considered the most important nontarget organisms exposed to pesticides' toxic effects **[3]**, **[4]**.

It should be noted that the bees are exposed to the effects of various environmental factors, but pesticide poisoning is one of the main decline reasons for the honey bee population **[5]**, **[6]**. In the future, beekeeping may be threatened by a considerable loss of the honey bee colonies since it may be difficult to restore apiaries after the loss of a large part of the bee colonies **[7]**, **[8]**.

In most countries, there are strict rules for the pesticides to be used in agricultural production, and the poisoning cases of honey bees are controlled by the applicable legislation **[9]**, **[10]**. However, bees' poisoning facts are recorded annually, which requires scientific research and the development of preventive measures for their pesticide poisoning **[11]**.

The poisoning facts of the bees with the pesticides, which were registered in different years, are known in Great Britain **[12]**, **[13]**. Furthermore, there is data on the poisoning of the bees during corn sowing in Germany because seeds were treated with plant defense agents **[14].** There are confirmed cases of pesticide poisoning of bees in Canada **[15]**. Therefore, the toxic effect risk of the pesticides on the bees should be always taken into account during their application **[16]**.

Suppose insecticides can cause the acute poisoning of the bees. In that case, it is necessary to point out that their effect was sometimes enhanced with the simultaneous combination of several active substances. Furthermore, pesticides are also found in environmental mixtures, so predicting their synergistic effects on the bees is difficult. The poisoning of the bees by highly toxic substances such as chlorpyrifos, deltamethrin, cypermethrin, and imidacloprid, as well as low-toxic ones such as prochloraz and thiacloprid, have been confirmed by studies **[17]**, **[18]**.

Some pesticides that are used to treat the crops against the pests mustn't kill any bees but make them vulnerable to mites and adverse environmental factors. Entomologists have also proven that the bees' memory and mental abilities deteriorate after being subjected to pesticides. Some pesticides cause epilepsy in the bees. The combined pesticides are the most dangerous for insects. It appeared those 4 days after the first contact with the pesticides, about 30% of the bees lost their ability to learn and began to undergo the remembering tests for flower smell. Previous studies have shown that glyphosate can affect the ability of the bees to learn and navigate in space **[19]**.

Honey made by the bees of the nectar of the plants, which are treated with pesticides, may contain toxic residues to the bee brood and harm the viability and productivity of the bee colonies. It should be noted that, according to the data of some scientists, honey can be a biological indicator of the use of pesticides on crops and their pollution of the environment **[20]**, **[21]**.

However, despite hundreds of approved pesticides being applied to the agricultural fields each year, only a small proportion of these organic compounds have been found in the honey and beeswax samples. This observation questioned the general suitability of bee products as an indicator for synthetic organic pesticides used when growing field crops **[22]**.

It was in studies revealed that the amount, frequency, and concentration of the pesticides in the bee honey were higher in the samples, which were collected from hives located in areas of intensive and high-tech agriculture. Insecticides that are the most dangerous for bees – neonicotinoids, organophosphorus compounds, herbicides, and fungicides were most often found in high concentrations **[23]**.

The pesticides of the neonicotinoid group require special attention due to their application almost worldwide. There is also growing concern about their negative effects as evidence accumulates of their impact on bee health and resistance. Scientists conducted tests on the distribution of these analytes in honey in many countries. As a result, their remains were found in most parts of the tested samples. Even though neonicotinoids were contained at levels considered safe for human consumption, a significant distribution of these pesticides in the bee habitat was established **[24]**. So, for example, during 2015-2017, honey studies in Poland revealed the remains of 21 pesticides. Acetamiprid and thiacloprid, quantified in 77 % of the samples, were most frequently detected **[25]**.

Assessing the risks to human health, Israeli scientists found that at least two pesticides were present in the samples of the examined bee products. Neonicotinoids and 2,4-dichlorophenoxyacetic acid were found in the honey samples, and more lipophilic pesticides were predominantly found in the beeswax **[26]**.

The pesticide distribution in the hive is a rather complex process mainly due to the interaction and food transfer between the colony members. That is why the presence of certain pesticides, as well as their concentration, are substantially different between the nectar, pollen, and other beekeeping products **[27]**.

The pesticide residues were also studied in the winter honey. Only eight residues were found: coumaphos, fluvalinate, boscalid, dimethoate, atrazine, bentazone, dichlorobenzene, and thymol. The honey from brood combs most often contained pesticide residues **[28]**, **[29]**.

