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Pesticides as a cause of honeybee (*Apis mellifera*) mortality and their persistence in honey

Y. A. Omelchun*, L. V. Shevchenko**, L. M. Nikitina***, V. V. Solomon**, V. M. Mykhalska**, S. V. Furman****, D. V. Lisohurska****, O. V. Lisohurska****

**State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise, Kyiv, Ukraine*
***National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine*
****National Scientific Center "Institute of Beekeeping named after P. I. Prokopovich" of the National Academy of Agrarian Sciences of Ukraine, Kyiv, Ukraine*
******Polissia National University, Zhytomyr, Ukraine*

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State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise, Donetska st., 30, Kyiv, 03151, Ukraine. Tel.: +38-067-420-06-50. E-mail: my_answer@ukr.net

National University of Life and Environmental Sciences of Ukraine, Heroiiv Oborony st., 15, Kyiv, 03041, Ukraine. Tel.: +38-050-193-10-29. E-mail: shevchenko_laris@ukr.net

National Scientific Center "Institute of beekeeping named after P.I. Prokopovich" of the National Academy of Agrarian Sciences of Ukraine, Zabolotny st., 19, Kyiv, 03680, Ukraine. Tel.: +38-097-557-17-62. E-mail: lesja.nikitina@gmail.com

Polissia National University, Staryi blvd., 7, Zhytomyr, 10008, Ukraine. Tel.: +38-067-993-86-10. E-mail: svitlana.furman@ukr.net

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Recently, there has been a widespread decline in honeybee (*Apis mellifera*) colonies globally, disrupting ecological balance and reducing the pollination capacity of many entomophilous plants. One of the primary causes of bee family deaths is the increasing use of pesticides, particularly insecticides, in agricultural practice. This study aimed to identify the causes of bee family mortality in various regions of Ukraine during 2021–2022 and to determine the breakdown potential of different pesticide groups in honey. Pesticide residues in biological samples were analyzed using liquid chromatography-mass spectrometry (UPLC-MS/MS) and gas chromatography-mass spectrometry (GC-MS/MS). In the bodies of dead bees, residues of various pesticides from different chemical groups, as well as their mixtures, were detected. In most cases, the cause of bee poisoning was mixtures of pyrethroids and neonicotinoids: thiamethoxam, clothianidin, and lambda-cyhalothrin; imidacloprid, lambda-cyhalothrin, and thiamethoxam; as well as clothianidin and lambda-cyhalothrin. The primary pesticides found in honey were neonicotinoids (58.8%), triazoles (29.6%), strobilurins (6.5%), and benzimidazoles (5.1%). Most pesticide levels did not exceed the maximum allowable levels in honey. The neonicotinoids detected in honey included thiacloprid, acetamiprid, imidacloprid, clothianidin, and thiamethoxam. The triazoles detected included tebuconazole, cyproconazole, flutriafol, and epoxiconazole; strobilurins included picoxystrobin, pyraclostrobin, and azoxystrobin; and benzimidazoles included carbendazim and thiophanate-methyl. Residues of neonicotinoids, triazoles, benzimidazoles, and strobilurins in honey did not undergo degradation over 12 months of storage at 4 °C. Storing honey at 20 °C after 12 months increased the degradation of thiacloprid by 21.2%, acetamiprid by 20.7%, and flutriafol by 36.3%. Between the 9th and 12th months at 20 °C, picoxystrobin concentration decreased by 24.5–38.0%, and carbendazim concentration decreased by 80.0% to a complete breakdown of residues in honey. The degradation of thiophanate-methyl in honey at 20 °C reached 28.0% by the 6th month, 46.0% by the 9th month, and 55.4% by the 12th month. This persistence of most pesticides in honey poses a significant risk of toxic effects on bee families as well as on human health.

Keywords: neonicotinoids; triazoles; strobilurins; benzimidazoles; insecticides; fungicides; beekeeping products.

Introduction

Honeybees are an integral part of ecosystem function and are the only pollinators responsible for 35% of global food production (Khalifa et al., 2021). The economic value of insect pollination in Europe and the United States alone is estimated at several hundred billion dollars annually (Schuhmann et al., 2022). However, modern agriculture, alongside the effectiveness of pollinating entomophilous crops, increasingly depends on the use of agrochemicals due to rising global food production (Jeschke, 2024). According to statistics, approximately 5.6 billion pounds of various agrochemicals are used in agricultural production, a significant portion of which are plant protection products. By 2050, the use of agrochemicals in agriculture is expected to double, increasing potential risks and adverse effects on non-target organisms, especially honeybees (Kozak et al., 2020; Faly et al., 2023; Faly & Brygadyrenko, 2024). Worldwide, over 1,600 active substances form the basis of plant protection products, potentially accumulating in various environmental sites and posing risks to beneficial insects (Zhao et al., 2022).

The use of insecticides to treat agricultural lands is especially hazardous, as it alters terrestrial invertebrate populations, reducing their taxonomic diversity and tolerance to adverse environmental factors (Faly et al., 2023). This, in turn, endangers bees with poisoning, reduces their viability and productivity, and may even cause mass mortality. Over the past two decades, the number of honeybee colonies worldwide has sharply declined, a phenomenon known as Colony Collapse Disorder (CCD) (Leska et al., 2021). The primary cause of CCD is pesticide side effects, which, along with infectious and invasive diseases, often lead to mass mortality of honeybee colonies (Haas et al., 2021).

