

Determination of pesticide residues in bee honey and pollen grains with their potential human health risks in the Nile Delta, Egypt

Asmaa El-Metwally Abd-Alla ¹, Rasha Adel Salem ², Abdulraouf Mohamed Amro ^{2,*}

¹Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University, Cairo 12613, Giza, EGYPT,

²Bee Research Department, Plant Protection Research Institute, Agricultural Research Center, Dokki 12619, Giza, EGYPT

*Corresponding author: Bee Research Department, Plant Protection Research Institute, Agricultural Research Center, Dokki 12619, Giza, EGYPT.

Email: Abdulraouf.abdelrahman@arc.sci.eg

A growing trend in understanding human health involves looking at the bigger picture by examining all potential environmental exposures that may cause health risks, with a particular focus on dietary intake of anthropogenic chemicals. This study investigated the presence of pesticide residues in honey and pollen samples collected randomly from ten locations in four agricultural governorates during the spring season of 2023 in the Nile Delta, Egypt. A QuEChERS extraction was employed for sample preparation before GC-MS analysis for pesticide residues. The human health risk associated with these residues were evaluated using hazard quotient (HQ). Our findings indicate that the detection rate and levels of pesticide residues are greater than previously reported. Giza governorate exhibited the highest content of residues in both honey and pollen samples, followed by El-Dakahlia, El-Qalyubia and Gharbia. Also, honey samples from El-Dakahlia, El-Qalyubia, and Giza contained the highest concentrations of aldrin, hexachlorocyclohexane (HCH) and chlorpyrifos, ranging from 10.45 to 19.6 $\mu\text{g kg}^{-1}$, 21.70 to 62.23 $\mu\text{g kg}^{-1}$, and 167.55 to 190.74 $\mu\text{g kg}^{-1}$, respectively. Pollen grain samples from Giza and El-Dakahlia showed high levels of chlorpyrifos (76.20 $\mu\text{g kg}^{-1}$) and HCH (33.60 $\mu\text{g kg}^{-1}$), respectively. Health hazard and quotient studies indicate that the residue levels of pesticides in all tested honey did not pose a significant risk for human consumption. Out of all pesticides, aldrin is the only one that requires further risk assessment to determine its potential impact on honeybee colonies.

Keywords: Pesticides residue; Honey; Pollen; *Apis mellifera* L; Aldrin; Toxicological risk.

1 Introduction

Honeybees are vital to agricultural ecosystems, acting as essential pollinators for a wide range of crops.¹ Its products, particularly honey, are invaluable in the food manufacturing, cosmetics, pharmaceutical, and alternative medicine industries.² Honey has been used as a folk remedy for certain infections.³ Given these multifaceted significances, ensuring the health of honeybee populations and maintaining the quality of their hive products are pressing challenges for specialists in the field.

One of the most serious global challenges in this regard is the unregulated use of pesticides in nature. Several investigations have pointed to extensive pesticide use as a primary culprit in the occurrence of the disastrous phenomenon of colony losses.^{4,5} Even so, there are current concerns about the prevalence of various pesticides, including insecticides,⁶ herbicides,⁷ fungicides,⁸ and acaricides⁹ in honey samples. Bees encounter these contaminants when collecting nectar and pollen from treated crops or through chemicals used by beekeepers to control pests and diseases.^{10,11} This contamination can harm both bees and colony products, reducing honey quality and diminishing its health benefits.¹² Also, the US Food and Drug Administration considers pesticide residues as a major food safety challenge,¹³ highly dangerous and linked to numerous serious diseases like cancer, posing a significant risk to human health.¹⁴

In Egypt, Eissa et al.,¹⁵ analyzed 46 honey samples from 18 apiaries, finding that more than half (55.6%) contained pesticide residues. Notably, most of these contaminants belonging to the organochlorine (OCs), and organophosphate insecticides (OPs). However, their study also documented that the estimated daily intake of these residues was below acceptable daily limits, suggesting minimal toxicological risk from honey consumption. In three northern governorates, located in and near the Nile Delta, Egypt, Malhat et al.,¹⁶ investigated one hundred honey samples to assess their quality and potential as environmental pollution indicators by OCs and pyrethroid pesticides. Hexachlorobenzene was the most common OCs found, followed by permethrin and heptachlor epoxide. Only one sample exceeded the maximum allowed level for a specific pesticide (c-HCH), while the detected pesticide levels are unlikely to cause health problems for consumers, the source of the OCs remains a concern. However, Shendy et al.,¹⁷ reported that only one out of 64 honey samples collected from different areas across Egypt contained tauflualinate at a level of 10.00 $\mu\text{g kg}^{-1}$. Also, Al Naggat et al.,¹⁸ investigated the potential health risks of pesticide residues in honey for Egyptian adults. They looked at 28 different compounds in both ideal and worst-case situations. Even in the worst scenario, the amount of exposure from honey was 15 times lower than the harmful levels. These studies suggest that people consuming honey contaminated with these insecticides are unlikely to

experience negative health effects. Recently, Nassar et al.,¹⁹ analyzed pesticide residues in honey, pollen and wax from different geographical locations in Egypt, and they found that the highest pesticide residue levels in samples collected during fall and winter seasons, with phenylconazole fungicides and OPs consistently identified in all samples. Notably, beeswax exhibited the highest concentration of residues, followed by pollen and honey.

Egypt's agricultural and public health sectors relied on OCs throughout the 1950s and 60s. Despite their eventual ban in 1988,²⁰ the lingering environmental and health consequences of these chemicals continue to be felt.^{16–19} A ministerial decree in 1996 prohibited the import and use of 80 pesticides, including several OCs.²¹ Despite this ban, the persistent presence of these harmful chemicals in the Egyptian environment suggests that illegal use continues to be a significant issue. The European Commission has established maximum residue levels (MRLs) for pesticide traces in food and animal feed, effectively monitoring these levels in honey remains an analytical challenge, despite its crucial role in human health, ecological well-being, and pollinator protection. Hence, this study aims to detect pesticide contaminants in honey and pollen samples collected from 10 different locations across the Egyptian Nile Delta. In addition, we identify potential health risks to humans, primarily those stemming from environmental pollution caused by anthropogenic use of pesticides such as aldrin, endrin, hexachlorocyclohexane (HCH), 4,4DDT, 4,4DDE, heptachlor, heptachlor epoxide, methoxychlor, dieldrin, endosulfan I, endosulfan II, endosulfan sulfate, chlordane, metalaxyl, and chlorpyrifos.