As far as is known, bee honey belongs to ready-made food products and does not need to be cooked. That is why the toxicants in it enter the human body without impediment. This, in turn, reduces its nutritional and medicinal value **[30]**.

Indirect ecological and economic losses, as a result of the use of pesticides, are related to the pollution of underground and surface water; destruction of beneficial microorganisms, insects, natural predators, and wild birds, poisoning of animals, contamination of products, and impact on human health. Furthermore, the pesticides combined with xenobiotics lead to a permanent global population decline of the honey bees-pollinators, and loss of crops and plant products, which well may trigger a food security crisis. In addition, an account must be taken of the costs of public funds for controlling the pesticide circulation in the environment and food products. Thus, it may be concluded that if the total environmental, social, and economic costs for the pesticides to be used could be measured as a whole, the profitability of the pesticides to be used would be substantially lower **[31]**.

The studies to improve the diagnostics methods for bee poisoning are still ongoing despite significant analytics progress in the last few years. It is quite a complicated task since the determination of the pesticide residuals (often equal to sublethal doses) and the simultaneous presence of a wide compound range with various physicochemical properties in such a complex matrix as the honey and the body of the honey bee, is a serious problem for modern laboratory practice and requires the use of the highly sensitive and selective methods. In that context, new sample preparation approaches are also becoming topical **[32]**, **[33]**.

QuEChERS is a universal sample preparation method characterized by specificity, selectivity, accuracy, sensitivity, low cost, and adequate speed. It is suitable for determining the pesticide content in less-understood beekeeping matrices such as royal jelly and propolis **[34]**, **[35]**.

With due regard to these scientific facts, as well as the fact that honey is a widely-used product, monitoring its safety, among other controlling the pesticide content in this product, is required to be continued. This, in turn, aims to ensure consumer safety and determine the pesticide exposure risks to the health of the pollinators, other nontarget organisms, the ecosystem, and their potential consequences for human health.

Scientific Hypothesis

The number of plant protection products used in Ukrainian agriculture is increasing. The conducted research, including the diagnosis of bee poisoning, is aimed at obtaining data on the list of pesticides and their residual content in honey to substantiate the need for their further monitoring and control therefore The impact of pesticides on the bee population has a significant impact on the safety of honey in Ukraine, as a result of changes in the level of production and changes in the quality of products, which can have various ecosystem consequences for the plant world, since bees are important pollinators of plants. The results of the conducted studies justify how the use of pesticides can affect the level of honey production due to the decline of bee populations and the quality of honey due to pesticide contamination.

MATERIAL AND METHODOLOGY

Samples

In 2021, during the honey flow period, the bee honey samples were taken from the private apiaries in the amount of 156 samples from 21 regions of Ukraine (Table 1). Furthermore, during 2021 and 2022 the samples of the dead bees and honey in the combs were obtained to conduct diagnostic studies for determining the pesticide poisoning of the bee. During the study period, 172 samples of bee honey and 40 samples of dead bees were taken from different regions of Ukraine.

Today, beekeeping is a developed industry in Ukraine. It ranks first in honey production and export in Europe **[37]**.

Most samples were taken from the Central regions of Ukraine, i.e. Vinnytsia, Poltava, Kirovohrad, Cherkasy, and Dnipropetrovsk regions, where a significant number of the apiaries are located, which provide a larger volume of the produced honey in comparison with other regions. Thus, 17,070 tons of honey were obtained in this region according to statistical data for 2021 **[36]**.

The beekeepers from 23 districts participated in the sampling. In the Vinnytsia region, the samples were taken from 8 districts, in Dnipropetrovsk – 6, Poltava – 5, Kirovohrad – 3, and Cherkasy – 1.

For the tests, 16 samples were used – from the Vinnytsia region, 13 each from the Kirovohrad and Poltava regions, 9 from the Dnipropetrovsk region, and 9 from the Cherkasy region. Their percentage ratio to the total number of honey samples was 10.3; 8.3, 8.3; 5.8, and 5.1%.

The Northern part of Ukraine is represented by Zhytomyr, Kyiv, Chernihiv, Sumy, Volyn and Rivne regions. 14,614 tons of honey, which is 2,456 tons less than in the central part, were obtained in this region in 2021 **[37]**.

The samples were taken from the apiaries in 16 districts of the following regions: Zhytomyr– 1 district, Kyiv – 4, Chernihiv – 2, Sumy – 6, Volyn – 2, Rivne – 1.

