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# Accumulation of a multi-component mixture of pesticides in soil, plants, bee organisms, and beekeeping products

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Environmental pollution worldwide is systemic in nature and is associated with the use of pesticides from various groups as plant protection products. Agricultural pesticides accidentally affect beneficial, non-target insects, particularly the honeybee (*Apis mellifera* L.), which leads to mass poisoning and contamination of beekeeping products with toxic compounds. The aim of the research was to determine the accumulation of pesticides from different groups in soil, plants, dead bees, and beekeeping products in apiaries in various regions of Ukraine during the mass deaths of bee colonies in 2021–2022. Pesticide content in biological samples was determined using liquid mass spectrometry (UPLC-MS/MS) and gas mass spectrometry (GC-MS/MS). The main pesticides that contaminate the soil, plants, and beekeeping products, causing the death of bee colonies, are insecticides and fungicides in various combinations. In isolated cases, bee colony deaths were registered from a single insecticide. More often, two or more pesticides were found in the soil, plants, dead bees, and beekeeping products. The total list of detected pesticides in soils, grain, plant biomass, bees, brood, bee bread, and honey included 23 compounds. The maximum number of pesticides forming a cocktail in biological samples included 5 substances. Most frequently, pesticide combinations that contaminated the soil and plants and caused bee deaths included insecticides from the pyrethroid group: lambda-cyhalothrin and cypermethrin, and from the neonicotinoid group: clothianidin and imidacloprid, as well as fungicides from the triazole group: tebuconazole, cyproconazole, and strobilurins: azoxystrobin in various concentrations. The cause of bee poisoning was the contamination of the inflorescences of non-target crops, such as maple and meadow grasses, as well as wind-pollinated and self-pollinated crops, including wheat, peas, soybeans, and potatoes, located within the flight radius of bees. Among entomophilous plants, rapeseed and sunflower were the main crops, the pesticide treatment of which contributed to soil contamination, plant biomass, and bee poisoning during honey collection. It was found that the concentration of certain pesticides in dead bees reached, and in some cases exceeded, the acute oral toxic dose $LD_{50}$ by tens of times. The results of the research could form the basis for determining the cumulative toxicity of combinations of different pesticides for beneficial insects and for developing biological plant protection products.

*Keywords*: pyrethroids; neonicotinoids; triazoles; strobilurins; insecticides; fungicides; honey; bee bread.

## Introduction

One of the significant problems of today is the disruption of the ecological balance on the planet due to contamination of soil, water, and air with toxicants of various origins, including pesticides. Among the consequences of such disruptions is the mass death of pollinators of entomophilous plants, particularly the honeybee, which is responsible for pollinating approximately 35% of global agricultural production (Papa et al., 2022). In recent decades, there has been a significant decline in the population of honeybees in many regions of the world, including Asia, the USA, and Europe (Chatzaki et al., 2023; Choi et al., 2024).

Poisoning of honeybees by pesticides is most often associated with foraging, which involves daily visits to about 2,000 flowering plants within a 5.5 km radius from the apiary (Abbasi et al., 2021). Despite the growing concern among scientists and practitioners about the decline in honeybee populations, the full scale of the circulation of pesticides and their toxic effects in the environment, not only from individual pesticides but also from their combinations, remains unclear. Pesticides are widely used as plant protection products, which has led to their significant spread in nature, and they are found in all environmental objects in various combinations, concentrations, and proportions (Cang et al., 2023). Currently, over 1,100 plant protection products (mostly fungicides, insecticides, and herbicides) are on the European market (El-Nahhal, 2020). These pesticides destroy living organisms and cause continuous harm to ecosystems, as they often do not reach the target species (Faly et al., 2023) or are accidentally targeted at beneficial insects, such as pollinators or even hydrobionts. It has been proven that between 80 and over 160 different plant protection products are detected in the bodies of bees and beekeeping products (He et al., 2023), with the primary ones being insecticides and fungicides (Omelchun et al., 2023). This indicates the constant influx of pesticide cocktails not only into the bodies of worker bees, which come into contact with them while foraging, but also other colony members who consume this food both during the development of larvae and in adulthood. Moreover, the contamination of beekeeping products with pesticides, which are food products and components of medicinal products for humans, creates an additional risk in ensuring the concept of One Health.

Pesticides can enter the bodies of honeybees through various pathways, including contact – when the toxic substance directly comes into contact with the body surface during the spraying of crops – and oral – through food and/or water during collection or consumption. It is believed that the main route of pesticide entry into bees' bodies is oral, through pollen, nectar, and guttation droplets produced by plants. This process is significantly influenced by the ability of plant protection products to accumulate in high concentrations not only in flowers after crop treatment but also after systemic treatments such as seed coating (Achimón et al., 2024). Systemic pesticides are absorbed by plants and transported into pollen, nectar, and guttation droplets. Since most plant protection products are highly persistent in the soil and groundwater, they can accumulate in the pollen and nectar of not only agricultural crops but also nearby plants, particularly trees and shrubs in shelterbelt areas. Pesticide residues can migrate, dissolve or wash away in irrigation waters, rivers, marshes, small puddles, and fishponds (El-Nahhal & El-Nahhal, 2021). This creates a circulation of pesticides in the environment that poses a threat not only to ecological systems but also to human health.

Recently, more and more scientists recognize that various combinations of pesticides, rather than individual pesticides, are often used as plant protection products (Nassar et al., 2024). This is due to the need to overcome the resistance of certain pathogens and pests to plant protection products. Additionally, the degradation of pesticide residues from different groups in the environment is prolonged, leading to the accumulation of not only the pesticides themselves but also their metabolites in the soil, water, and plants, and as a result, their entry into the bodies of bees (Lichtenstein et al., 2021). It should be noted that honeybees, collecting contaminated nectar and pollen from different plants, receive a cocktail of pesticides with varying concentrations and toxicity both temporally and spatially.

It is important to note that most ecotoxicological assessments and chemical risk assessments concern the impact of individual pesticides on different biological systems. However, the behavior of different chemical groups in mixtures may not correspond to the predictions regarding individual pesticides in various environmental objects. This process is influenced by the interactions of components in a complex mixture, which can cause unpredictable changes in both living organisms and the soil, water, and air (Lichtenstein et al., 2021).