2 Materials and methods

2.1 Study areas

Egypt's agricultural heartland located within the Nile Delta, a fan-shaped region where the Nile River meets the Mediterranean Sea. The Nile River Valley, culminating in the fertile Nile Delta, forms the backbone of Egyptian agriculture. In spring 2023, sixty samples (thirty honey samples and thirty pollen grain samples) were collected randomly from ten locations across four agricultural governorates within the Nile Delta, Egypt as follows: three apiaries in Giza (AL-Qanater 30°11'18" N 31°07'35"E, Kerdasah 30°01'12"N 31°07'29"E and EL-Hawamdeya 29°54'28"N 31°13'55"E), three apiaries in El-Dakahlia (Mansoura 30°08'04"N 31°05'50"E, Mit-Ghamr 30°43'08"N 31°17'31"E and EL-Sembellawein), two apiaries in El-Qalyubia (Banha 30°26'32"N 31°12'56"E and Toukh 30°19'29"N 31°12'39"E), and two apiaries in Gharbia (Basioun 30°55'27"N 30°49'03"E and Tanta 30°49'18"N 31°02'59"E) (Fig. 1).

2.2 Sample collection and preparation

Apis mellifera carnica is the dominant bee species reared in the Delta apiaries in Egypt from mid-April to early June depending heavily on clover (*Trifolium* spp) pollen and nectar.²² While, in the summer, maize, vegetables, cotton, and pumpkins are their primary sources. High honey production coincides with peak clover bloom season, making it ideal for our study. Five hives were randomly selected from each apiary. Five blocks of honeycombs (25 × 25 cm) from each chosen hive were carefully transferred to the laboratory in clean Ziplock bags for honey collection. These samples were initially stored at –20 °C, subsequently, transferred in a refrigerated box with ice to the Food Safety and Quality Control Laboratory for extraction and analysis.

To determine whether the honey samples are primarily clover or alfalfa honey, their pollen contents were analyzed using Louveaux et al.²³ method. Ten grams were dissolved in 20 mL of warm water to prepare the honey samples, then were centrifuged at 3500 rpm for 10 min, and the pellets were resuspended in fresh water and were centrifuged again for 10 min. The entire sediment was mounted on a slide and spread to cover an area of approximately 20 × 20 mm. After drying by gentle heating at 40 °C, glycerin gelatin was added, and the preparation was examined under a light microscope (Olympus –CH20i BIMU). Melissopalynological studies were used as a reference for estimating pollen grain frequencies. The following terms are used to categorize these frequencies: > 45%, very frequent; 16–45%, frequent; 3–15%, rare; and < 3%, sporadic.²⁴

For collecting pollen samples in the same period, three pollen traps were attached to five different colonies within each apiary. Pollen analysis began with cleaning, weighing, and color separation of fresh pollen pellets collected from each trap. A small drawing brush facilitated the color separation. Groups containing a single dominant color (> 70% of the total) were identified as the major pollen source.²⁵ Following this initial sorting, the pollen groups were further classified into specific pollen types based on individual grain structure,²⁶ under a light microscope (LM) equipped with an Axiocam 512 color camera. To create reference slides for comparison, pollen grains were directly collected from open clover flowers around the apiary during the corresponding flowering period. Finally, the morphology of each pollen species on the reference slides was compared to the morphology of the pollen pellets obtained from the traps to ensure that is clover pollen grain. The samples were transferred and stored in the same way as honey samples.

2.3 Pesticides detection

The honey and pollen grain samples were analyzed to identify and quantify residues of 15 pesticides, aldrin, endrin, HCH, 4,4DDT, 4,4DDE, heptachlor epoxide, heptachlor, dieldrin, methoxychlor, endosulfan II, endosulfan I, endosulfan sulfate, chlordane, chlorpyrifos, and metalaxyl.

2.4 Extraction procedure

One gram sample of honey and pollen was first combined with 7 mL of Milli-Q[®] water in a centrifuge tube and vortexed for one minute. After centrifugation, 10 milliliters of a 5% formic acid solution in acetonitrile were added to the residue. The mixture was then agitated by hand shaking and further disrupted using an ultrasonic water bath for 10 min. Four grams of a magnesium sulfate (MgSO₄) and sodium acetate (NaAc) mixture (4:1 W/W) were added to the solution and shaken for 30 seconds. The mixture was then centrifuged again at 5,000 rpm for 15 min at 4 °C. For extract purification, the solution was fortified with 0.15 grams of MgSO₄, 0.05 grams each of primary secondary amine (PSA) and zirconium silicate (Z-Sep) per milliliter. Finally, the extract was passed through a 0.22 micrometer nylon filter for further cleanup before chromatographic analysis.

2.5 Quantification analysis

Quantification of OC and xylylalanine in the honey and pollen samples was performed by gas chromatography–mass spectrometry (GC–MS) (Agilent5977A) after sample extraction by QuEChERS-method at the Food Safety and Quality Control Laboratory. For the separation, a 30-meter HP-5MS column (Agilent, Torrance, CA, USA) with a 0.25 mm inner diameter and 0.25 μm film thickness