From the Zhytomyr region, 3.8 % of the total number of honey samples were taken for analysis. From Kyiv – 6.4%, Chernihiv – 1.9%, Sumy – 5.1%, Volyn – 2.6%, Rivne – 3.2%. The total number of the taken samples by region was 6, 10, 3, 8, 4, and 5 samples, respectively.

A significant number of the apiaries of Odesa, Mykolaiv, Kherson, and Zaporizhzhia regions, which produced 14,106 tons of bee honey in 2021, which is 508 tons less than in the Northern part of the country, operate in the Southern part of Ukraine **[38]**.

Table 1 Number of taken bee honey samples by regions of Ukraine.

Region	District	Number of samples	Number of taken samples by region	Percentage of the total amount, %
Volyn	Volodymyr-Volynskyi	1	4	2.6
	Manevytskyi	3		
	Novomyrhorodskyi	2		
Kirovohrad	Kropyvnytskyi	7	13	8.3
	Novoukrainskyi	4		
	Murovanokurylovetskyi	2		
	Kalynivskyi	1		
	Orativskyi	1		
Vinnytsia	Barskyi	1	16	10.3
	Tyvrivskyi	2		
	Vinnytskyi	1		
	Apostolivskyi	2		
	Yuryivskyi	2		
	Kryvorizkyi	1		
	Dnipropetrovskyi	2		
Dnipropetrovsk	Tomakivskyi	2	9	5.8
	Zhytomyrskyi	6		
	Mukachivskyi	1		
Zhytomyr	Khustskyi	1	6	3.8
	Uzhhorodskyi	1		
Zakarpattia	Kamyansko-Dniprovskyi	3	3	1.9
	Obukhivskyi	1		
Zaporizhzhia	Bilotserkivskyi	4	3	1.9
	Bohuslavskyi	3		
Kyiv	Brovarskyi	2	10	6.4
	Khmelnytskyi	3		
	Kamianets-Podilskyi	3		
	Shepetivskyi	3		
Khmelnytskyi	Mykolaivskyi	6	9	5.8
	Yelanetskyi	2		
	Bereznehuvatskyi	1		
Mykolaiv	Voznesenskyi	1	10	6.4
	Podilskyi	2		
	Lymanskyi	1		
	Mykolaivskyi	1		
Odesa	Odeskyi	2	6	3.8
	Hadyatskyi	1		
	Poltavskyi	6		
	Myrhorodskyi	1		
	Kremenchutskyi	4		
Poltava	Lubenskyi	1	13	8.3
	Hoshchanskyi	5		
	Konotopskyi	2		
Rivne	Sumskyi	1	5	3.2
	Romenskyi	1		
Sumy	Okhtyrskyi	1	8	5.1
	Trostyanetskyi	1		
	Shostkynskyi	2		

The honey from the beekeeping of 8 districts were included in the study for the pesticide residues, in particular, from 4 districts of Odesa and 4 districts of Mykolaiv region, 3 districts from Kherson region, and 1 district from Zaporizhzhia region.

6 samples of the honey, or 3.8 % of the total samples, were taken from the Odesa region, 10 samples (6.4%) – from Mykolaiv, and 3 samples each (1.9%) – from Kherson and Zaporizhzhia.

The western part of Ukraine is represented by the apiaries of Lviv, Zakarpattia, Chernivtsi, and Ternopil regions, mostly breeding honey bees. The total honey it produces is much lower than in other regions. An exception is the Khmelnytskyi region, where a fairly large amount of honey is produced – 5,437 tons out of 10,059 tons **[38]**, obtained in the region as a whole in 2021. Therefore, the largest honey samples were taken from the beekeeping of 3 districts of the Khmelnytskyi region in the amount of 9 samples, corresponding to 5.8%. Out of 5 districts of Lviv it is 7 (4.5%), 2 districts of Ternopil – 5 (3.2%), 3 districts of Zakarpattia – 3 (1.9%), and 1 district of Chernivtsi region – 1.3% (2 samples).

In 2021, 3,181 tons of honey were pumped out in the Kharkiv region in the Eastern part of Ukraine **[38]**. For the tests, 12 samples of the bee honey were received, the relative amount of which was 7.7%, from 5 districts of Kharkiv region.