The toxic effects of pesticides on honeybees depend on the route of entry into the body. Some cause toxicity on contact, while others, such as neonicotinoids, are more toxic when ingested (Pashte & Patil, 2018; Hrynko et al., 2021). Among the most widely used pesticides in agricultural production are neonicotinoids, a class of neuroactive insecticides chemically similar to nicotine, first developed in the 1980s. Their mechanism involves continuous stimulation of the insects' nicotinic acetylcholine receptors in the nervous system. The neonicotinoid family includes pesticides such as acetamiprid, clothianidin, imidacloprid, nitenpyram, nithiazine, thiacloprid, and thiamethoxam. In some cases, neonicotinoid pesticides do not cause immediate bee death but instead weaken the immune and nervous systems, making the insects more susceptible to infections.

Another problem is that when pesticides do not cause immediate bee death, they enter the hive along with nectar and bee bread, contaminating bee brood and bee products (Kadlikova et al., 2021; Omelchun et al., 2023).

Compared to organophosphate and carbamate insecticides, neonicotinoids are less toxic to birds and mammals. Although the use of neonicotinoids has significantly reduced mammalian and avian toxicity over the past few decades, these pesticides still harm beneficial insects, especially bees, due to physiological similarities to pests.

Since the introduction of neonicotinoids, beekeepers have reported mass bee deaths, including disorientation, memory impairment, and decreased reproduction. In 2013, the European Union and several neighboring countries restricted neonicotinoid use on certain crops (Hrynko et al., 2021; Yang et al., 2023). In 2018, the EU banned three major neonicotinoids (clothianidin, imidacloprid, and thiamethoxam) from all outdoor uses. Some U.S. states have also limited neonicotinoid use due to concerns about their toxic effects on pollinators. Despite restrictions, seed treatment with neonicotinoids remains permitted in many countries outside the EU. Banning neonicotinoids in agriculture would increase the use of other insecticide classes, particularly pyrethroids, which are necessary to control plant disease vectors. Therefore, all EU member states are required to take measures to control agricultural pest populations using non-chemical methods wherever possible.

In Ukraine, regulatory documents governing pesticide use in agriculture and their residues in food products need review and alignment with current European legislation. Given the intensive use of pesticides for crop protection, it is essential to develop measures to prevent honeybee poisoning. This involves investigating all cases of mass bee family deaths in agricultural areas where they gather pollen and nectar from crop fields or within the flight radius of foraging bees.

Since honey and other bee products may contain pesticide residues along with residues from medicines (Bayer et al., 2017; Bayer et al., 2018), there is a risk of their synergistic toxic effects on humans. This necessitates monitoring pesticide residues not only during production but also throughout the storage period of bee products (Lamas et al., 2023). Therefore, this study aimed to quantify pesticides during investigations of mass bee deaths in different regions of Ukraine and to determine the degradation rate of detected pesticides in honey under different storage conditions.

Materials and methods

The study consisted of two stages. In the first stage, an investigation into the causes of honeybee family mortality in various regions of Ukraine was conducted over 2021–2022. Samples of dead bees and honey from apiaries in different regions,where mass bee mortality had been recorded were analyzed for pesticide residues across different groups. A total of 156 honey samples and 40 samples of dead bees were examined. The biological samples were analyzed for the presence of pesticides from the following chemical groups: pyrethroids, organophosphate insecticides, neonicotinoids, triazoles, strobilurins, and benzimidazoles.

In the second stage, honey containing pesticide residues was divided into two batches for storage. The first batch was stored at 4 °C, and the second at 20 °C for 12 months. For each pesticide, one or two concentrations most frequently found in honey were selected. The honey was analyzed for residual pesticide content one month after storage, then every three months thereafter. The tests were conducted at the gas chromatography laboratory of the Research Department of Chemical Toxicology at the State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise.

For determining the residual pesticide content in honey, an internal method was used based on the QUECHERS sample preparation approach, employing liquid chromatography-mass spectrometry (UPLC-MS/MS) and gas chromatography-mass spectrometry (GC-MS/MS) (Omelchun et al., 2022). Reagents used for the analyses were Sigma-Aldrich PH-MS grade (Chromasolv, 99.9%).

Samples of 5 g of honey and 10 g of dead bees were collected for analysis. Residual pesticide content in the honey samples was determined after solvent extraction, cleaning the extracts with dispersive solid-phase extraction, and identifying analytes using MRM (Multiple Reaction Monitoring) mode based on specific ion transitions. Quantitative pesticide content in honey was determined by the external standard method using peak area. Given that values below 1.0 µg/kg approached the detection limit of the method and considering standard deviation, only analyte concentration levels of 1.0 µg/kg or more were used for further data analysis.

The study was conducted using a "Waters Xevo TQ-XS" liquid triple quadrupole tandem mass spectrometer (Waters Corporation, Medford, USA, 2019) and a "Thermo TSQ 9000" gas triple quadrupole tandem mass spectrometer (Thermo Fisher Scientific Inc., Waltham, USA, 2022). Chromatography parameters for the "Waters Xevo TQ-XS": ionization type: ES+; ES-; UPLC system: ACQUITY UPLC I – Class; column: Acquity UPLC BEN C18, 100 x 2.1 mm x 1.7 µm (Waters, USA); column thermostat temperature: 40 ºC; injection volume: 10 µL; sample injection mode: automatic; chromatography duration: 17 minutes; software: MassLynx (Waters, USA); mobile phase: A – 2 mM ammonium formate with 0.1% formic acid in water-acetonitrile (95:5), B – 0.025% formic acid in acetonitrile.