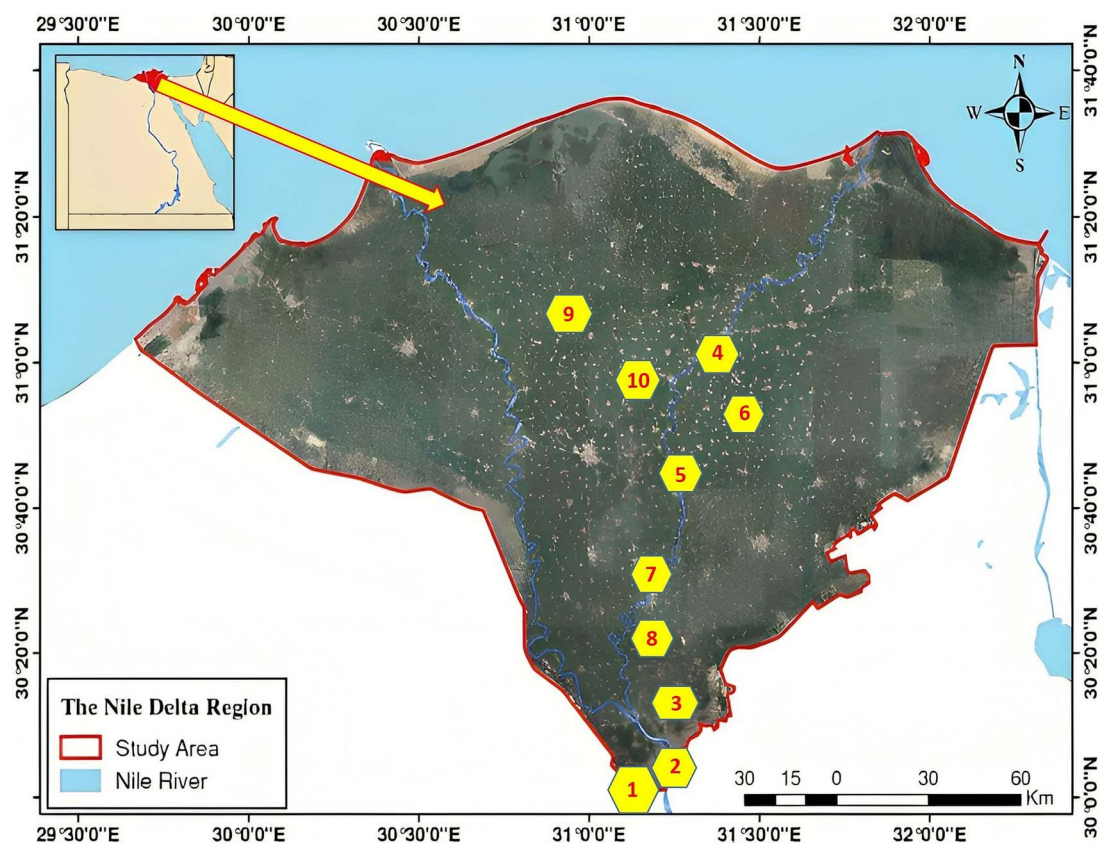


Fig. 1. Map of honey and pollen sampling sites in Giza, El-Dakahlia, El-Qalyubia and Gharbia governorate, Egypt.

was employed. High-purity helium gas (99.995%) served as the carrier gas, flowing at a constant rate of 1.4 mL/minute.

Chromatographic separation utilizes a specific set of parameters to achieve optimal results. For injection onto the chromatography column, 2 microliter aliquots of the extracts were introduced into a single-tapered glass liner packed with deactivated glass wool. The autosampler employed a pulsed splitless mode with a pressure of 40 psi for the initial 0.75 min to achieve optimal sample introduction. The injection port temperature was maintained at 250 °C. The column temperature program consisted of two ramps. Initially, the system held the temperature at 70 °C for 1 min. Then, the first ramp increased the temperature at a rate of 25 °C per min to reach 280 °C, which was held for an additional 7 min. Finally, a second ramp increased the temperature even faster, at 39 °C per minute, to reach a final temperature of 300 °C. These specific conditions facilitated the separation of components within the samples. The entire analysis process was completed in 15.5 min. Mass spectrometry employed electron impact ionization for detection, set at an ionization energy of 70 eV. To optimize analyte detection, the ion source and transfer line were maintained at 230 °C and 280 °C, respectively. Full scan mode (m/z 50–600) was initially used to acquire a complete picture of the sample components, including their retention times and mass spectra. For quantitative analysis, the method then shifted to the Selected Ion Monitoring (SIM) mode. In SIM mode, each analyte was identified by a unique combination of one target ion and two qualifier ions.

2.6 Quality optimization and validation

To ensure adherence to Codex quality principles for multi-residue analysis, the performance of the developed technique was rigorously assessed according to the Codex guidelines

outlined in 1993.²⁷ The method's limit of quantification (LOQ) was determined as the lowest concentration that can be consistently quantified with acceptable accuracy (between 70% and 120%) and adequate precision (relative standard deviation <20%). Honey samples were spiked with a pesticide blend standard at increasing concentration levels ranging from 0.01 mg/kg to 0.05 mg/kg to assess recovery rates and LOQ. Since blank pollen samples were not readily available, the LOQ was determined by analyzing existing samples containing target pesticides. This approach ensures the LOQ reflects the performance achievable with real-world samples and aligns with the SANTE criteria by considering the lowest validated spike level.²⁸

2.7 Evaluation of dietary intake, potential health risks and hazard quotient in humans

To determine whether pesticide residues in honey pose a health risk or not, we compare the amount people consume daily (EDI) with the acceptable daily intake (ADI) established by the FAO/WHO JMPR. Health risk assessment for dietary exposure to pesticides relies on the integration of pesticide residue analysis data and food consumption data. This approach aims to represent realistic dietary intake patterns of the general population, typically assumed to have an average body weight of 60 kg. To understand what people consume, we used dietary data from cluster B diets within the World Health Organization's GEMS/Food program.²⁹ Juan-Borrás et al.³⁰ defined a metric called Estimated Daily Intake (EDI), measured in micrograms per kilogram of body weight per day ($\mu\text{g kg}^{-1} \text{d}^{-1}$), to quantify how much pesticide residue people might ingest daily as follows:

$$EDI = \frac{C * Con}{BM}$$

Table 1. Concentrations ($\mu\text{g kg}^{-1}$ wm) of pesticides detected in honey samples collected from different locations in Egypt Delta with their EU maximum residue limits (MRLs) during spring 2023.