Chemicals

All chemicals were of analytical grade and were purchased from Sigma-Aldrich, i.e. solvents - acetonitrile, methanol and deionized water with LC-MS grade (Chromasolv, 99.9%); reagents – ammonium formate, magnesium sulfate, sodium chloride, sodium citrate dihydrate, sodium hydrocitrate 1,5-hydrate with purity 99%, A.C.S. reagent; sorbents: a mixture of primary and secondary amines (PSA), cat. number 52738-U and octadecyl modified silica gel (C18), cat. number 97727-U.

Animals, Plants, and Biological Materials

Laboratory and farm animals were not used directly during the studies.

Instruments

Liquid triple-quadrupole tandem mass spectrometer (Waters Xevo TQ-XS, USA).

Gas triple quadrupole mass spectrometer (Thermo TSQ 9000, USA).

Laboratory Methods

The internal method, which was used for the studies, was developed with the use of the QUECHERS sample preparation approach and the methods of liquid mass spectrometry (UPLC-MS/MS) and gas mass spectrometry (GC-MS/MS) **[39]**.

Description of the Experiment

Sample preparation: The selected material was prepared for the study, and 10 g was taken from each sample.

Number of samples analyzed: 172 samples of bee honey and 40 samples of dead bees were taken during the study period.

Number of repeated analyses: All measurements were performed 3 times.

Number of experiment replications: The number of replicates of each experiment to determine one value was 5 times.

Design of the experiment: The pesticide residues were determined after their appropriate extraction from the sample with a solvent, purification of extracts with the use of dispersion solid-phase extraction, and identification of analytes by retention time and the ratio of the mass of corresponding ions to their charge, quantitative determination – by the external standard method in terms of the peak area according to the method **[39]**.

Statistical Analysis

Statistical analysis of the results of experimental studies was performed in five replicates using standard methods of research of organoleptic, physical, physicochemical, microbiological, and other indicators. The obtained results of experimental research are processed using modern analytical integrated systems Microsoft Excel 2016 and Statistica 13.3. Adequacy of decision-making was carried out according to the criteria of Fisher, Cochran, and Student.

RESULTS AND DISCUSSION

Most scientists focus on the determination of the pesticide residues in honey products, but much less attention has been paid to the pesticide contamination of the honey bees and their deaths.

Most scientists in their scientific works, focus on the determination of pesticide residues in honey products, but much less attention is paid to pesticide contamination of honey bees and their death. Several scientific works are devoted to similar experimental and theoretical studies, in particular:

- research of pesticide residues in sunflower honey **[40]**, **[41]**;
- research of pesticide residues in rapeseed honey **[42]**, **[43]**;
- study of pesticide residues in alfalfa honey **[44]**, **[45]**;
- research on pesticide residues in acacia honey **[46]**, **[47]**;
- research of pesticide residues in buckwheat honey **[48]**, **[49]**.

We investigated the distribution area of the pesticide poisoning of honey bees by the regions of Ukraine for 2021–2022 (Table 2).

Table 2 Distribution area of pesticide poisoning of honey bees.

Region	District	Number of poisoning cases	Detected active substances of the 1st toxicity class	Number of dead bee colonies
		2021		
Vinnytsia	Vinnytskyi	1	Permethrin	10
	Zhmerynskyi	3	Thiamethoxam	300
Khmelnytskyi	Khmelnytskyi	1	Lambda-Cyhalothrin	42
Ivano-Frankivsk	Ivano-Frankivskyi	2	Imidacloprid	59
Poltava	Poltavskyi	2	Thiamethoxam, clothianidin, lambda-Cyhalothrin	223
Rivne	Rivnenskyi	1	Imidacloprid	26
Sumy	Shostkynskiy	2	Clothianidin	100
Total:		16		760
		2022		
Kirovohrad	Novoukrainskyi	2	tau-fluvalinate*	20
Vinnytsia	Vinnytskyi	2	Clothianidin, lambda-Cyhalothrin	177
	Khmilnytskyi	1		18
Dnipropetrovsk	Dniprovskyi	1	lambda-Cyhalothrin	10
Kyiv	Obukhivskyi	1	Thiamethoxam, clothianidin	10
	Bilotserkivskyi	1	lambda-Cyhalothrin	15
Khmelnytskyi	Khmelnytskyi	2	alpha-Cypermethrin	33
Odesa	Berezivskyi	2	Acetamiprid*	320
	Odeskyi	1	lambda-Cyhalothrin	46
Poltava	Poltavskyi	6	Imidacloprid, lambda-Cyhalothrin, thiamethoxam	342
Rivne	Dubenskyi	2	Clothianidin	74
Ternopil	Ternopilskyi	1	lambda-Cyhalothrin, clothianidin	45
Cherkasy	Zolotoniskyi	2	Cypermethrin, chlorpyrifos	20
Total:		24		1130

Note: Acetamiprid and tau-fluvalinate belong to the third toxicity class for the bees.