The complexity of determining the cause of poisoning and death of honeybees is also due to the fact that foraging bees travel long distances, crossing various agricultural lands and mixed landscapes, encountering numerous pesticides even during a single foraging period. During this time, they may come into contact with both individual pesticides along their foraging path and their mixtures in a single flower (Ma et al., 2024), a single puddle from which they drink, or aerosols they encounter during crop spraying. Honeybees can accumulate dozens of different pesticides in varying concentrations (Keodara et al., 2024), and it is difficult to predict which one will be lethal. These mixtures are either directly collected by foragers from pollen or nectar, or are fed to the brood or colony inside the hive, which may have long-term effects. Despite the high likelihood of the impact of pesticide mixtures, honeybees have rarely been tested for the adverse effects of their combinations, and the sources of these chemicals entering the bees' bodies are not always clearly identified. Furthermore, investigations into bee colony deaths primarily focus on studying pesticide residues in dead bees and beekeeping products, while the soil and plants that are the sources of these protection products are not always considered, making it difficult to objectively assess the risk to the ecological system.

The most common pesticide cocktail responsible for bee deaths consists of a combination of insecticides and fungicides, as both groups are often used to treat agricultural crops either sequentially with certain intervals or simultaneously in mixtures. In this case, it is necessary to assess the actual risk of combining and potentially accumulating pesticide mixtures in soils, plants, bee bodies, and beekeeping products. Therefore, the aim of the research was to determine the contamination of soil, grain, green mass of target and non-target plants, the bodies of bees, and beekeeping products when investigating cases of mass honeybee colony deaths in various regions of Ukraine.

**Materials and methods**

The study results were obtained during investigations into the causes of mass honeybee colony deaths in various regions of Ukraine. These cases were reported to the laboratory of the State Scientific Research Institute of Laboratory Diagnostics and Veterinary-Sanitary Expertise during 2021–2022.

Samples analyzed included dead bees, brood, bee bread, pollen loads, and honey from apiaries experiencing mass colony losses. Additionally, soil, grain, and green plant material samples from areas within the foraging radius of worker bees were examined. Pesticides were confirmed in 181 biological material samples.

All analyses were conducted in the gas chromatography laboratory of the Research Chemical-Toxicology Department at the State Scientific Research Institute of Laboratory Diagnostics and Veterinary-Sanitary Expertise in Kyiv, Ukraine. Pesticide content in biological samples was determined using an in-house method based on the QUECHERS sample preparation approach. Liquid chromatography-mass spectrometry (UPLC-MS/MS) and gas chromatography-mass spectrometry (GC-MS/MS) techniques were employed (Omelchun et al., 2022). All reagents used for pesticide residue analysis were of LC-MS grade (Chromasolv, 99.9%) from Sigma-Aldrich.

Samples analyzed included 5 g each of soil, grain, green plant material, honey, and bee bread, and 10 g of dead bees and brood. Pesticide residues were identified following extraction with a solvent and subsequent purification of the extracts using dispersive solid-phase extraction. Analyte identification utilized the MRM (multiple reaction monitoring) mode based on specific ion transitions. Quantitative pesticide analysis in biological samples was performed using peak area and an external standard method. The detection limit for pesticides in biological material samples was 0.1 μg/kg.

For the analyses, a liquid three-quadrupole tandem mass spectrometer "Waters Xevo TQ-XS" (Waters Corporation, Medford, USA, 2019) was used. Chromatographic parameters: 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; chromatographic run time: 17 minutes; software: MassLynx (Waters, USA); mobile phase: A: 2 mM ammonium formate with 0.1% formic acid in water-acetonitrile solution (95:5), B: 0.025% formic acid in acetonitrile.

Individual studies of pesticide content in biological samples were carried out using a gas three-quadrupole tandem mass spectrometer "Thermo TSQ 9000" (Thermo Fisher Scientific Inc., Waltham, USA, 2022). Chromatographic parameters: 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, hold for 5 minutes; injection volume: 1 μL; sample injection mode: automatic; chromatographic run time: 40 minutes; software: TraceFinder 4.1 EFSGC (Thermo, USA).

Statistical processing of the obtained results was performed using one-way analysis of variance. Data in the tables are presented as x ± SD (mean ± standard deviation).

**Results**

In the soil, grain, plant, dead bee, and beekeeping product samples from different regions of Ukraine during 2021–2022, 23 pesticides were identified, belonging to various groups of agricultural products. The majority of the pesticides responsible for the mass death of bee colonies were insecticides from the pyrethroid and neonicotinoid groups, as well as fungicides from the triazole and strobilurin groups (Table 1). Among the pyrethroids, lambda-cyhalothrin was found most frequently – in 33 cases, and cypermethrin – in 13 cases; from the neonicotinoids group, clothianidin – in 30 cases, and imidacloprid – in 7 cases. The main representatives of the triazole group were tebuconazole – 21 cases, cyproconazole – 11 cases, and from the strobilurin group, azoxystrobin – 14 cases. Most of the pesticides were found in biological material samples from the Cherkasy, Poltava, Vinnytsia, Kharkiv, Ivano-Frankivsk, Odesa, Khmelnytskyi, Ternopil, Rivne, Sumy, and Kyiv regions, in combinations ranging from binary to pentanary.

The death of bee colonies in apiaries in Khmelnytskyi region due to poisoning by a single pesticide was identified in two cases. In the first case, it was an insecticide from the pyrethroid group – alpha-cypermethrin, which was detected in maple blossoms. In the second case, the mass death of bee colonies was caused by another representative of the same chemical group as alpha-cypermethrin – lambda-cyhalothrin. The latter was found in the green mass of a nectar-producing plant, rapeseed (Tables 2 and 3).