Chromatography parameters for the "Thermo TSQ 9000": ionization type: EI – 70 eV; MS Transfer Line Temp – 300 °C; Source Temperature – 320 °C; GC system – ThermoTrace 1310; column – TG-5SILMS, 30 m x 0.25 mm x 0.25 µm (Thermo, USA); column thermostat temperature – 90 °C for 3 minutes, 25 °C/min to 180 °C, 5 °C/min to 280 °C, 10 °C/min to 300 °C for 5 minutes; injection volume: 1 µL; sample injection mode – automatic; chromatography duration – 40 minutes; software – TraceFinder 4.1 EFSGC (Thermo, USA).

During the toxicological studies on honey, residues of plant protection agents from insecticide and fungicide groups were frequently detected. In comb honey samples, the primary pesticides were neonicotinoids, with 163 occurrences, making up 58.8% of the total. Triazoles were frequently detected in honey, with 82 occurrences, comprising 29.6% of the total, while strobilurins accounted for 18 cases (6.5%). Benzimidazoles were found in 14 cases of bee deaths, representing 5.1% of the total (Table 1). The content of most detected pesticides was within the maximum allowable level (MAL) for beekeeping products, which is less than 50 µg/kg, except for benzimidazoles, which have a MAL of 1000 µg/kg. Thiacloprid is an exception, with a MAL in honey of 200 µg/kg, and in some samples, it reached this limit. Among neonicotinoid insecticides found in honey, thiacloprid, acetamiprid, imidacloprid, clothianidin, and thiamethoxam were detected. Thiacloprid and acetamiprid were the most frequently identified in honey, with concentrateons varying greatly across samples (Table 1). In some cases, acetamiprid levels significantly exceeded the MAL.

Table 1
Range of detected pesticide concentrations in honey samples

No.	Active substance	Production classification	Chemical classification	Number of cases	Concentration, µg/kg
1	Thiacloprid	insecticide	neonicotinoid	54	1.1–190.8
2	Acetamiprid	insecticide	neonicotinoid	49	1.0–510.6
3	Imidacloprid	insecticide	neonicotinoid	27	1.2–15.4
4	Clothianidin	insecticide	neonicotinoid	18	1.1–12.4
5	Thiamethoxam	insecticide	neonicotinoid	15	1.4–9.5
6	Tebuconazole	fungicide	triazole	35	1.3–22.9
7	Cyproconazole	fungicide	triazole	25	1.0–16.4
8	Flutriafol	fungicide	triazole	18	1.1–4.8
9	Epoxiconazole	fungicide	triazole	4	0.8–1.4
10	Picoxystrobin	fungicide	strobilurin	8	2.6–16.4
11	Pyraclostrobin	fungicide	strobilurin	7	1.8–9.2
12	Azoxystrobin	fungicide	strobilurin	4	0.9–2.5
13	Carbendazim	fungicide	benzimidazole	11	1.5–29.3
14	Thiophanate-methyl	fungicide	benzimidazole	4	0.7–9.4

Neonicotinoids classified as toxicity class 1 for bees were present at much lower concentrations in honey compared to class 3 compounds. The levels of imidacloprid, clothianidin, and thiamethoxam in honey confirm this. Like other fungicides such as strobilurins and benzimidazoles, triazoles are classified as toxicity class 3 for bees. The following triazoles were found in honey: tebuconazole, cyproconazole, flutriafol, and epoxiconazole. Strobilurins in honey included picoxystrobin, pyraclostrobin, and azoxystrobin, while benzimidazoles included carbendazim and thiophanate-methyl. The levels of these pesticides in honey did not exceed the MAL.

Statistical processing of the test results was conducted using one-way analysis of variance (ANOVA). The degradation intensity of certain pesticides was determined using correlation and regression analysis, with data presented as x ± SD (mean ± standard deviation) in the tables. Differences between groups were considered significant using the Tukey test at P < 0.05.

Results

Honeybee (Apis mellifera) mortality from pesticide poisoning in various regions of Ukraine. The highest honeybee colony losses in Ukraine during 2021–2022 were caused by both individual pesticides from different chemical groups, such as thiamethoxam, acetamiprid, and clothianidin, and combinations of two or three pesticides. The most frequently identified combinations included pyrethroids and neonicotinoids: thiamethoxam + clothianidin + lambda-cyhalothrin; imidacloprid + lambda-cyhalothrin + thiamethoxam; and clothianidin + lambda-cyhalothrin.

The greatest number of bee colony deaths from pesticide poisoning was recorded in Poltava (565), Vinnytsia (487), and Odesa (366) regions. Slightly fewer cases were registered in Rivne (100), Sumy (100), Kharkiv (77), and Khmelnytskyi (75) regions. The lowest numbers of honeybee colony deaths were reported in Dnipropetrovsk, Ivano-Frankivsk, Kyiv, Kirovohrad, Ternopil, and Cherkasy regions (Fig. 1).

Persistence of pesticides in honey under different storage conditions. Storing honey with neonicotinoid residues at 4 °C for 12 months did not affect their concentrations (Table 2). No correlation was found between the initial concentration of neonicotinoids – thiacloprid, acetamiprid, imidacloprid, clothianidin, and thiamethoxam – and their levels in honey throughout the storage period.

At a temperature of 20 °C, residues of neonicotinoids such as imidacloprid, clothianidin, and thiamethoxam in honey did not undergo degradation over 12 months. It was found that the 20 °C storage regime promotes an increase in the degradation rate of thiacloprid and acetamiprid, particularly at high concentrations in honey. Specifically, a reduction in thiacloprid content by 21.2% and in acetamiprid content by 20.7% was observed by the 12th month of storage compared to the initial values (Table 3).