Kiljanek et al. focused their studies on bee poisoning with pesticides. Thus, out of 70 samples of the bees, which were suspected of chemical toxicosis, 57 samples, containing the pesticides and their metabolites, were found **[50]**.

In 2021, cases of pesticide poisoning of the honey bees were found in 7 regions of Ukraine: Vinnytsia, Khmelnytskyi, Ivano-Frankivsk, Poltava, Rivne, Sumy, and Kharkiv. A total of 837 bee colonies died. The large majority of the poisoning was registered in the central regions, where the largest number of bee colonies in Ukraine is located. Thus, 310 bee colonies died in the Vinnytsia region, their share of the total number was 37.0%. A significant part of the bee colonies also died in the Poltava region – 223, which corresponds to 26.6% of the total number. The fewest cases of the poisoning were found in the Rivne region, namely 26 dead bee colonies, i.e. 3.1%. It is necessary to point out that this area belongs to those with a small number of bee colonies in Ukraine **[8]**, **[18]**.

The bees died due to insecticides such as lambda-cyhalothrin, thiamethoxam, imidacloprid, clothianidin, and permethrin (Table 2, Figure 1). They all belong to the 1st toxicity class for the bees. lambda-Cyhalothrin was used alone and in combination with other insecticides and led to the death of 342 bee colonies in our country's Western, Eastern, and Central parts. It is necessary to point out that the bee poisoning, caused by imidacloprid, led to the death of 31 bee colonies in the North and South West of Ukraine. Permethrin and thiamethoxam in the Central region killed 10 and 523 bee colonies, respectively. Clothianidin was found in the samples of the dead bees in the North, Center, and West of the country. The total number of bee colonies that died as a result of the toxic effect of clothianidin is quite high and reaches 400. However, it's worth mentioning that the obtained data may not fully reflect the real situation in Ukraine, since not all facts of the bee poisoning have been confirmed by studies **[3]**, **[4]**.

If the facts of the pesticide poisoning of the honey bees are analyzed for 2022, an increase of 8 cases in comparison with the previous year was found, that is, their number reached 24. As a result, 1,130 bee colonies died, which is 293 colonies more than in 2021. The number of dead bee colonies prevailed in the Odesa region and amounted to 366. Most cases of pesticide poisoning of the bees were observed in the Poltava region, i.e. the number of dead bee colonies was 342.

In 2022 the honey bees were poisoned with 7 insecticides of Ist toxicity class, that is, lambda-cyhalothrin, thiamethoxam, imidacloprid, clothianidin, alpha-cypermethrin, cypermethrin, chlorpyrifos (Table 2, Figure 2). Lambda-cyhalothrin, widely used as a plant defense agent throughout Ukraine in 2021 and 2022, was most often found. 653 bee colonies in 6 regions died due to this pesticide poisoning. The use of insecticides with the active substance - clothianidin, which led to the death of 325 bee colonies in Ukraine, is quite popular **[9]**, **[10]**.

Similar to the previous year, thiamethoxam was found in 2 samples of dead bees from the Central region of our country. It caused the death of 352 bee colonies. Similar in quantitative meaning was the bee death due to imidacloprid, which amounted to 342 bee colonies. The dead bees due to alpha-cypermethrin have been found in 2 regions of Ukraine, resulting in the death of 78 bee colonies. Cypermethrin, chlorpyrifos, acetamiprid, and tau-fluvalinate were the less common poisoning causes, where 1 case was recorded, respectively. Among the mentioned pesticides, acetamiprid and tau-fluvalinate belong to the 3rd toxicity class for bees.

Acetamiprid led to the death of 320 bee colonies. Even though it is considered to be slightly toxic, in synergy with other pesticides it can cause the heavy mortality of the bees, which is confirmed by the studies of other scientists **[39]**, **[40]**.

16 insecticides of the 3rd toxicity class were found in the studied samples of the dead bees for 2021-2022 (Table 3). These include: acetamiprid, cyproconazole, tebuconazole, azoxystrobin, permethrin, promethrin, carbendazim, prothioconazole, propiconazole, difenoconazole, epoxyconazole, pyraclostrobin, picoxystrobin, tau-fluvalinate, hexythiazox, pyridaben.