**Table 1**
List of pesticides detected in soils, plants, dead bees, and bee products

| | Active substance | Production classification | Chemical classification | Toxicity class for bees | Positive samples detected |
|---|---|---|---|---|---|
| 1. | Alpha-cypermethrin | insecticides | pyrethroids | 1 | 2 |
| 2. | Cypermethrin | insecticides | pyrethroids | 1 | 13 |
| 3. | Permethrin | insecticides | pyrethroids | 1 | 3 |
| 4. | Lambda-cyhalothrin | insecticides | pyrethroids | 1 | 33 |
| 5. | Tau-fluvalinate | insecticides | pyrethroids | 3 | 2 |
| 6. | Chlorpyrifos | insecticides | organophosphates | 1 | 3 |
| 7. | Imidacloprid | insecticides | neonicotinoids | 1 | 7 |
| 8. | Clothianidin | insecticides | neonicotinoids | 1 | 30 |
| 9. | Thiamethoxam | insecticides | neonicotinoids | 1 | 5 |
| 10. | Acetamiprid | insecticides | neonicotinoids | 3 | 3 |
| 11. | Prometryn | herbicides | pyrethroids | 3 | 4 |
| 12. | Tebuconazole | fungicides | triazoles | 3 | 21 |
| 13. | Cyproconazole | fungicides | triazoles | 3 | 11 |
| 14. | Propiconazole | fungicides | triazoles | 3 | 3 |
| 15. | Flutriafol | fungicides | triazoles | 3 | 2 |
| 16. | Epoxiconazole | fungicides | triazoles | 3 | 3 |
| 17. | Difenoconazole | fungicides | triazoles | 3 | 3 |
| 18. | Azoxystrobin | fungicides | strobilurins | 3 | 14 |
| 19. | Pyraclostrobin | fungicides | strobilurins | 3 | 3 |
| 20. | Picoxystrobin | fungicides | strobilurins | 3 | 4 |
| 21. | Carbendazim | fungicides | benzimidazoles | 3 | 6 |
| 22. | Hexythiazox | acaricides | carbamides | 3 | 3 |
| 23. | Pyridaben | acaricides | pyridazines | 3 | 3 |

**Table 2**
Pesticide content in dead bees (x ± SD)

| Region | Study period | Pesticide name | Concentration, μg/kg |
|---|---|---|---|
| Khmelnytskyi region | April 2021 (n = 3) | Lambda-cyhalothrin | 52.6 ± 7.7 |
| | April 2022 (n = 3) | Alpha-cypermethrin | 31.8 ± 3.5 |
| Cherkasy region | May 2022 (n = 3) | Tebuconazole | 1.01 ± 0.06 |
| | | Cypermethrin | 66.9 ± 3.2 |
| | May 2022 (n = 8) | Clothianidin | 3.85 ± 0.77 |
| | | Imidacloprid | 2.72 ± 0.21 |
| | | Tebuconazole | 6.35 ± 0.64 |
| | | Tau-fluvalinate | 5552 ± 826 |
| | June 2022 (n = 5) | Chlorpyrifos | 19.07 ± 2.04 |
| | | Cypermethrin | 7.13 ± 1.21 |
| Poltava region | September 2021 (n = 3) | Clothianidin | 5.63 ± 0.26 |
| | | Lambda-cyhalothrin | 40.3 ± 3.2 |
| | June 2022 (n = 3) | Clothianidin | 3.05 ± 0.71 |
| | | Lambda-cyhalothrin | 2.61 ± 0.24 |
| | July 2022 (n = 7) | Thiamethoxam | 4.38 ± 0.11 |
| | | Cyproconazole | 50.7 ± 16.5 |
| | | Azoxystrobin | 63.3 ± 5.4 |
| | | Lambda-cyhalothrin | 95.1 ± 28.3 |
| Vinnytsia region | May 2021 (n = 4) | Clothianidin | 3.17 ± 0.09 |
| | | Thiamethoxam | 1.72 ± 0.13 |
| | | Prometryn | 1.83 ± 0.22 |
| | September 2021 (n = 3) | Permethrin | 8074 ± 522 |
| | June 2022 (n = 7) | Clothianidin | 7.37 ± 0.25 |
| | | Tebuconazole | 3.90 ± 0.27 |
| | | Difenoconazole | 2.71 ± 0.09 |
| | | Azoxystrobin | 1.33 ± 0.08 |
| | | Lambda-cyhalothrin | 50.0 ± 6.98 |
| Kharkiv region | July 2021 (n = 4) | Clothianidin | 2.31 ± 0.58 |
| | | Tebuconazole | 2.85 ± 0.14 |
| | | Lambda-cyhalothrin | 0.91 ± 0.22 |
| | July 2021 (n = 6)[Bb] | Clothianidin | 0.47 ± 0.13 |
| | | Tebuconazole | 0.46 ± 0.17 |
| | | Lambda-cyhalothrin | 0.17 ± 0.02 |
| Ivano-Frankivsk region | July 2021 (n = 6) | Imidacloprid | 806.5 ± 66.1 |
| | | Cyproconazole | 1002 ± 320 |
| | | Azoxystrobin | 341.5 ± 78.2 |
| Odesa region | May 2022 (n = 5) | Acetamiprid | 54.6 ± 11.1 |
| | | Tebuconazole | 13.25 ± 1.58 |
| | | Propiconazole | 83.7 ± 13.5 |
| | | Azoxystrobin | 204.1 ± 25.29 |

| Region | Study period | Pesticide name | Concentration, µg/kg |
|---|---|---|---|
| Ternopil region | July 2022 (n = 7) | Clothianidin | 17.7 ± 2.71 |
| | | Carbendazim | 67.1 ± 14.5 |
| | | Cypermethrin | 234.2 ± 87.9 |
| | | Lambda-cyhalothrin | 458.8 ± 88.1 |
| Rivne region | May 2022 (n = 4) | Clothianidin | 3.31 ± 0.23 |
| | | Tebuconazole | 134.5 ± 21.3 |
| | July 2022 (n = 8) | Clothianidin | 3.75 ± 0.28 |
| | | Epoxiconazole | 56.3 ± 15.7 |
| | | Pyraclostrobin | 10.21 ± 1.13 |
| | | Cypermethrin | 20.02 ± 4.19 |
| | | Lambda-cyhalothrin | 98.9 ± 14.3 |
| Kyiv region | August 2022 (n = 3) | Cyproconazole | 0.38 ± 0.03 |
| | | Picoxystrobin | 0.48 ± 0.12 |
| | | Lambda-cyhalothrin | 97.8 ± 10.1 |
| | | Hexythiazox | 1.36 ± 0.66 |
| | | Pyridaben | 8.03 ± 1.01 |
| Sumy region | May 2021 (n = 4) | Clothianidin | 1.85 ± 0.22 |
| | | Tebuconazole | 0.91 ± 0.13 |

*Note*: Bb – Bee brood.