| Name of pesticides | Uses | Chemical class | Location of honey samples | | | | | | | | | | EU MRLs $\mu\text{g kg}^{-1}$ |
|--|-------------|-----------------|---|--------|--------|---------------------|-------|--------|---------------------|-------|-----------------|-------|-------------------------------|
| | | | Concentration of pesticides $\mu\text{g kg}^{-1}$ | | | | | | | | | | |
| | | | Giza (n = 9) | | | El-Dakahlia (n = 9) | | | El-Qalyubia (n = 6) | | Gharbia (n = 6) | | |
| I* | II* | III* | I** | II** | III** | I*** | II*** | I**** | II**** | | | | |
| Aldrin | Insecticide | Organochlorine | ND | 19.6 | 10.45 | < LOQ | 12.51 | 14.15 | ND | 11.48 | ND | < LOQ | 10 |
| Endrin | Insecticide | Organochlorine | ND | ND | ND | ND | ND | ND | ND | ND | ND | < LOQ | 10 |
| HCH (Hexachlorocyclohexane) | Insecticide | Organochlorine | ND | 21.7 | 49.725 | 37.22 | < LOQ | 46.595 | 62.23 | 55.99 | ND | 55.97 | 10 |
| 4,4DDT | Insecticide | Organochlorine | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 10 |
| 4,4DDE | Insecticide | Organochlorine | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 50 |
| Heptachlor | Insecticide | Organochlorine | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 10 |
| Heptachlor epoxide | Insecticide | Organochlorine | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 10 |
| Methoxychlor | Insecticide | Organochlorine | ND | ND | ND | < LOQ | ND | < LOQ | ND | < LOQ | ND | ND | 10 |
| Dieldrin | Insecticide | Organochlorine | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 10 |
| Endosulfan I (Hexachlorocyclopentadiene) | Insecticide | Organochlorine | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 10 |
| Endosulfan II | Insecticide | Organochlorine | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 10 |
| Endosulfan sulfate | Insecticide | Organochlorine | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 10 |
| Chlordane | Insecticide | Organochlorine | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 10 |
| Metalaxyl | Fungicide | Xylylalanine | ND | < LOQ | < LOQ | ND | < LOQ | ND | < LOQ | < LOQ | ND | ND | 500 |
| Chlorpyrifos | Insecticide | Organophosphate | ND | 190.74 | 167.55 | ND | < LOQ | < LOQ | ND | ND | ND | ND | 10 |

- Honey samples location in Giza: (I* = AL-Qanater, II* = Kerdasah, III* = EL-Hawamdeya), in El-Dakahlia: (I** = Mansoura, II** = Mit-Ghamr, III** = EL-Sembellawein), in El-Qalyubia: (I*** = Banha, II*** = Toukh) and in Gharbia: (I**** = Basioun, II**** = Tanta). - LOQ: limit of quantification, ND: none detected.

where C is the level of a specific pesticide residue found within honey in $\mu\text{g kg}^{-1}$; Con is the daily average amount of honey consumed by adult individual in $\text{kg person}^{-1} \text{d}^{-1}$; BM represents body mass in kg person^{-1} . For each pesticide, a Hazard Quotient (HQ) was determined as described by Al Naggar et al.¹⁸ This HQ is calculated by dividing the estimated daily intake (EDI) by the acceptable daily intake (ADI), both expressed in micrograms per kilogram of body weight per day ($\mu\text{g kg}^{-1} \text{d}^{-1}$):

$$\text{HQ} = \frac{\text{EDI}}{\text{ADI}}$$

3 Results

Pesticide residues in honey samples collected from different areas within the Nile Delta region of Egypt, along with their corresponding European Union maximum residue limits (MRLs) and chemical structures are portrayed in Table 1. All pesticides listed in the table belong to the OCs, except for xylylalanine (fungicide), and chlorpyrifos (OPs). The maximum allowable level for all depicted pesticides is $10 \mu\text{g kg}^{-1}$, except for metalaxyl, which is $500 \mu\text{g kg}^{-1}$, and $50 \mu\text{g kg}^{-1}$ for 4,4DDE.

Limit of quantification, presence or absence, and centile concentrations of OC and xylylalanine residues ($\mu\text{g kg}^{-1}$ wet mass) in honey samples collected from ten locations in four different governorates (Giza, El-Dakahlia, El-Qalyubia and Gharbia) in Egypt during spring 2023 are reported in Table 1. No pesticides were detected (ND) in honey samples that were collected from AL-Qanater site (Giza) and Basioun site (Gharbia). Notably, analysis of pesticide residues in honey samples from all study areas revealed that there were no detectable levels of these pesticides: 4,4DDT, 4,4DDE, heptachlor epoxide, heptachlor, endosulfan I, dieldrin, endosulfan II, chlordane endosulfan, sulfate. Six pesticides, including aldrin, endrin HCH, methoxychlor, metalaxyl, and

chlorpyrifos, were found below the limit of quantification (LOQ) in the honey samples which were collected from different sites in Giza, El-Dakahlia, El-Qalyubia and Gharbia governorate. Within El-Dakahlia, three honey samples from Mit-Ghamr site, two from EL-Sembellawein, and two from Mansoura showed levels of aldrin, HCH, methoxychlor, metalaxyl, and chlorpyrifos below the LOQ. Other pesticides were detected in some samples at levels above the LOQ. For example, aldrin was detected in honey samples from El-Dakahlia, El-Qalyubia, and Giza at concentrations ranging from 10.45 to $19.6 \mu\text{g kg}^{-1}$. HCH was also detected in honey samples from all studded areas ranging from 21.70 to $62.23 \mu\text{g kg}^{-1}$. Finally, chlorpyrifos was only detected in two honey samples collected from Giza recording 167.55 and $190.74 \mu\text{g kg}^{-1}$ in EL-Hawamdeya and Kerdasah sites, respectively.