Fig. 1. Mortality of honeybee colonies in various regions of Ukraine due to pesticide poisoning in 2021–2022: the numbers represent the frequency of pesticide detections in the bodies of dead bees: red – clothianidin, green – thiamethoxam, black – imidacloprid, dark red – acetamiprid, pink – cypermethrin, purple – alpha-cypermethrin, light purple – permethrin, blue – tau-fluvalinate, gray – chlorpyrifos, light brown – lambda-cyhalothrin; no dead bee samples for pesticide residue analysis were received in 2021–2022 from the Zakarpattia, Volyn, Lviv, Chernivtsi, Donetsk, Chernihiv, Luhansk, Mykolaiv, and Kherson regions

Table 2
Residual content of neonicotinoids in honey stored at 4 °C (x ± SD, μg/kg)

Pesticide name	n	Honey storage period, months					
		initial concentration	1	3	6	9	12
Thiacloprid	4	23.35 ± 1.70	23.35 ± 2.88	23.93 ± 1.18	23.45 ± 2.37	22.91 ± 2.48	23.91 ± 2.34
	4	177.23 ± 14.24	175.37 ± 14.21	172.76 ± 11.41	176.47 ± 18.79	173.72 ± 9.50	173.29 ± 12.16
Acetamiprid	13	3.17 ± 0.71	3.12 ± 0.65	3.02 ± 0.66	3.11 ± 0.81	3.13 ± 0.88	3.09 ± 0.69
	4	22.08 ± 1.38	22.18 ± 0.63	21.37 ± 1.07	21.42 ± 2.32	22.83 ± 0.57	22.58 ± 1.45
Imidacloprid	6	6.97 ± 1.32	6.91 ± 1.34	7.13 ± 1.42	7.03 ± 1.52	6.85 ± 1.29	6.38 ± 1.14
Clothianidin	5	7.16 ± 1.35	7.38 ± 1.37	7.55 ± 1.19	7.13 ± 1.53	6.78 ± 1.41	6.68 ± 1.33
Thiamethoxam	7	6.77 ± 1.62	6.46 ± 1.53	6.79 ± 1.85	6.54 ± 1.31	6.51 ± 1.32	6.76 ± 1.51

Note: no significant differences were found between the values.

The residual content of triazoles, specifically tebuconazole, cyproconazole, flutriafol, and epoxiconazole, in honey stored at 4 °C for 12 months did not undergo significant changes. The initial concentration of tebuconazole and cyproconazole in honey also did not affect their degradation rate (Table 4).

Storing honey containing triazole residues at 20 °C for 12 months did not affect the levels of tebuconazole, cyproconazole, and epoxiconazole. However, a 36.3% degradation of flutriafol in honey was observed by the 12th month of storage compared to the initial concentration (Table 5).

Residual content of strobilurins in honey stored at 4 °C for 12 months strobilurins, including picoxystrobin, pyraclostrobin, and azoxystrobin, did not exhibit significant changes in residual content in honey stored at 4 °C for 12 months (Table 6).

Table 3
Residual content of neonicotinoids in honey stored at 20 °C (x ± SD, μg/kg)

Pesticide name	n	Honey storage period, months					
		initial concentration	1	3	6	9	12
Thiacloprid	4	23.35 ± 1.70^{a}	24.83 ± 1.49^{a}	24.88 ± 2.41^{a}	23.40 ± 3.95^{a}	22.05 ± 4.35^{a}	20.63 ± 2.95^{a}
	4	177.23 ± 14.24^{a}	178.81 ± 14.23^{a}	167.07 ± 2.88^{a}	180.47 ± 2.83^{a}	170.62 ± 2.51^{a}	139.73 ± 7.84^{b}
Acetamiprid	13	3.17 ± 0.71^{a}	3.25 ± 0.66^{a}	3.04 ± 0.73^{a}	3.01 ± 0.54^{a}	2.74 ± 0.56^{a}	2.52 ± 0.62^{a}
	4	22.08 ± 1.38^{a}	22.05 ± 2.21^{a}	22.33 ± 1.85^{a}	21.43 ± 1.65^{a}	19.80 ± 1.51^{ab}	17.50 ± 0.96^{b}
Imidacloprid	6	6.97 ± 1.32^{a}	7.03 ± 1.25^{a}	6.98 ± 1.31^{a}	7.01 ± 0.93^{a}	6.52 ± 1.26^{a}	6.37 ± 1.18^{a}
Clothianidin	5	7.16 ± 1.35^{a}	7.14 ± 1.16^{a}	7.68± 0.96^{a}	7.02 ± 1.58^{a}	6.66 ± 1.27^{a}	6.10 ± 1.34^{a}
Thiamethoxam	7	6.77 ± 1.62^{a}	6.44 ± 2.01^{a}	6.03 ± 0.78^{a}	7.36 ± 1.48^{a}	6.87 ± 1.74^{a}	6.14 ± 1.73^{a}

Note: different superscript letters indicate values that significantly differ within the same row of the table ($P < 0.05$) based on comparisons using the Tukey test.