The most common cause of mass bee colony deaths in various regions of Ukraine was the mixture of two pesticides in different combinations. Tebuconazole – a triazole fungicide – and cypermethrin – a pyrethroid insecticide – were detected in the green mass of wheat. The concentration of tebuconazole in the green mass of wheat exceeded its concentration in the soil from Cherkasy region by 10.52 times, while the concentration of cypermethrin exceeded it by 159.72 times. Both pesticides were found in the dead bees on the apiary grounds (Table 2).

In another case, the cause of mass bee deaths was a mixture of insecticides belonging to different chemical groups – chlorpyrifos, an organophosphorus pesticide, and cypermethrin, a pyrethroid pesticide. Both insecticides were detected in the green mass of rapeseed and the soil in Cherkasy region. In this instance, the concentration of cypermethrin in the soil and the green mass of rapeseed was at the same level, while the concentration of chlorpyrifos in the green mass of rapeseed was 2.82 times higher than in the soil (Tables 3, 4).

Another source of bee poisoning in Cherkasy region was a mixture of clothianidin and lambda-cyhalothrin, which were detected in the soil and the green mass of soybeans. These insecticides belong to different chemical groups: neonicotinoids and pyrethroids. The concentration of clothianidin in the green mass of soybeans exceeded its level in the soil by 5.43 times, while the concentration of lambda-cyhalothrin exceeded it by 11.91 times.

**Table 3**
Pesticide accumulation in plants (x ± SD)

| Region | Study period | Plant species | Pesticide name | Concentration, µg/kg |
|---|---|---|---|---|
| Khmelnytskyi region | April 2021 (n = 5) | Green mass of rapeseed (*Brassica napus* L.) | Lambda-cyhalothrin | 890.3 ± 135.7 |
| | April 2022 (n = 3) | Flowers of Norway maple (*Acer platanoides* L.) | Alpha-cypermethrin | 125.9 ± 11.3 |
| Rivne region | May 2022 (n = 3) | Corn grain (*Zea mays* L.) | Clothianidin | 36900 ± 1287 |
| | | Soybean grain (*Glycine max* L. (Merr.)) | Tebuconazole | 133.3 ± 27.8 |
| | July 2022 (n = 5) | Green mass of rapeseed (*Brassica napus* L.) | Clothianidin | 576.2 ± 99.3 |
| | | | Epoxiconazole | 784.3 ± 78.6 |
| | | | Pyraclostrobin | 253.2 ± 73.3 |
| | | | Lambda-cyhalothrin | 731.9 ± 95.6 |
| | July 2022 (n = 4) | Green mass of potato (*Solanum tuberosum* L.) | Clothianidin | 131.1 ± 69.9 |
| | | | Cypermethrin | 227.0 ± 67.5 |
| | | | Lambda-cyhalothrin | 1603 ± 258 |
| Cherkasy region | May 2022 (n = 3) | Green mass of wheat (*Triticum* L.) | Tebuconazole | 6760 ± 523 |
| | | | Cypermethrin | 21483 ± 989 |
| | June 2022 (n =3) | Green mass of rapeseed (*Brassica napus* L.) | Chlorpyrifos | 208.2 ± 27.9 |
| | | | Cypermethrin | 692.7 ± 99.5 |
| Poltava region | September 2021 (n = 4) | Green mass of soybean (*Glycine max* L. (Merr.)) | Clothianidin | 998.4 ± 115.9 |
| | | | Lambda-cyhalothrin | 1111 ± 186 |
| | June 2022 (n = 3) | Green mass of sunflower (*Helianthus* L.) | Clothianidin | 554.1 ± 102.3 |
| | | | Lambda-cyhalothrin | 218.3 ± 75.8 |
| | July 2022 (n = 7) | Green mass of pea (*Pisum sativum* L.) | Thiamethoxam | 301.9 ± 63.2 |
| | | | Cyproconazole | 865.2 ± 164.6 |
| | | | Azoxystrobin | 385.2 ± 87.4 |
| | | | Lambda-cyhalothrin | 359.1 ± 99.2 |
| Vinnytsia region | September 2021 (n = 5) | Green mass of meadow herbs | Permethrin | 75934 ± 1589 |
| | | | Prometryn | 75939 ± 2005 |
| | June 2022 (n = 5) | Green mass of sunflower (*Helianthus* L.) | Clothianidin | 460.3 ± 95.2 |
| | | | Tebuconazole | 11.22 ± 3.15 |
| | | | Difenoconazole | 57.7 ± 6.7 |
| | | | Azoxystrobin | 29.6 ± 4.9 |
| | | | Lambda-cyhalothrin | 1182 ± 252 |
| Kharkiv region | July 2021 (n = 4) | Green mass of pea (*Pisum* L.) | Clothianidin | 1000 ± 289 |
| | | | Tebuconazole | 3.42 ± 0.19 |
| | | | Lambda-cyhalothrin | 97.4 ± 9.5 |
| Ivano-Frankivsk region | July 2021 (n = 6) | Green mass of sunflower (*Helianthus* L.) | Imidacloprid | 2078 ± 322 |
| | | | Cyproconazole | 2275 ± 195 |
| | | | Azoxystrobin | 428.4 ± 96.5 |
| Odesa region | May 2022 (n = 3) | Green mass of rapeseed (*Brassica napus* L.) | Acetamiprid | 1727 ± 305 |
| | | | Tebuconazole | 1492 ± 299 |

| Region | Study period | Plant species | Pesticide name | Concentration, µg/kg |
|---|---|---|---|---|
| | | | Propiconazole | 3602 ± 887 |
| | | | Azoxystrobin | 1079 ± 211 |
| Ternopil region | July 2022 (n = 8) | Green mass of soybean (*Glycine max* L. (Merr.)) | Clothianidin | 774.9 ± 100.1 |
| | | | Carbendazim | 822.3 ± 115.2 |
| | | | Cypermethrin | 745.6 ± 90.2 |
| | | | Lambda-cyhalothrin | 10728 ± 586 |
| Kyiv region | August 2022 (n = 3) | Green mass of soybean (*Glycine max* L. (Merr.)) | Cyproconazole | 1121 ± 369 |
| | | | Picoxystrobin | 131.3 ± 55.4 |
| | | | Lambda-cyhalothrin | 3047 ± 524 |
| | | | Hexythiazox | 409.1 ± 63.5 |
| | | | Pyridaben | 133.2 ± 43.3 |
| Sumy region | May 2021 (n = 3) | Green mass of rapeseed (*Brassica napus* L.) | Clothianidin | 69.7 ± 6.9 |
| | | | Flutriafol | 13.52 ± 1.63 |
| | | | Carbendazim | 3.62 ± 0.94 |
| | | | Tebuconazole | 646.4 ± 85.1 |