Table 2 presents the limit of quantification (LOQ), detection status, and centile concentrations of OC, OP and xylylalanine residues ($\mu\text{g kg}^{-1}$ wet mass) in pollen grain samples collected from ten locations across four Egyptian governorates (Giza, El-Dakahlia, El-Qalyubia, and Gharbia) during spring 2023. Unlike honey, all collected pollen grain samples contained detectable pesticide residues, ranging from below LOQ to exceeding MRLs. Meanwhile, similar to honey, endrin, 4,4DDT, 4,4DDE, heptachlor epoxide, heptachlor, endosulfan I, dieldrin, endosulfan II, endosulfan sulfate, and chlordane were not detected in any pollen samples.

Three pesticides, including HCH, methoxychlor, and metalaxyl, were found at levels below LOQ in pollen grain samples from different site across all four governorates. Within Giza, all pollen samples from AL-Qanater, Kerdasah and EL-Hawamdeya showed levels of those pesticides below the LOQ except for HCH in the pollen sample from EL-Hawamdeya which recorded $18.83 \mu\text{g kg}^{-1}$. For xylylalanine, a fungicide, all samples recorded levels below the LOQ except for a single pollen sample from Kerdasah, which was not detected.

Table 2. Concentrations ($\mu\text{g kg}^{-1}$ wm) of pesticides detected in pollen grain samples collected from different locations in Egypt Delta with their EU maximum residue limits (MRLs) during spring 2023.

| Name of pesticides | Uses | Chemical class | Location of pollen samples | | | | | | | | | | MRLs $\mu\text{g kg}^{-1}$ |
|--|-------------|-----------------|---|-------|-------|---------------------|-------|-------|---------------------|-------|-----------------|-------|----------------------------|
| | | | Concentration of pesticides $\mu\text{g kg}^{-1}$ | | | | | | | | | | |
| | | | Giza (n = 9) | | | El-Dakahlia (n = 9) | | | El-Qalyubia (n = 6) | | Gharbia (n = 6) | | |
| I* | II* | III* | I** | II** | III** | I*** | II*** | I**** | II**** | | | | |
| Aldrin | Insecticide | Organochlorine | 14.62 | 7.23 | 14.88 | ND | ND | 10.93 | 18.85 | ND | ND | ND | 10 |
| Endrin | Insecticide | Organochlorine | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 10 |
| HCH (Hexachlorocyclohexane) | Insecticide | Organochlorine | < LOQ | < LOQ | 18.83 | < LOQ | 52.82 | 29.16 | ND | < LOQ | ND | ND | 10 |
| 4,4DDT | Insecticide | Organochlorine | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 10 |
| 4,4DDE | Insecticide | Organochlorine | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 50 |
| Heptachlor | Insecticide | Organochlorine | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 10 |
| Heptachlor epoxide | Insecticide | Organochlorine | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 10 |
| Methoxychlor | Insecticide | Organochlorine | ND | < LOQ | < LOQ | ND | < LOQ | ND | ND | ND | ND | ND | 10 |
| Dieldrin | Insecticide | Organochlorine | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 10 |
| Endosulfan I (Hexachlorocyclopentadiene) | Insecticide | Organochlorine | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 10 |
| Endosulfan II | Insecticide | Organochlorine | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 10 |
| Endosulfan sulfate | Insecticide | Organochlorine | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 10 |
| Chlordane | Insecticide | Organochlorine | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 10 |
| Metalaxyl | Fungicide | Xylylalanine | < LOQ | ND | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | 500 |
| Chlorpyrifos | Insecticide | Organophosphate | 128.9 | 97.98 | 82.52 | 67.07 | ND | 69.8 | ND | ND | 10.7 | ND | 10 |

- Pollen samples location in Giza: (I* = AL-Qanater, II* = Kerdasah, III* = EL-Hawamdeya), in El-Dakahlia: (I** = Mansoura, II** = Mit-Ghamr, III** = EL-Sembellawein), in El-Qalyubia: (I*** = Banha, II*** = Toukh) and in Gharbia: (I**** = Basioun, II**** = Tanta). - LOQ: limit of quantification, ND: none detected.

Aldrin and chlorpyrifos presence in pollen grain samples from all study areas raises significant concerns. Aldrin was detected in pollen grain samples from Giza in all tested sites at concentrations ranging from 7.23 to 14.88 $\mu\text{g kg}^{-1}$. Aldrin also was present in one pollen grain sample from EL-Sembellawein (10.93 $\mu\text{g kg}^{-1}$), and Banha sites (18.85 $\mu\text{g kg}^{-1}$) in both El-Dakahlia and El-Qalyubia governorates, respectively. In contrast, aldrin was not detected in any pollen samples in the Gharbia governorate. In the Giza governorate, chlorpyrifos levels were significantly high in all studied areas. Notably, chlorpyrifos residues in pollen grain samples from AL-Qanater, Kerdasah, and EL-Hawamdeya exceeded MRLs by more than 13, 10, and 8 folds, respectively, meanwhile, it was absent in all sites of El-Qalyubia governorate.

Table 3 presents the EDI, ADIs, and overall HQ of pesticide residues detected in honey and pollen samples that were collected in Egypt's middle Delta during spring 2023. The estimated amount of the aldrin insecticide residue a person might consume each day is recorded as $4 \times 10^{-2} \mu\text{g kg}^{-1}$ bw/day, which is lower than the ADI (0.1 $\mu\text{g kg}^{-1}$ bw/day), and the HQ is 4×10^{-1} ; suggesting a high potential health risk. The EDI for HCH in honey ($1 \times 10^{-4} \mu\text{g kg}^{-1}$ bw/day) is much lower than the ADI (5 $\mu\text{g kg}^{-1}$ bw/day), and the HQ is 2×10^{-5} ; suggesting a very low potential health risk. On the other hand, the EDI for HCH in pollen ($8 \times 10^{-3} \mu\text{g kg}^{-1}$ bw/day) is lower than the ADI (5 $\mu\text{g kg}^{-1}$ bw/day), and the HQ is 1.6×10^{-3} ; suggesting a very low potential health risk.