Table 4
Residual content of triazoles in honey stored at 4 °C (x ± SD, μg/kg)

Pesticide name	n	Honey storage period, months					
		initial concentration	1	3	6	9	12
Tebuconazole	7	13.03 ± 2.66	12.94 ± 2.93	12.87 ± 3.35	13.19 ± 2.67	12.43 ± 2.75	12.37 ± 2.27
	5	20.82 ± 1.81	20.02 ± 1.56	20.91 ± 1.83	21.37 ± 2.58	20.47 ± 2.76	18.73 ± 3.01
Cyproconazole	5	8.33 ± 1.07	9.23 ± 1.54	8.27 ± 0.97	8.17 ± 1.65	8.10 ± 0.72	8.47 ± 0.73
	5	14.70 ± 1.75	14.53 ± 3.31	14.39 ± 2.23	14.03 ± 1.69	14.87 ± 1.48	13.97 ± 1.33
Flutriafol	12	2.78 ± 0.31	2.74 ± 0.78	2.77 ± 0.89	2.91 ± 1.03	2.80 ± 0.84	2.57 ± 0.66
Epoxiconazole	4	1.18 ± 0.26	1.13 ± 0.17	1.18 ± 0.31	1.33 ± 0.43	1.19 ± 0.32	1.13 ± 0.21

Note: no significant differences were found between the values.

Table 5
Residual content of triazoles in honey stored at 20 °C (x ± SD, μg/kg)

Pesticide name	n	Honey storage period, months					
		initial concentration	1	3	6	9	12
Tebuconazole	7	13.03 ± 2.66^{a}	13.49 ± 2.68^{a}	13.51 ± 2.69^{a}	12.84 ± 2.55^{a}	11.73 ± 2.53^{a}	11.24 ± 2.57^{a}
	5	20.82 ± 1.81^{a}	21.27 ± 2.15^{a}	21.70 ± 2.51^{a}	21.69 ± 3.16^{a}	20.53 ± 2.93^{a}	18.67 ± 2.84^{a}
Cyproconazole	5	8.33 ± 1.07^{a}	8.41 ± 1.04^{a}	8.67 ± 1.03^{a}	7.63 ± 0.50^{a}	7.57 ± 0.68^{a}	6.90 ± 0.69^{a}
	5	14.70 ± 1.75^{a}	14.87 ± 2.11^{a}	15.13 ± 1.42^{a}	14.01 ± 1.87^{a}	13.32 ± 1.71^{a}	12.07 ± 1.59^{a}
Flutriafol	12	2.78 ± 0.31^{a}	2.67 ± 0.82^{ab}	2.71 ± 0.81^{ab}	2.57 ± 0.79^{ab}	2.16 ± 0.77^{ab}	1.77 ± 0.38^{b}
Epoxiconazole	4	1.18 ± 0.26^{a}	1.25 ± 0.34^{a}	1.11 ± 0.33^{a}	1.01 ± 0.23^{a}	0.88 ± 0.25^{a}	0.78 ± 0.17^{a}

Note: see Table 3.

Table 6
Residual content of strobilurins in honey stored at 4 °C (x ± SD, μg/kg)

Pesticide name	n	Honey storage period, months					
		initial concentration	1	3	6	9	12
Picoxystrobin	5	4.32 ± 0.58	3.54 ± 0.61	3.90 ± 0.55	3.21 ± 0.56	3.64 ± 0.45	3.16 ± 0.47
Pyraclostrobin	4	7.98 ± 1.14	7.87 ± 1.39	8.91 ± 1.54	8.49 ± 1.38	7.63 ± 1.19	7.96 ± 1.28
Azoxystrobin	4	1.85 ± 0.79	1.77 ± 0.72	1.93 ± 0.82	1.88 ± 0.83	1.68 ± 0.76	1.93 ± 0.67

Note: no significant differences were found between the values.

Storage of honey at 20 °C did not affect the degradation of pyraclostrobin or azoxystrobin over 12 months. However, a notable decrease in picoxystrobin concentration was observed: a reduction of 24.5% by the 9th month and 38.0% by the 12th month compared to the initial value (Table 7). The residual content of benzimidazoles, specifically carbendazim and thiophanate-methyl, in honey stored at 4 °C did not change significantly during 12 months of storage (Table 8). Increasing the storage temperature of honey to 20 °C accelerated the degradation of carbendazim, reducing its content by 80.0% by the 9th month, and leaving no detectable residues by the 12th month (Table 9). Thiophanate-methyl also exhibited a higher degradation rate at 20 °C, with its concentration decreasing by 28.0% by the 6th month, 46.0% by the 9th month, and 55.4% by the 12th month compared to the initial concentration.

Correlation and regression analysis indicates that the residual content of carbendazim in honey exhibits a strong inverse relationship with the storage duration at 20 °C ($r = –0.832 \pm 0.051$, $P < 0.01$). The regression line confirms a linear inverse relationship between the residual content of carbendazim and the storage duration of honey (Fig. 2).

Table 7
Residual content of strobilurins in honey stored at 20 °C (x ± SD, μg/kg)

Pesticide Name	n	Honey storage period, months					
		initial concentration	1	3	6	9	12
Picoxystrobin	5	4.32 ± 0.58^{a}	3.44 ± 0.45^{ab}	3.38 ± 0.37^{b}	3.64 ± 0.55^{ab}	3.26 ± 0.48^{b}	2.68 ± 0.41^{b}
Pyraclostrobin	4	7.98 ± 1.14^{a}	7.78 ± 1.32^{a}	8.63 ± 1.27^{a}	8.53 ± 1.38^{a}	8.01 ± 0.93^{a}	6.97 ± 1.32^{a}
Azoxystrobin	4	1.85 ± 0.79^{a}	2.05 ± 0.99^{a}	1.94 ± 0.93^{a}	1.75 ± 0.68^{a}	1.66 ± 0.74^{a}	1.38 ± 0.37^{a}

Note: see Table 3.