Pollution of soil and sunflower green mass with a mixture of two insecticides from the pyrethroid and neonicotinoid groups, namely clothianidin and lambda-cyhalothrin, was identified as the cause of poisoning and mass death of bees at one of the apiaries in Poltava region. Clothianidin was predominantly found in the sunflower green mass, with the soil being somewhat less contaminated. Lambda-cyhalothrin was present in trace amounts in the soil, while its main concentration was found in the sunflower green mass. Both pesticides were present in the dead bees at nearly the same levels.

Pollution of the green mass of meadow mixed grass from Vinnytsia region, submitted for examination, simultaneously with two pyrethroids from the group of insecticides and herbicides, permethrin and prometryn, exceeded their concentration in the soil by 9.41 times. At the same time, no prometryn residues were detected in the dead bees, while the content of permethrin ranged within the same level as in the soil (Tables 2–4).

Another case revealed a combination of the insecticide clothianidin from the neonicotinoid group and the fungicide tebuconazole from the triazole group, which caused bee mortality at one of the apiaries in Rivne region.

The source of bee poisoning with clothianidin was treated corn grain, while tebuconazole poisoning was linked to soybean grain.

**Table 4**
Accumulation of pesticides in soil (x ± SD)

| Region | Study period | Pesticide name | Concentration, µg/kg |
|---|---|---|---|
| Cherkasy region | May 2022 (n = 3) | Tebuconazole | 642.5 ± 73.1 |
| | | Cypermethrin | 134.5 ± 21.3 |
| | June 2022 (n = 5) | Chlorpyrifos | 73.8 ± 10.7 |
| | | Cypermethrin | 878.5 ± 133.3 |
| Poltava region | September 2021 (n = 3) | Clothianidin | 140.4 ± 27.4 |
| | | Lambda-cyhalothrin | 1.22 ± 0.17 |
| | June 2022 (n = 3) | Imidacloprid | 183.7 ± 12.6 |
| | | Lambda-cyhalothrin | 93.3 ± 18.4 |
| | July 2022 (n = 7) | Thiamethoxam | 249.5 ± 74.2 |
| | | Cyproconazole | 794.8 ± 115.1 |
| | | Azoxystrobin | 609.0 ± 93.7 |
| | | Lambda-cyhalothrin | 206.7 ± 55.4 |
| Vinnytsia region | May 2021 (n = 4) | Clothianidin | 8.71 ± 0.45 |
| | | Thiamethoxam | 145.5 ± 33.6 |
| | | Prometryn | 0.82 ± 0.18 |
| | September 2021 (n = 3) | Permethrin | 8127 ± 825 |
| | | Prometryn | 8139 ± 902 |
| | June 2022 (n = 7) | Clothianidin | 282.6 ± 74.1 |
| | | Tebuconazole | 9.31 ± 1.17 |
| | | Difenoconazole | 336.3 ± 58.4 |
| | | Azoxystrobin | 288.6 ± 55.1 |
| | | Lambda-cyhalothrin | 30.7 ± 3.22 |
| Kharkiv region | July 2021 (n = 4) | Clothianidin | 265.3 ± 50.1 |
| | | Tebuconazole | 3.16 ± 0.72 |
| | | Lambda-cyhalothrin | 0.11 ± 0.03 |
| Ivano-Frankivsk region | July 2021 (n = 6) | Imidacloprid | 253.2 ± 57.9 |
| | | Cyproconazole | 540.5 ± 72.4 |
| | | Azoxystrobin | 444.1 ± 89.3 |
| Odesa region | May 2022 (n = 5) | Acetamiprid | 99.7 ± 15.2 |
| | | Tebuconazole | 477.4 ± 63.7 |
| | | Propiconazole | 928.8 ± 112.3 |
| | | Azoxystrobin | 514.5 ± 96.3 |
| Ternopil region | July 2022 (n = 7) | Clothianidin | 287.1 ± 64.2 |
| | | Carbendazim | 140.8 ± 85.4 |
| | | Cypermethrin | 186.0 ± 82.5 |
| | | Lambda-cyhalothrin | 88.8 ± 19.5 |
| Rivne region | July 2022 (n = 8) | Clothianidin | 361.5 ± 63.9 |
| | | Epoxiconazole | 450.3 ± 75.6 |
| | | Pyraclostrobin | 237.6 ± 51.2 |
| | | Cypermethrin | 9.21 ± 2.54 |
| | | Lambda-cyhalothrin | 50.9 ± 5.8 |
| Kyiv region | August 2022 (n = 3) | Cyproconazole | 30.71 ± 3.91 |
| | | Picoxystrobin | 14.82 ± 1.23 |

| Region | Study period | Pesticide name | Concentration, µg/kg |
|---|---|---|---|
| | | Lambda-Cyhalothrin | 11.12 ± 0.99 |
| | | Hexythiazox | 4.78 ± 0.26 |
| | | Pyridaben | 14.94 ± 2.32 |

A more complex combination of three pesticides was identified in soil samples and dead bees from Vinnytsia region. This combination consisted of clothianidin, thiamethoxam (insecticides of the neonicotinoid group), and prometryn (a herbicide from the pyrethroid group) (Tables 2, 4). No plant samples were submitted for this study.

The combination of clothianidin, tebuconazole, and lambda-cyhalothrin caused contamination of pea green mass and the death of bees and bee broods in one apiary in Kharkiv region. In this case, while the concentration of clothianidin in the soil and pea green mass was higher than that of the other pesticides, the concentrations of all three pesticides in dead bees and bee broods were almost identical (Tables 2–4).