4 Discussions

Analysis of pesticide residue in honey serves two important purposes: it safeguards consumer health by identifying potential risks and sheds light on the pesticide practices employed in surrounding crop fields. Fifteen pesticides were assessed in honey samples from the Egyptian Nile Delta, all of them are OCs and OPs, with one exception, metalaxyl, which is a fungicide. These pesticides

were widely used in the past but now banned or restricted in many countries due to concerns about their safety and environmental impacts.^{31,32} However, the presence of those pesticides in Egyptian honey is a recurring issue, as multiple studies have shown them to be the most frequently detected contaminants in samples from various regions including dieldrin and endosulfan I¹⁵, chlorpyrifos,^{18,19} metalaxyl, HCH, 4,4DDE, endrin and methoxychlor,³³ chlorpyrifos, heptachlor epoxide, endrin, aldrin, and HCH.^{16,34}

The present study revealed high concentrations of aldrin, HCH, chlorpyrifos (insecticides), and metalaxyl (fungicide) in honey samples. The substantial presence of these compounds, particularly aldrin, HCH, and chlorpyrifos, suggests their potential insecticide applications to surrounding crops during winter season. Across nine hives sampled in Giza governorate, the average aldrin concentration average was 15.03 $\mu\text{g kg}^{-1}$, and 13.33 $\mu\text{g kg}^{-1}$ in all hives located in El-Dakahlia which aligns with previous studies reported by.^{16,17,34} Also, higher levels of HCH and chlorpyrifos were found in honey samples from two sites in Giza (Kerdasah and EL-Hawamdeya), two sites in El-Dakahlia (Mansoura and EL-Sembellawein), two sites of El-Qalyubia (Banha and Toukh), and one site in Gharbia (Tanta) governorates, suggesting its increased use as an insecticide in all the studied areas. This consistent with our findings from El-Qalyubia governorate, Egypt, ranged from 0.06 to 18.70 ng/g¹⁶. On the other hand, Al Naggar et al.,³⁵ recorded chlorpyrifos as the most frequently detected organophosphorous insecticides in pollen grain collected from Delta apiaries in Egypt. Recently, Nassar et al.,¹⁹ reported chlorpyrifos and profenofos residues in honey and wax samples from various Egyptian regions. Chlorpyrifos concentrations ranged from 0.07 to 39.16 ng/g, while profenofos levels were between 1.94 and 17.00 ng/g.

The present results recorded three pesticides (aldrin, HCH, and chlorpyrifos) in both honey and pollen samples exceeded the maximum residue limits (MRLs) that documented by the European Union (EU), as established by the European Food

Table 3. Estimated daily intake EDI ($\mu\text{g}/\text{kg}$ body weight/day), acceptable daily intake ADIs ($\mu\text{g}/\text{kg}$ body weight/day), overall hazard quotient (HQ), and health risk of pesticide residues found in honey and pollen grains collected during spring 2023 from Nile Delta governorates of Egypt.

| Name of pesticide | Honey | | | | Pollen | | | |
|-------------------|---|--|--------------------|-------------|--|--|----------------------|-------------|
| | ADI* ($\mu\text{g}/\text{kg}$ b w/day) | EDI** ($\mu\text{g}/\text{kg}$ b w/day) | HQ*** | Health risk | ADI ($\mu\text{g}/\text{kg}$ b w/day) | EDI ($\mu\text{g}/\text{kg}$ b w/day) | HQ | Health risk |
| Aldrin | 0.1 | 4×10^{-2} | 4×10^{-1} | Yes | 0.1 | 3×10^{-4} | 3×10^{-3} | No |
| HCH | 5 | 1×10^{-4} | 2×10^{-5} | No | 5 | 8×10^{-3} | 1.6×10^{-3} | No |
| Chlorpyrifos | 10 | 5×10^{-4} | 5×10^{-5} | No | 10 | 2×10^{-3} | 2×10^{-4} | No |

^aADI: Developed by the Codex Alimentarius Commission in collaboration with the JMPR (Joint FAO/WHO Meeting on Pesticide Residues), EPA (Environmental Protection Agency), and EFSA (European Food Safety Authority) to regulate pesticide residues. ^bEDI = $C \times \text{Con}/\text{BM}$, where C is the mean levels of a specific pesticide residue found within honey samples ($\mu\text{g kg}^{-1}$), Con (kg person⁻¹ d⁻¹) is the daily average intake per person (5.5 g honey and 1.2 g pollen per person approximately), and BM = body mass (kg person⁻¹), the mean body weight, which was considered 60 kg. ^cHQ, hazard quotient = EDI/ADI.

Safety Authority (EFSA). Also, the MRLs for these pesticides are $10 \mu\text{g kg}^{-1}$, in Kerdasah site (Giza), while the measured levels of aldrin, HCH, and chlorpyrifos in honey samples were 19.6, 21.7, and $190.74 \mu\text{g kg}^{-1}$, respectively. In addition, the same previous pesticides in Banha site, (El-Qalyubia) Mit-Ghamr site (El-Dakahlia), and AL-Qanater site (Giza), were recorded at very high and unacceptable levels which were 18.85, 52.82 and $128.9 \mu\text{g kg}^{-1}$ in pollen samples, respectively. Worryingly, pollen samples collected across all study areas contained residues of aldrin and chlorpyrifos, both insecticides exceeding MRLs. Honey safety is protected by the default level of MRLs. Comparing these limits to measured pesticide levels in honey samples helps identify potential risks. However, even if levels stay below the MRLs, chronic exposure to low levels might still pose health concerns.³⁶ MRLs are based on short-term effects and may not fully capture long-term risks. Continuous development of food safety regulations by organizations like EFSA is crucial.³⁷