Analyzing the study analyses for 2021, it should be noted that most of the samples of the dead bee contained clothianidin residues. It was found in 11 poisoning cases out of 16, which is 68.75%.

The insecticide lambda-cyhalothrin was registered quite often in the bodies of the dead bees: 6 cases out of 16, or 37.5%. The same number of the studied samples of dead bees contained tebuconazole. Thiamethoxam was present in 25%, and imidacloprid, cyproconazole, azoxystrobin, and promethrin – in 18.75% of the death cases of the honey bees. Other analytes, which we identified, caused fewer bee poisonings.

In 2022, a similar trend was observed regarding the causes of the pesticide poisoning of honey bees. Most samples of the dead bees contained lambda-cyhalothrin – 14 out of 24 analyzed in total, which corresponds to 58.33%. Such analytes as clothianidin, tebuconazole, and azoxystrobin were found in 41.67% of the dead honey bees' studied samples, corresponding to 10 cases of toxicosis caused by these insecticides. Thiamethoxam and cyproconazole were present in 6 dead bee samples, corresponding to 25% of their total amount. Cypermethrin was found in 5 samples (20.83%), and imidacloprid in 4 samples (16.67%) of the dead bees. The rest of the pesticides, that we studied, were less frequently detected in the samples of the dead bees.

The pesticide content in the bodies of the dead bees for 2021-2022 is presented in Table 3.

Table 3 Pesticide content in bodies of dead bees (2021-2022).

List of detected pesticides	Number of conducted studies	Number of detected cases	Concentration, µg/kg	Toxicity class for bees
2021				
Clothianidin	16	11	1.6-4.0	1
lambda-Cyhalothrin		6	0.8-52.6	1
Tebuconazole		6	0.5-5.1	3
Thiamethoxam		4	1.0-28.3	1
Imidacloprid		3	194.2-1166.0	1
Cyproconazole		3	4.5-1463.8	3
Permethrin		3	0.9-2.8	3
Azoxystrobin		3	10.4-375.6	3
Acetamiprid		2	5.1-63.1	3
Thiacloprid		1	1.0	1
Permethrin		1	8074.6	3
2022				
lambda-Cyhalothrin	24	14	17.7-458.8	1
Clothianidin		10	1.8-34.8	1
Azoxystrobin		10	1.6-217.1	3
Tebuconazole		10	1.0-21.8	3
Thiamethoxam		6	1.7-270.3	1
Cyproconazole		6	0.38-110.8	3
Cypermethrin		5	7.0-234.2	1
Imidacloprid		4	2.2-9.0	1
Difenoconazole		3	1.4-4.7	3
Acetamiprid		2	11.9-97.4	3
Propiconazole		2	74.7-92.7	3
Epoxyconazole		2	43.2-56.3	3
alpha-Cypermethrin		2	28.6-35.0	1
tau-Fluvalinate		2	776.1-10328.8	3
Chlorpyrifos		1	19.7	1
Carbendazim		1	67.1	3
Prothioconazole		1	102.9	3
Pyraclostrobin		1	10.2	3
Picoxystrobin		1	0.48	3
Hexythiazox		1	1.36	3
Pyridaben		1	8.03	3

The study results of the bee honey samples, and the pesticide residues found in them are shown in Table 4. A total of 172 honey samples were studied, 156 of which were taken from the apiaries in different regions of Ukraine to detect the pesticide residues, and 16 honey samples that were taken from the combs were obtained together with the dead bees to establish the fact of the pesticide poisoning of the bees. As a result of the tests, 11 insecticides, 11 fungicides, and 2 acaricides and herbicides were found. There were 425 detection cases of insecticides, 285 fungicides, 8 acaricides, and 3 herbicides **[19]**, **[30]**.

The combined use of the different classes of pesticides (insecticides, herbicides, fungicides) causes deep concern among scientists worldwide **[15]**.

As we can see from the test results of the dead bees and bee honey, insecticides of the neonicotinoid group were quite often detected. Such data were also obtained by scientists from other countries **[22]**, **[23]**, **[24]**.

Thus, the analysis of the pesticides in the bee honey, obtained in the Western regions of Mexico, showed the presence of 14 pesticides in different concentrations in 63% of the studied samples. The pesticides most frequently found in higher concentrations were insecticides (neonicotinoids, then organophosphates), herbicides, and fungicides.

Table 4 Pesticide residues in bee honey.