A mixture of imidacloprid (a neonicotinoid insecticide), cyproconazole (a triazole fungicide), and azoxystrobin (a strobilurin fungicide) was found in soil and sunflower green mass. This mixture caused bee deaths in apiaries in Ivano-Frankivsk region. In this case, the accumulation of azoxystrobin in soil and sunflower green mass was similar, while the levels of imidacloprid and cyproconazole in sunflower green mass exceeded their levels in the soil by 8.21 and 4.21 times, respectively. All three pesticides were simultaneously detected at high concentrations in dead bees, accompanied by their accumulation in honey (Tables 2, 5).

A multicomponent mixture of four pesticides belonging to different production and chemical groups caused mass bee deaths in apiaries across many regions throughout the entire growing season of agricultural crops (Table 2). For example, in soil and rapeseed green mass from Odesa region, a mixture of acetamiprid (a neonicotinoid insecticide), tebuconazole and propiconazole (triazole fungicides), and azoxystrobin (a strobilurin fungicide) was detected. Based on the concentration of all four pesticides, rapeseed was treated with these substances during its flowering, which coincided with the mass poisoning and death of bees.

In another case, a mixture of clothianidin (a neonicotinoid insecticide), flutriafol and tebuconazole (triazole fungicides), and carbendazim (a benzimidazole fungicide) was found in rapeseed green mass from Sumy region. However, only residues of clothianidin and tebuconazole were found in dead bees, while bee bread contained residues of all four pesticides (Table 5).

**Table 5**
Pesticide content in beekeeping products (x ± SD)

| Region | Study period | Product | Pesticide name | Concentration, µr/kg |
|---|---|---|---|---|
| Cherkasy region | May 2022 (n = 3) | Bee bread | Clothianidin | 35.5 ± 5.44 |
| | | | Imidacloprid | 13.21 ± 0.99 |
| | | | Tebuconazole | 6.32 ± 0.62 |
| | | | Tau-fluvalinate | 17.55 ± 1.28 |
| Poltava region | July 2022 (n = 7) | Honey | Lambda-cyhalothrin | 20.1 ± 12.4 |
| Vinnytsia region | June 2022 (n = 5) | Honey | Clothianidin | 0.70 ± 0.23 |
| | | | Tebuconazole | 0.73 ± 0.32 |
| | | | Azoxystrobin | 1.65 ± 0.55 |
| | | | Lambda-cyhalothrin | 18.23 ± 1.03 |
| Ivano-Frankivsk region | Jule 2021 (n = 6) | Honey | Imidacloprid | 22.5 ± 1.75 |
| | | | Cyproconazole | 70.75 ± 23.14 |
| | | | Azoxystrobin | 156.6 ± 42.2 |
| Ternopil region | Jule 2022 (n = 8) | Pollen | Carbendazim | 4.81 ± 1.02 |
| | | | Cypermethrin | 895.9 ± 92.1 |
| | | | Lambda-cyhalothrin | 483.3 ± 55.4 |
| | Jule 2022 (n = 6) | Honey | Clothianidin | 0.15 ± 0.02 |
| | | | Lambda-cyhalothrin | 6.02 ± 0.45 |
| Kyiv region | August 2022 (n = 3) | Honey | Cyproconazole | 0.11 ± 0.04 |
| | | | Picoxystrobin | 0.05 ± 0.00 |
| | | | Lambda-cyhalothrin | 42.10 ± 3.29 |
| Sumy region | May 2021 (n = 3) | Bee bread | Clothianidin | 3.77 ± 0.23 |
| | | | Flutriafol | 0.52 ± 0.08 |
| | | | Carbendazim | 0.34 ± 0.11 |
| | | | Tebuconazole | 2.55 ± 0.17 |

Another pesticide combination proved lethal to bees from an apiary in Cherkasy region. It consisted of clothianidin, imidacloprid, tebuconazole, and tau-fluvalinate, i.e., three insecticides from the neonicotinoid and pyrethroid groups and one fungicide from the triazole group. The highest concentration in the bodies of the dead bees was tau-fluvalinate. All four pesticides were detected in the bee bread samples from the apiary, where mass bee deaths were observed. The source of these pesticides entering the bees' bodies could not be determined.

The fourth combination of four pesticides found in the soil and pea green mass from Poltava region consisted of two insecticides and two fungicides from different groups, namely thiamethoxam, cyproconazole, azoxystrobin, and lambda-cyhalothrin. In most cases, the pesticide content in the soil closely matched the content in the pea green mass. All four pesticides were detected in the bodies of the dead bees at varying concentrations, while only lambda-cyhalothrin was found in the honey (Tables 2, 5).

In the fifth case of mass bee deaths at apiaries in Ternopil region, three insecticides and one fungicide were found in soil and soybean green mass: clothianidin, carbendazim, cypermethrin, and lambda-cyhalothrin. In soybean green mass, the concentration of clothianidin exceeded that in the soil by 2.70 times, carbendazim by 5.84 times, cypermethrin by 4.01 times, and lambda-cyhalothrin by 120.81 times. The highest concentrations in the bodies of the bees and bee bread were found for two insecticides: lambda-cyhalothrin and cypermethrin. The pesticides contaminating the honey in this case were clothianidin and lambda-cyhalothrin.

A combination of five pesticides in the soil caused mass bee deaths at apiaries in Rivne region. The mixture consisted of three insecticides – cypermethrin, lambda-cyhalothrin, and clothianidin (two pyrethroid group and one neonicotinoid group) and two fungicides – epoxiconazole and pyraclostrobin (from the triazole and strobilurin groups, Table 2). All pesticides except cypermethrin were detected in the green mass of rapeseed, while only the fungicides epoxiconazole and pyraclostrobin were absent from the potato green mass. In contrast, all six pesticides were found in the dead bees (Table 3).

In a case of mass bee poisoning at an apiary in Vinnytsia region, a mixture of five pesticides was identified. This mixture included two insecticides from the neonicotinoid and pyrethroid groups – clothianidin and lambda-cyhalothrin – and three fungicides: two from the triazole group – tebuconazole and difenoconazole – and one from the strobilurin group – azoxystrobin. All these pesticides were detected not only in the dead bees but also in the soil and green mass of sunflowers. A similar pesticide contamination pattern was found in honey, with the exception of difenoconazole (Tables 2–5).