A comparison of EDI with ADI, established by FAO in 1965²⁹ is crucial for assessing potential health risks. In this study, the EDI for chlorpyrifos in honey ($5 \times 10^{-4} \mu\text{g kg}^{-1}$ bw/day) is much lower than the ADI ($10 \mu\text{g kg}^{-1}$ bw/day), and the HQ is 5×10^{-5} . This suggests a very low potential health risk from chlorpyrifos in honey. The same trend was recorded for aldrin, HCH, and chlorpyrifos in the collected pollen grain samples. Our results revealed that the HQ in honey samples ranged from 4×10^{-1} to 5×10^{-5} , and in pollen samples from 3×10^{-3} to 2×10^{-4} during spring 2023. These values are significantly lower than the thresholds typically associated with adverse health effects. However, an exception was found for aldrin in honey samples, where the HQ indicated a negligible risk of negative health outcomes from honey consumption. The current evaluation of potential adverse effects from aldrin, HCH, and chlorpyrifos in Egyptian honey yielded higher HQ values compared to previous studies.^{15,16,19,34} These variations might be due to factors such as targeted pesticide analysis, differing national ADI values and consumption patterns, seasonal fluctuations, and HQ calculation methods. OCs, known for their resistance to degradation and their tendency to accumulate in soil³¹ and fatty tissues,³⁸ pose a significant environmental threat. The long half-lives of compounds like DDT, aldrin, heptachlor, and chlorpyrifos³⁹ coupled with the potential for illegal use, explain their continued presence in the local environment.

5 Conclusions

This study highlights the presence of various pesticides in honey and pollen samples. Higher pesticide residue levels were found in

samples collected from Giza and El-Dakahlia governorates compared to El-Qalyubia and Gharbia governorates. These findings suggest a potential trend of greater pesticide use in southern Nile Delta region of Egypt. Aldrin, HCH (OCs), and chlorpyrifos (OPs) were frequently detected in the honey and pollen grain samples analyzed. The HQ for aldrin in honey samples indicated a negligible risk of negative health effects from honey consumption. While, most detected pesticides in honey or pollen grain samples were not found or present at levels below the MRLs. However, the levels of pesticides in the honey samples were below the MRLs, there is still some concern about the potential health effects of long-term exposure to low levels of pesticides. Further research is needed to determine the safety of consuming honey containing low levels of pesticides.

Acknowledgments

Not applicable.

Author contributions

The authors of this manuscript contributed equally to the design and/or execution of the experiments described in the manuscript. AEA, conducted the work and the analysis of data; RAS and AMA, prepared and edited the final version of this manuscript. All authors approved the final manuscript.

Funding

This work was not supported by any funding body, but personally financed.

Conflict of interest statement: All authors declare that they have no conflicts of interest.

References

1. Amro AM. Pollinators and pollination effects on three canola (*Brassica napus* L.) cultivars: a case study in upper Egypt. *J King Saud Univ Sci.* 2021;33(1):101240.
2. Hirpara P. Honey: a functional food and its application in food products (doctoral dissertation). *J Xidian Univ.* 2023;782:10.37896/jxu17.10/071.
3. Kowalczyk I, Gębski J, Stangierska D, Szymańska A. Determinants of honey and other bee products use for culinary, cosmetic, and medical purposes. *Nutrients.* 2023;15(3):737.