Table 8
Residual content of benzimidazoles in honey stored at 4 °C (x ± SD, µg/kg)

Pesticide name	n	Honey storage period, months					
		initial concentration	1	3	6	9	12
Carbendazim	6	2.01 ± 0.37	1.88 ± 0.44	2.05 ± 0.49	2.11 ± 0.28	1.75 ± 0.19	1.79 ± 0.43
Thiophanate-methyl	3	6.43 ± 0.71	6.52 ± 0.75	6.33 ± 0.51	5.72 ± 0.63	5.47 ± 0.86	6.22 ± 0.74

Note: no significant differences were found between the values.

Table 9
Residual content of benzimidazoles in honey stored at 20 °C (x ± SD, µg/kg)

Pesticide name	n	Honey storage period, months					
		initial concentration	1	3	6	9	12
Carbendazim	6	2.01 ± 0.37^{a}	1.93 ± 0.66^{a}	1.62 ± 0.67^{a}	1.33 ± 0.74^{a}	0.40 ± 0.29^{b}	0.00 ± 0.00^{b}
Thiophanate-methyl	3	6.43 ± 0.71^{a}	6.02 ± 0.51^{a}	5.57 ± 0.64^{a}	4.63 ± 0.51^{b}	3.47 ± 0.22^{b}	2.87 ± 0.27^{b}

Note: see Table 3.

The residual content of thiophanate-methyl in honey shows a moderate inverse relationship with storage duration at 20 °C ($r = –0.644 \pm 0.101$, $P < 0.01$). Regression analysis indicates a linear relationship between the residual content of thiophanate-methyl and the storage duration of honey (Fig. 3).

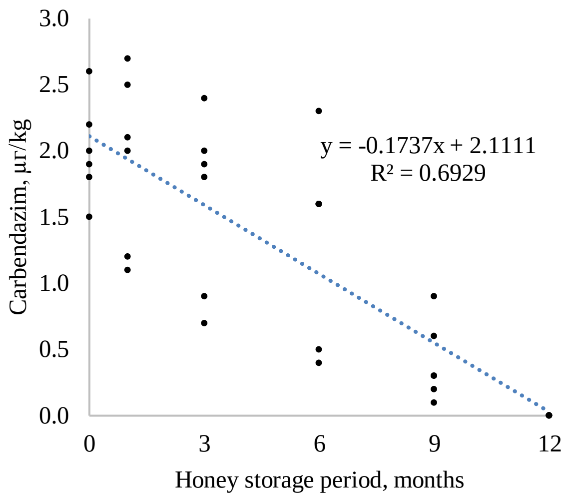

Fig. 2. Dependence of residual carbendazim content in honey stored at 20 °C, n = 36

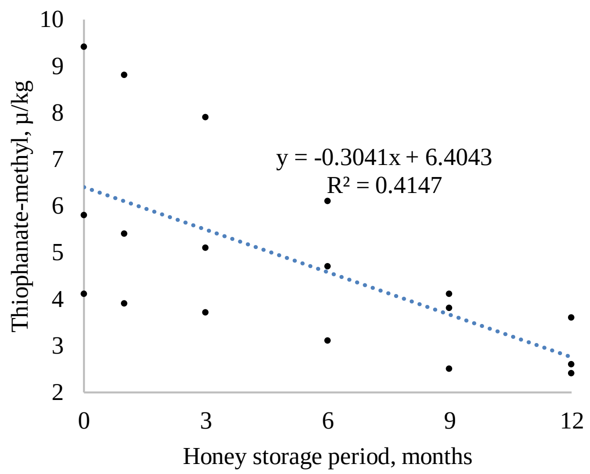

Fig. 3. Dependence of residual thiophanate-methyl content in honey stored at 20 °C, n = 18

Discussion

The death of the honeybee (A. mellifera) due to pesticide poisoning in different regions of Ukraine. It has been found that between 2006 and 2015, the number of honeybee colonies worldwide decreased by 25% (Zattara & Aizen, 2021). The mass death of bee colonies on various continents raises concerns about the disruption of the biodiversity balance of beneficial insects and the emerging risks of ecological catastrophe in the near future due to human economic activity (Abay et al., 2023). Among the main causes of honeybee colony deaths, pesticides from various groups and classes, widely used in agricultural practice, play an important role. To reduce the risks of bee deaths from poisoning, pesticide manufacturers are required to assess the harmfulness of their products to bees. Regulation (EC) No. 1107/2009 of the European Parliament and Council of 21 October 2009 on the placing of plant protection products on the market sets out the framework for actions by the Community to achieve sustainable pesticide use and is the main legislative act in the European Union governing issues related to the comprehensive protection of pollinating insects, including honeybees, from the harmful effects of pesticides (Leska et al., 2021).

It is also impossible to ignore the fact that agricultural production without the use of plant protection products can increase crop losses by up to 80%. Therefore, although some countries are introducing restrictions on the use of pesticides, they are still used and will continue to be used in the near future due to the lack of reliable alternatives. One of the most common pesticides used for plant protection is neonicotinoids, which belong to the neurotoxic class of insecticides (Zuščíková et al., 2023; Rondeau & Raine, 2024). Neonicotinoids act as agonists of nicotinic acetylcholine receptors (nAChR) and are used in urban landscaping, veterinary medicine, and for crop protection in agriculture (Dukhnytskyi et al., 2024). This group of chemicals targets parasitic bloodsucking insects, soil insects, and pests that feed on agricultural crops. In veterinary medicine, they are used to kill ectoparasites in domestic animals. The application methods of neonicotinoids are varied, but the most common are soil or seed treatment for agricultural crops (Tasman et al., 2021).