Name of the active substance	Production classification	Number of detected cases	Concentration, µg/kg	Method detection limit (LOD), µg/kg
Clothianidin	Insecticides	55	0.1-12.4	0.1
Imidacloprid	Insecticides	74	0.1-43.7	0.1
Thiacloprid	Insecticides	89	0.2-190.8	0.1
Acetamiprid	Insecticides	96	0.1-510.6	0.1
Thiamethoxam	Insecticides	38	0.3-9.5	0.1
Chlorpyrifos	Insecticides	12	0.2-1.1	0.1
Dimethoate	Insecticides	22	0.1-4.5	0.1
Cypermethrin	Insecticides	4	0.8-2.2	0.1
alpha-Cypermethrin	Insecticides	5	0.6-3.4	0.1
lambda-Cyhalothrin	Insecticides	28	0.4-42.1	0.1
Tau-fluvalinate	Insecticides	2	29.2-36.9	0.1
Carbendazim	Fungicides	12	1.5-29.3	0.1
Flutriafol	Fungicides	76	0.2-4.8	0.1
Cyproconazole	Fungicides	81	0.1-112.2	0.1
Tebuconazole	Fungicides	63	0.5-2.9	0.1
Prothioconazole	Fungicides	1	102.9	0.1
Propiconazole	Fungicides	6	0.5-3.8	0.1
Difenoconazole	Fungicides	5	0.1-0.8	0.1
Epoxyconazole	Fungicides	9	0.3-1.4	0.1
Azoxystrobin	Fungicides	16	0.2-298.5	0.05
Pyraclostrobin	Fungicides	8	1.8-9.2	0.05
Picoxystrobin	Fungicides	8	2.6-16.4	0.05
Hexythiazox	Acaricides	4	1.0-3.8	0.1
Pyridaben	Acaricides	4	0.8-1.6	0.1
Promethrin	Herbicides	1	3.7	0.1
Metribuzin	Herbicides	2	2.5-6.4	0.1

These study results underline the need for continued monitoring of the pollutant substances in this product to determine the risks of pesticide exposure to the health of the pollinator, particularly the honey bees, ecosystems, and their potential consequences for human health and other nontarget organisms **[21]**, **[31]**.

Table 5 Pesticide contamination depending on species composition of bee honey.

Type of honey	Number of samples	% of total amount
poly floral	132	76.7
sunflower	15	8.7
rapeseed	12	7.0
buckwheat	7	4.1
acacia	3	1.7
honeydew	2	1.2
white	1	0.6

Among the studied honey samples in which the pesticide residues were found, the highest percentage was the bee poly floral honey – 76.7%, sunflower, and rapeseed honey – 8.7 and 7.0%, respectively. The smallest honey samples contaminated with pesticides were found in buckwheat honey – 4.1%, acacia – 1.7%, honeydew honey – 1.2%, and white honey – 0.6% (Table 5). This regularity is related to the flowering seasonality of these honey plants and the treatment of cultivated honey plants with the plant defense agents, compared with wild-growing plants. This is especially observed in the case of monofloral types of honey, such as acacia and white, which could be contaminated with pesticides in protective forest strips near the crops of the cultivated plants.

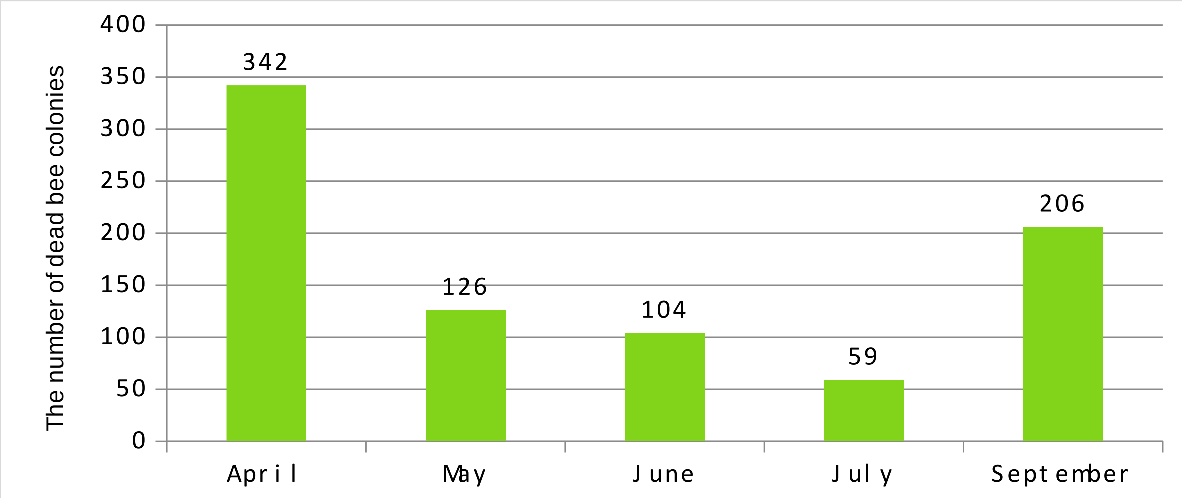

Figure 1 Number of dead bee colonies for 2021 by months.