4. Evans JD, Chen YJ. Colony Collapse Disorder and Honey Bee Health. In: Kane TR, Faux CM, editors. *Honey Bee Medicine for the Veterinary Practitioner*. Hoboken, NJ, USA: John Wiley & Sons, Ltd., 2021; 2, pp. 229–234. <https://doi.org/10.1002/9781119583417.ch19>.
5. Asritha C, Satwika M, Rajashekhar M, Bharghavi K, Priyanka N, Keerthana B, Ramesha NM. Bee poisoning-a potential threat to biodiversity. *Chron Biosour Manage*. 2024;8(1):009–011. <https://ojs.pphouse.org/index.php/>.
6. Kerkich K, Bouargane B, El Laghdach A, Souhail B, Kadmi Y. Recent advances in the extraction, purification and analysis of emerging pesticides in honey products: a review. *J Food Compos Anal*. 2023;127:105947.
7. Mohamed IA, Omar EM, Tawfik AI, Amro AM, Al Naggar Y. Sublethal effects of herbicides clethodim, haloxyfop-P-methyl, and their mixture on honey bee health. *Apidologie*. 2023;54(1):2–17.
8. Rondeau S, Raine NE. Fungicides and bees: a review of exposure and risk. *Environ Int*. 2022;165:107311. <https://doi.org/10.1016/j.envint.2022.107311>.
9. Albero B, Miguel E, García-Valcárcel AI. Acaricide residues in beeswax. Implications in honey, brood and honeybee. *Environ Monit Assess*. 2023;195(4):454.
10. Bogdanov S. Contaminants of bee products. *Apidologie*. 2006;37(1):1–18.
11. Hester KP, Stoner KA, Eitzer BD, Koethe RW, Lehmann DM. Pesticide residues in honey bee (*Apis mellifera*) pollen collected in two ornamental plant nurseries in Connecticut: implications for bee health and risk assessment. *Environ Pollut*. 2023;333:122037.
12. Leska A, Nowak A, Nowak I, Górczyńska A. Effects of insecticides and microbiological contaminants on *Apis mellifera* health. *Molecules*. 2021;26(16):5080.
13. Liang CP, Sack C, McGrath S, Cao Y, Thompson CJ, Robin LP. US Food and Drug Administration regulatory pesticide residue monitoring of human foods: 2009–2017. *Food Addit Contam: Part A*. 2021;38(9):1520–1538.
14. Ashraf SA, Mahmood D, Abd Elmoneim OE, Siddiqui AJ, Khan MI, Ashfaq F, Patel M, Snoussi M, Kieliszek M, Adnan M. Exposure to pesticide residues in honey and its potential cancer risk assessment. *Food Chem Toxicol*. 2023;180:114014.
15. Eissa F, El-Sawi S, Zidan NEH. Determining pesticide residues in honey and their potential risk to consumers. *Pol J Environ Stud*. 2014;23(5):1573–1580.
16. Malhat FM, Haggag MN, Loutfy NM, Osman MA, Ahmed MT. Residues of organochlorine and synthetic pyrethroid pesticides in honey, an indicator of ambient environment, a pilot study. *Chemosphere*. 2015;120:457–461.
17. Shendy AH, Al-Ghobashy MA, Mohammed MN, Alla SAG, Lotfy HM. Simultaneous determination of 200 pesticide residues in honey using gas chromatography–tandem mass spectrometry in conjunction with streamlined quantification approach. *J Chromatogr A*. 2016;1427:142–160.
18. Al Naggar Y, Codling G, Giesy JP. Human dietary intake and hazard characterization for residues of neonicotinoides and organophosphorus pesticides in Egyptian honey. *Toxicol Environ Chem*. 2017;99(9–10):1397–1408.
19. Nassar AM, Salim YM, Nour-Eldeen E, Younis MS, Kelany MM, Shebl MA, Shafey AS, Abou-Shaara HF. Seasonal screening of pesticide residues in beehive products collected from different districts in Egypt. *Environ Monit Assess*. 2024;196(3):297.
20. Barakat AO, Mostafa A, Wade TL, Sweet ST, El Sayed NB. Distribution and ecological risk of organochlorine pesticides and polychlorinated biphenyls in sediments from the Mediterranean coastal environment of Egypt. *Chemosphere*. 2013;93(3):545–554.
21. Mansour SA. Environmental impact of pesticides in Egypt. *Rev Environ Contam toxicol*, vol 196. New York, NY: Springer, 2008, 1–51. https://doi.org/10.1007/978-0-387-78444-1_1.
22. Abou-Shaara H. Potential honey bee plants of Egypt. *Cercetari Agronomice in Moldova*. 2015;48(2):99–108. <https://repository.uaiasi.ro/xmlui/handle/20.500.12811/1530>.
23. Louveaux J, Maurizio A, Vorwhol G. Methods of melissopalynology. *Bee World*. 1978;59(4):139–157.
24. Maurizio A. Microscopy of honey. In: Crane E, editors. *Honey: a comprehensive survey*. London: Heinemann in cooperation with the International Bee Research Assassination; 1975. pp. 240–257.
25. Kirk WDJ. *A colour guide to pollen loads of the honeybee*. Cardiff: International Bee research Association; 1994 p. 54.
26. Dimou M, Thrasyvoulou A. A comparison of three methods for assessing the relative abundance of pollen resources collected by honeybee colonies. *J Apic Res*. 2007;46(3):143–147.
27. Codex. *Codex Alimentarius commission-pesticides residues in food*. 2nd ed. Joint: FAO/WHO food standards Programme; 1993.
28. Guidance SANTE 11312/2021 V2. Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed. European Commission. Rome, Italy: Accredia, 2021, SANTE 11312, pp. 1–57. https://www.eurl-pesticides.eu/docs/public/tmpl_article.asp?CntID=727.
29. Evans RM, Scholze M, Kortenkamp A. Examining the feasibility of mixture risk assessment: a case study using a tiered approach with data of 67 pesticides from the joint FAO/WHO meeting on pesticide residues (JMPPR). *Food Chem Toxicol*. 2015;84:260–269.
30. Juan-Borrás M, Periche A, Domenech E, Escriche I. Routine quality control in honey packaging companies as a key to guarantee consumer safety. The case of the presence of Sulfonamides analyzed with LC-MS-MS. *Food Control*. 2015;50:243–249.
31. Mukiibi SB, Nyanzi SA, Kwetegyeka J, Olisah C, Taiwo AM, Mubiru E, Tebandeke E, Matovu H, Odongo S, Abayi JJM, et al. Organochlorine pesticide residues in Uganda's honey as a bioindicator of environmental contamination and reproductive health implications to consumers. *Ecotoxicol Environ Saf*. 2021;214:112094.
32. Morariu ID, Avasilcai L, Vieriu M, Lupu VV, Ioniuc I, Morariu BA, Lupu A, Morariu PC, Pop OL, Burduoiu VM, et al. A comprehensive narrative review on the hazards of bee honey adulteration and contamination. *J Food Qual*. 2024;3512676:1–13.
33. Abdallah OI, Hanafi A, Abdel Ghani SB, Ghisoni S, Lucini L. Pesticides contamination in Egyptian honey samples. *J Consum Prot Food Saf*. 2017;12(4):317–327.
34. Abdel-Rahman TA, Asmaa AE, Hatem MM. Assessment of pesticide residues in honey and their prospective risk to consumers in Egypt. *Egypt J Plant Prot Res Inst*. 2020;3(4):1028–1034.
35. Al Naggar Y, Codling G, Vogt A, Naiem E, Mona M, Seif A, Giesy JP. Organophosphorus insecticides in honey, pollen and bees (*Apis mellifera* L.) and their potential hazard to bee colonies in Egypt. *Ecotoxicol Environ Saf*. 2015;114:1–8.
36. Khaled O, Ryad L, Nagi M, Eissa F. Development and validation of a multiclass method for the determination of veterinary drug residues in honey. *Int J Environ Anal Chem*. 2024;1-18:1–18.
37. European Food Safety Authority (EFSA). International framework dealing with human risk assessment of combined exposure to multiple chemicals. *EFSA J*. 2013;11(7):3313.
38. Ruiz-Toledo J, Vandame R, Castro-Chan RA, Penilla-Navarro RP, Gómez J, Sánchez D. Organochlorine pesticides in honey and

pollen samples from managed colonies of the honey bee *Apis mellifera* Linnaeus and the stingless bee *Scaptotrigona mexicana* Guérin from southern Mexico. *Insects*. 2018;9(2):54.

39. Ritter L, Solomon KR, Forget J, Stemeroff M, O'Leary C. Persistent organic pollutants, an assessment report on DDT, Aldrin, Dieldrin, Endrin, Chlordane, Heptachlor, Hexachlorobenzene, Mirex,

Toxaphene, Polychlorinated biphenyls, Dioxins and Furans. *The International Programme on Chemical Safety (IPCS)*. Intergovernmental Negotiating Committee for an internationally legally binding instrument for implementing international action on certain persistent organic pollutants, First session, Montreal, 1998: PCS 95.39. UNEP/POPS/INC.1/INF/10.