Our research has shown that neonicotinoids, both alone and in combination with other pesticide groups, are widely used and accumulate in the environment, causing the death of honeybee colonies in most regions of Ukraine. This is consistent with similar studies by other researchers (Wang et al., 2022; Hisamoto et al., 2024). Despite low doses, neonicotinoids exhibit toxic effects both acutely and chronically. It has been established that 1/5 of the LC_{50} of thiamethoxam, which belongs to this pesticide group, significantly impairs learning and memory ability after both acute and chronic exposure in *A. cerana* (Shi et al., 2023).

The results of our studies confirm the toxic effect of neonicotinoid insecticides, and in some cases in synergy with fungicides, on plant pollinators – honeybees (*A. mellifera*). Moreover, the results of the study of 156 honey samples from different regions of Ukraine show the presence of various plant protection product residues commonly used in modern agriculture. Contact between bees and pesticides in the environment, in their various combinations, is undoubted (Crall & Raine, 2023; Raine et al., 2024). This is also confirmed by research on pollen, where 79 different pesticides were found. Insecticides were found more frequently in pollen than fungicides or herbicides, and up to six compounds could be present in one mixture (Ostiguy et al., 2019). The impact of mixtures of pesticides from different groups on bees may be due to the intentional mixing of various pesticides in application tanks for crop treatments, but most often it results from their contact with different pesticides during foraging and flying activities. Pesticide-treated arable lands, including fruit orchards, technical crops, or vegetable fields, can increase both the quantity and diversity of pesticides to which bees are exposed through nectar, pollen, and soil (Graham et al., 2021).

During toxicological studies of selected honey samples, the most common pesticide residues found were from the neonicotinoid class, which belongs to the group of insecticides, as well as fungicides such as triazoles, strobilurins, and benzimidazoles, consistent with findings from other researchers (Zioga et al., 2023).

Our data also align with the findings of Anson et al. (2020), which indicate that the most common cause of bee poisoning is neonicotinoids, followed by triazoles, with the remaining cases attributed to strobilurins and benzimidazoles. According to data from Chmiel et al. (2020), the LD_{50} for neonicotinoids in oral intake, adjusted for bees, is 0.003 µg for clothianidin, 0.05 µg for imidacloprid, 11.81 µg for acetamiprid, and 19.9 µg for thiacloprid.

Most pesticides detected in honey from apiaries did not exceed the maximum allowable level, except for thiacloprid and benzimidazoles – carbendazim and thiophanate-methyl. This is because these compounds are less toxic to bees, and insects typically bring them into the hive in nectar and pollen. The harmfulness of low doses of pesticide mixtures has been confirmed, indicating the potential for long-term effects that may lead to an increase in bee lifespan, but also slow down and reduce the efficiency of worker bees (Prado et al., 2019; Gándara et al., 2024). In cases of poisoning by pesticides classified in the first toxicity class, bees die on their way to the apiary or within the apiary itself, with only non-lethal doses of pesticides reaching the honey. Among neonicotinoid insecticides, which are classified in the first toxicity class for bees, we recorded thiacloprid, acetamiprid, imidacloprid, clothianidin, and thiamethoxam at much lower concentrations in honey than those from the third toxicity class. The concentrations of imidacloprid, clothianidin, and thiamethoxam were almost at the same level. Thiacloprid and acetamiprid were most frequently detected in honey. It is worth noting that, despite acetamiprid being significantly less toxic than clothianidin and imidacloprid (Chmiel et al., 2020), we found evidence of its involvement in honeybee mortality.

Triazoles, like other fungicides – strobilurins and benzimidazoles – are classified in the third toxicity class for bees. During our honey analysis, we detected several triazole compounds: tebucanazole, cyproconazole, flutriafol, and epoxiconazole. From the strobilurin group, we identified picoxystrobin, pyraclostrobin, and azoxystrobin, while from the benzimidazole group, we detected carbendazim and thiophanate-methyl. As we can see, the levels of these analytes did not exceed the maximum permissible residues for bee products. Oral and contact LD_{50} concentrations for fungicides are generally four orders of magnitude higher than the concentrations of fungicides detected in bee feeds available in the commercial market. Therefore, fungicides are considered harmless to honeybees, though their toxic effect has been confirmed in several studies (Christen, 2023). However, there is evidence that fungicides, which inhibit the sterol biosynthesis pathway, may greatly increase the toxicity of neonicotinoids and pyrethroids (Schuhmann et al., 2022), especially when they enter the bodies of bees in mixtures (Knuth et al., 2024).

Persistence of pesticides in honey under different storage conditions. The physical and chemical stability of pesticides in the ecosystem is well-documented in the literature (Mishra et al., 2022; Lamas et al., 2023). Most literature focuses on the potential use of pesticides, their classification according to their properties and toxicity, and their adverse impacts on natural systems such as soil, water, plants (growth, metabolism, genotype and phenotype changes, and impact on defense systems), human health (genetic changes, cancer, allergies, and asthma), as well as in preserved food products (Pathak et al., 2022). The issue of pesticide stability in bee products, particularly honey during storage, has not been fully explored. This is an especially relevant and promising area of research, given the synergistic toxicity of pesticides to living organisms, including humans (Ashraf et al., 2023). During storage, natural physical-chemical processes occur in honey, which can change its organoleptic properties, such as crystallization, separation, and color changes. It should be noted that these changes in honey do not diminish its biological and nutritional value, and its high acidity, sugar content, and low moisture ensure that it can be stored for extended periods (Ashraf et al., 2023). Thus, if stored properly, honey can remain intact for decades. Considering the specifics of honey storage conditions, it can be concluded that honey is almost impervious to the main physical factors of spoilage – light, oxygen, and moisture. General recommendations for honey storage suggest temperatures from –6 to +20 °C. It is important to note that the most optimal temperature range for long-term honey storage is from –6 to +4 °C. In this context, a significant scientific interest lies in the influence of temperature regimes on the stability of pesticide residues in honey.