Another case of mass bee poisoning occurred at an apiary in Kyiv region, involving a mixture of five pesticides. In the soil, soybean green mass, and dead bees, two fungicides were found: one from the triazole group – cyproconazole, and one from the strobilurin group – picoxystrobin. Three insecticides were also detected: one from the pyrethroid group – lambda-cyhalothrin, one from the carboxamides group – hexythiazox, and one from the pyridazinone group – pyridaben (Tables 2, 3). In the soybean green mass, the concentration of cyproconazole exceeded that in the soil by 36.51 times, picoxystrobin by 8.85 times, lambda-cyhalothrin by 274.54 times, hexythiazox by 85.59 times, and pyridaben by 8.94 times. The highest concentration of the listed pesticides in the bodies of bees and honey was lambda-cyhalothrin. All pesticides were accumulated in the honey, except for hexythiazox and pyridaben (Tables 2–5).

**Discussion**

An analysis of pesticides found in components of the soil-plant-bee-beekeeping products system across different regions of Ukraine revealed that the primary pollutants were insecticides and fungicides. Herbicide accumulation in the soil and dead bees was detected only once. Among insecticides, pyrethroids and neonicotinoids were the most common, and among fungicides, triazoles and strobilurins were prevalent, which aligns with previous data obtained by our team (Omelchun et al., 2023).

The results of the study show that bee poisoning by a single pesticide is quite rare, with only two such cases identified. In one case, bee poisoning with alpha-cypermethrin was linked to its presence in the inflorescence of maple trees, which are early nectar plants and quite attractive to bees. In this case, the maple was not considered a target plant for pesticide treatment. It is likely that the maple plantation was located near agricultural crops being treated with this plant protection product and within the flight radius of the bees. Given that the average weight of a worker bee is 100 µg, the residue of alpha-cypermethrin found in the bodies of dead bees corresponded to a dose of 0.003 µg/bee, which is 1/7 of the $LD_{50}$.

Another pyrethroid insecticide, permethrin, was detected in dead bees only in one instance at a very high dose, exceeding its $LD_{50}$ by 14 times (0.057 µg/bee) (Piccolomini et al., 2018). This indicates that meadow herbs as a source of permethrin were located very close to the apiary, as such a dose would cause bee deaths either at the foraging site or on the way back to the apiary. Similarly to permethrin, the pesticide tau-fluvalinate from the same group was found in one case in combination with other pesticides in the bodies of dead bees, with a dose of 1.2 $LD_{50}$, which is an average of 10.7 µg/bee (Benito-Murcia et al., 2024).

One of the most common pesticides among pyrethroid insecticides was lambda-cyhalothrin. It was detected in soil, plant samples, dead bees, and beekeeping products both alone and in complex mixtures with other pesticides. It caused bee deaths in 9 cases due to its presence in rapeseed and sunflower, which are actively used by bees as nectar and pollen sources during the flowering period. The presence of lambda-cyhalothrin in the green mass of legumes such as soybeans and peas, as well as nightshades such as potatoes, which are not entomophilous plants and do not serve as a food source for bees, indicates that these plantings are within the bees' flight radius. Lambda-cyhalothrin is registered as an insecticide with an acute contact $LD_{50}$ of 0.038 µg/bee and an acute oral $LD_{50}$ of 0.91 µg/bee. It is also toxic to bee brood (Choi et al., 2024). The maximum concentration of lambda-cyhalothrin found in dead bees was up to 1/4 of the acute contact $LD_{50}$ and 1/91 of the acute oral $LD_{50}$. Despite the fact that soybeans and peas do not require entomophilous pollination, bees can enter crop fields and come into contact with pesticides while crossing their planted areas.

The second most common insecticide among pyrethroids, detected in mixtures with pesticides from other groups, was cypermethrin. It was found in soil, green mass of wheat, rapeseed, soybeans, and potatoes, as well as in dead bees. The highest concentration of cypermethrin was detected in the bodies of dead bees, which corresponded to the $LD_{50}$, i.e., 20 ng/bee.

Neonicotinoids were found with the same frequency in dead bees as pyrethroids. In 9 cases of bee deaths, mixtures of neonicotinoid insecticides, specifically clothianidin, were detected in various combinations with other pesticides. The oral $LD_{50}$ for neonicotinoids in bees is as follows: clothianidin – 0.003 µg, imidacloprid – 0.05 µg, acetamiprid – 11.81 µg, and thiacloprid – 19.9 µg per bee (Chmiel et al., 2020). The highest dose of clothianidin found in dead bees in our study was 0.002 µg/bee, which was very close to the $LD_{50}$. Even at doses typically found in the range of 1/10 of the $LD_{50}$, the toxicity of clothianidin could be enhanced when combined with other pesticides. Imidacloprid was the second most frequently found pesticide in mixtures with others in dead bees, with the highest detected dose being 1.6 $LD_{50}$. Imidacloprid was most often used to treat sunflower crops. It is believed that the effects of neonicotinoids on bees extend beyond adult bees, affecting the brood as well, specifically causing toxicity in larvae and increasing their mortality (Claus et al., 2021).

Among the fungicides found in soil, plants, and dead bees in combination with other pesticides, the most common was tebuconazole. Tebuconazole is a systemic fungicide from the triazole group, detected in 7 cases in the green mass of wheat, peas, and soybean grains. Although these plants are not entomophilous, the pesticides were found in dead bees on the apiary site, likely due to the positioning of these crops within the flight radius of the bees. As for rapeseed and sunflower green mass, their involvement in bee poisoning by tebuconazole through the collection of pollen and nectar is indisputable. Tebuconazole has acute toxicity for bees at a dose of 200 µg/bee (Raimets et al., 2022). Although such a dose was not detected in our study, when combined with other pesticides from different groups, it could show a synergistic toxic effect not only on adult worker bees but also on other members of the colony, as it was found in dead brood, pollen, and honey (Raimets et al., 2022).