If we consider the bee death to be seasonality, the largest number died in April and September 2021. From May to June 2021, the death of the bee colonies due to pesticide poisoning was approximately at the same level, while the lowest percentage of bee death was observed in July (Figure 1).

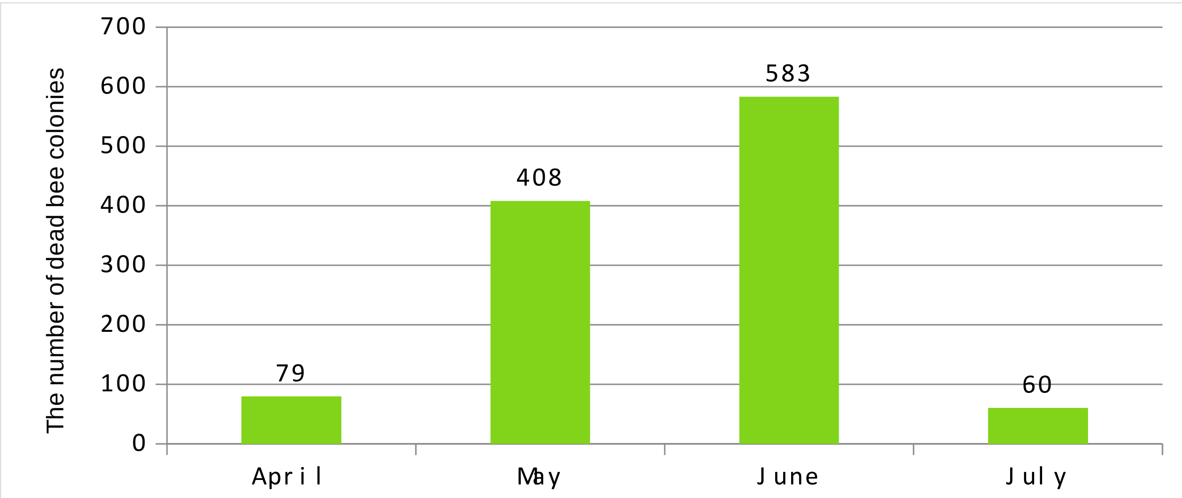

Figure 2 Number of dead bee colonies for 2022 by months.

In 2022, the highest mortality of the bee colonies was observed from May to June (Figure 2), while it was minimal in April and July, and in September, no bee samples with suspected pesticide poisoning were received in the laboratory. It relates to the weather conditions, which in the spring of 2022 were characterized by a large precipitation amount, and the adverse weather conditions in the fall, which did not contribute to the bee flight.

CONCLUSION

The use of pesticides for crops to be treated causes the poisoning of the bees during the period of the honey flow. It has been established that the use of pesticides on the territory of Ukraine causes significant death of the bee colonies and contamination of the honey. In 2021 and 2022, 837 and 1130 bee colonies died due to pesticide poisoning, respectively. The largest number of dead bee colonies in Ukraine was caused by 5 pesticides such as thiamethoxam, clothianidin, lambda-cyhalothrin, imidacloprid, and acetamiprid. The honey, which was made by the bees, contained 11 insecticides, 11 fungicides, and 2 acaricides and herbicides. It was proven that insecticides, fungicides, acaricides, and herbicides accumulated in the honey, and 16 insecticides of the 3rd toxicity class were found in the dead bees. The pesticide accumulation in bee honey depends on its species' origin and is related to the seasonal flight activity of the bees. With due regard to the wide use of plant defense agents in agricultural production, the safety risks for food products, including bee honey, are constant. Taking into consideration the fact that the bees are important subjects for the ecological balance, for the use and registration of agricultural chemicals in any country it is necessary to carry out the normative evaluation of the danger to these insects, as well as to develop the effective preventive measures for their poisoning of the bees.

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