It is important to note that over 12 months of storage at +4 °C, all the pesticides from the groups of neonicotinoids, triazoles, strobilurins, and benzimidazoles detected in honey maintained stable concentrations, indicating that under these conditions, the toxicological safety of honey remains almost unchanged.

The stability of pesticides depends on the physicochemical properties of their molecules and the composition of food products. In agricultural practice, the stability of pesticides is determined by their half-life, which varies depending on the nature of the pesticide and can range from hours to years. Pesticides can decompose through various mechanisms, such as hydrolysis, reduction, oxidation, microbial metabolism, or changes in temperature and pH levels. There is very little data on the half-life of pesticides in honey, and the available information does not provide objective data on the toxicological safety of honey during storage. However, several researchers claim that the predicted degradation of pesticides in soils occurs much more slowly than previously thought, and they exhibit significant persistence (Froger et al., 2023). Overall, residues of about 80% of all applied pesticides have been detected in soils, with half of them identified as degradation products that persist for more than ten years (Aurea et al., 2017).

It is believed that hydrophilic compounds, including neonicotinoids, may accumulate in honey. Neonicotinoids are chemically stable and typically remain in the environment for extended periods, raising concerns about their neurotoxicity for both bee colonies and humans (Ramirez-Cando et al., 2023; Averill et al., 2024).

At a storage temperature of 20 °C, partial degradation of pesticides from nearly all the groups we studied occurred, though in some cases, it was minimal, which does not guarantee the complete removal of toxic residues from honey. From the neonicotinoid group found in honey, only thiacloprid and acetamiprid underwent partial degradation, but only after 12 months and at high concentrations, while in samples with low pesticide content, this degradation was less noticeable. Imidacloprid, clothianidin, and thiamethoxam did not degrade in honey at 20 °C, even after 12 months. Among the triazoles, we observed a similar pattern as with neonicotinoids: at 20 °C, tebucanazole, cyproconazole, and epoxiconazole did not degrade, unlike flutriafol, which showed degradation at the 12-month mark.

The residues of strobilurins in honey at 20 ^{0}C exhibited high stability, with the exception of picoxystrobin, which started degrading from the 9th month of honey storage. However, by the 12th month, residues of picoxystrobin were still present in the honey.

It is worth noting that among all the pesticide groups studied, benzimidazoles underwent the fastest and most significant regression, particularly when stored at 20 ^{0}C The residual content of thiophanate-methyl in honey began to decrease from the 6th month of storage and continued until the 12th month. The most intense degradation was observed for carbendazim, whose content sharply decreased starting from the 9th month, and by the 12th month, its concentration reached the detection limit.

Conclusions

Among the main causes of mass honeybee colony deaths in Ukraine during 2021–2022 were pesticide poisoning, with the following groups involved: neonicotinoids, triazoles, strobilurins, benzimidazoles, pyrethroids, and organophosphorus insecticides. The most frequent cause of bee deaths was the mixture of two or more pyrethroids and neonicotinoids in various ratios. The highest number of bee colonies killed was found in the following regions: Poltava – 565, Vinnytsia – 487, and Odesa – 366. In Rivne and Sumy regions, 100 bee colonies were lost, in

Kharkiv – 77, and in Khmelnytskyi – 75. The honey from the bee colonies primarily contained residues of plant protection products from the groups of insecticides (neonicotinoids) and fungicides (triazoles, strobilurins, and benzimidazoles). Neonicotinoids made up 58.8% of the pesticide content in honey, triazoles – 29.6%, strobilurins – 6.5%, and benzimidazoles – 5.1%. Most of the pesticides detected in the honey were within the maximum allowable limits, with the exception of acetamiprid, whose residue in some cases exceeded the maximum allowable level.

Residues of neonicotinoids, triazoles, strobilurins, and benzimidazoles in honey at 4 ^{0}C remained stable throughout 12 months of storage. Under conditions of 20 ^{0}C, imidacloprid, clothianidin, and thiamethoxam remained stable in honey over 12 months. At 20 ^{0}C, the degradation of thiacloprid increased by 21.2% and acetamiprid by 20.7% by the 12th month of storage. Triazoles such as tebuconazole, cyproconazole, and epoxiconazole showed no degradation in honey at 20 ^{0}C whereas flutriafol degraded by 36.3% by the 12th month.

Residues of strobilurins, particularly pyraclostrobin and azoxystrobin, did not undergo degradation in honey at 20 ^{0}C over 12 months, with the exception of picoxystrobin, which degraded by 38.0% from the 9th to the 12th month of storage. Benzimidazoles showed degradation at 20 ^{0}C in honey, with an inverse linear relationship to the storage time. Carbendazim degraded by 80.0% by the 9th month at 20 ^{0}C, and by the 12th month, it had completely degraded. Thiophanate-methyl began to degrade from the 6th to the 12th month by 55.4% compared to the initial value.

The study results indicate the need for monitoring pesticide residues in the bodies of dead bees and honey during investigations of mass bee colony deaths. The obtained data justify the search and development of reliable biopreparations as an alternative to chemical pesticides in agriculture.

The authors declare that they have no potential conflict of interest.

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