The second most common fungicide among triazoles found in various combinations with other pesticides was cyproconazole. It contaminated the green mass of sunflower, peas, and soybeans, caused bee deaths, and accumulated in honey. It is believed that its oral $LD_{50}$ is greater than 100 µg/bee. The highest dose recorded in our study when bees were poisoned by a pesticide mixture containing cyproconazole was 1/1000 of the $LD_{50}$. Although the dose received by the bees was minimal, when combined with neonicotinoids and strobilurins, it could exhibit a synergistic effect (Lv et al., 2023).

Among the fungicides in the strobilurin group, the most frequently detected in soil, plants, and dead bees was the combination of azoxystrobin with other pesticides. Although its acute toxicity is 57.5 µg/bee, and some studies report it as 100 µg/bee, when combined with other pesticides, it could enhance their effect (Serra et al., 2023). Additionally, in this case, there is a possibility of delayed effects of pesticides on the bee colony. Research indicates the chronic toxicity of azoxystrobin for honeybee larvae and pupae, affecting their susceptibility to pathogens and other external stressors during development (Duan et al., 2024).

Pesticides that were detected in rare cases in various complex combinations in dead bees, plants, and soils include chlorpyrifos (an organophosphorus pesticide), hexythiazox (carboxamides), and pyridaben (pyridazinones). Their toxicity to bees increases significantly in mixtures, particularly the toxicity of the chlorpyrifos and atrazine mixture, which is 2.5 times higher (Fellows et al., 2022). It has been found that the combination of imidacloprid, thiamethoxam, and chlorpyrifos significantly increases bumblebee mortality (Zhang et al., 2022).

Regarding the combination of two insecticides from the neonicotinoid group and fungicides from the triazole group – thiacloprid and cyproconazole – our findings align with the research of Lv et al. (2023). It has also been confirmed that combinations of sublethal doses of tetrachlorantraniliprole and tebuconazole (Cang et al., 2023), thiamethoxam and flusilazole (Wang et al., 2023) exhibit synergistic toxicity for bees. Experimentally, it has been proven that in 98 combinations of thiamethoxam with dimethoate, methomyl, zeta-cypermethrin, cyfluthrin, permethrin, esfenvalerate, and tetraconazole – ranging from binary to octonary combinations – 29.6% showed a synergistic toxic effect via oral exposure in bees (Li et al., 2023). The synergistic toxicity of binary mixtures of chlorpyrifos, acephate, and tetraconazole with nine pyrethroid insecticides was also confirmed for honeybees (*Apis mellifera* L.) (Wang et al., 2020). The more pesticides are included in the mixture in a biological sample, the more challenging it becomes to determine their origin. In a study conducted in the United States, a combination of over 13 pesticides from different groups was found in pollen from a nursery in Connecticut for decorative plants pollinated by bees, including pesticides that were not used for treating plants in that nursery (Hester et al., 2023). In the pollen of rapeseed grown in China, 48 pesticides were found, while only 34 chemical substances were detected in nectar samples (Wen et al., 2021).

The wide range of plant protection products used during the cultivation of various agricultural crops contributes to their unpredictable combinations not only in the soil and plants (Cappellari et al., 2024) but also in the bodies of bees and beekeeping products that are used for human food, as well as in the pharmaceutical and cosmetic industries (Lamas et al., 2023). For most pesticides, parameters of acute and chronic oral and contact toxicity have been established. However, in the case of their combinations, this does not always provide objective information about the real toxicity of the mixture in each specific case of poisoning. This is influenced by the chemical structure of each pesticide, its half-life, concentration in the environment, and its ability to synergize with other pesticides.

In this regard, several scientists express concern about the significant gaps in data regarding sublethal (71%) and combined (~99%) toxicity of pesticides. The greatest risks in terms of sublethal (chlorothalonil, pimetrozine, glyphosate; neonicotinoids) and combined (combinations of tau-fluvalinate; acetylcholinesterase inhibitors and neonicotinoids) effects may affect human health. Although regulatory limits for certain pesticides (chlorothalonil, pimetrozine, neonicotinoids) have been established in some countries, most of them are still widely used worldwide (for example, glyphosate) (Tosi et al., 2022).

When bees are poisoned by pesticides, it is important to consider the risks associated with the migration of their residues into the environment. In addition to the soil, plants, their pollen, and nectar collected by bees and other pollinators, pesticides enter the air with dust, are washed away by precipitation, and get into puddles and water bodies (Schuhmann et al., 2022), which bees and other animals drink from. This complicates the pathways of their entry into organisms and broadens the range of toxic effects (Guo et al., 2024). When assessing the synergistic toxicity of pesticide mixtures for bees, the presence of other chemical compounds, including antibacterial drugs that enter the bodies of bees during prophylactic or therapeutic treatments, must also be taken into account (Bayer et al., 2017, 2018). Thus, there is a need to search for alternatives to chemical plant protection products based on an assessment of the complex impact on non-target insect species and the development of biological control agents for plant pests and bee diseases.

## Conclusions

The use of plant protection products leads to the accumulation of their residues in soils, the vegetative form of target and non-target plants, which during foraging causes poisoning and death of bee colonies. The main pesticides responsible for the mass death of bee colonies in Ukraine in 2021–2022 are insecticides and fungicides, both alone and in various combinations. The largest number of cases of bee colony deaths is associated with the combined poisoning by insecticides from the pyrethroid and neonicotinoid groups, as well as fungicides from the strobilurin and triazole groups. The intake of 23 pesticides into the bodies of bees, as well as beekeeping products (honey, bee bread), is linked to their accumulation in the soil, grain, and flowering plants. At the same time, biological samples contained from 1 to 5 pesticides of various agricultural purposes. Pesticide combinations accumulated in the inflorescences of non-target crops (maple and meadow grasses), as well as wind-pollinated and self-pollinated agricultural crops (wheat, peas, soybeans, and potatoes). Among entomophilous agricultural crops, the main sources of pesticide poisoning are rapeseed and sunflower. The content of certain pesticides in dead bees approached and often exceeded the lethal oral dose $LD_{50}$. The results of the research indicate the necessity of considering the risks of mass poisoning of bees as non-target insects when pesticides of various origins accumulate in soils, grain, the vegetative form of entomophilous and non-entomophilous plants, as well as contamination of beekeeping products intended for human use.

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

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