Pesticide residues in different honey types and public health risk assessment

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Abstract

Honeybees and humans are endangered by pesticides in daily agricultural production. The aim of this research was to investigate pesticide residues in different honey types and to assess the risk to public health. A total of 88 honey samples originating from pine, multifloral, sunflower, acacia, linden, and canola were collected and analysed by a QuEChERS method. The hazard quotient (HQ) was used to evaluate the risk of detected pesticide residues. Analysis of pine honey did not detect any residue of investigated pesticides. The most frequently detected pesticides in the honey samples were chlorpyrifos ranging between 15.1 µg/kg (linden honey) to 22.3 µg/kg (multifloral honey), clothianidin ranging between 12.0 µg/kg (acacia honey) to 22.0 µg/kg (canola honey), dimethoate ranging between 8.9 µg/kg (multifloral honey) to 18.9 µg/kg (canola honey), and thiamethoxam ranging between 4.2 µg/kg (linden honey) to 15.6 µg/kg (canola honey), respectively. The lowest estimated daily intake (EDI) of 128×10^{-3} µg/kg of body weight per day was found in acacia honey, and the highest EDI of 265×10^{-3} µg/kg of body weight per day was found in canola honey. Similar values of EDI were determined for multifloral, sunflower, and linden honey (186×10^{-3}, 187×10^{-3}, and 183×10^{-3}), respectively. The HQ value for pine honey was 0 indicating that this honey is the safest for consumption, however, the other types of honey investigated in this study posed no risk to humans after potential consumption.

Honeybees, food safety, food analysis, honey, QuEChERS, environment

It is well established that pesticides play a beneficial role in agriculture. Though small amounts of pesticide residue remain in the food supply, they help combat a variety of pests that destroy crops (Mukherjee 2009). Honeybees are good biological indicators due to two factors: the analyte content of bees that died as a result of pesticide poisoning; and the residues present in their bodies or in beehive products that may be detected by laboratory analyses (Hung and Yiin 2023). Checking for pesticides such as bifenazate, bupirimate, buprofezin, cyprodinil, cyazofamid, and others in honey can provide information about the use of pesticides in and near crop fields (Prasanth et al. 2022). Due to their different chemical structures, pesticides belong to different classes and chemical groups. Their overuse or incorrect use can pose a threat to human health and the environment because of their chronic and subacute toxicity (Jepson et al. 2020). Organophosphorus and carbamates are the most widely used pesticides replacing organochlorine pesticides. Pesticide residues are transferred to honey by bees that feed on contaminated blossoms (Lika et al. 2021). Products made from honey are perceived as natural, healthy, and clean (Tauber et al. 2019), especially in developing countries, where honey is commonly consumed by adults and children. For this reason, honey must not be contaminated with chemicals and must be safe for human consumption (Vapa Tankosić et al. 2022). There

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are, however, several environmental problems associated with over-reliance on pesticides, such as pesticide residues in food (Wilkowska and Biziuk 2011; Bursić et al. 2021). Even if small amounts of pesticide residues remain in the food supply, they constitute a potential risk to human health because of their subacute and chronic toxicity (Anaduaka et al. 2023). Since some pesticides are carcinogenic and others can cause dysfunctions in the nervous and reproductive systems, they can be extremely harmful to human health, even at low concentrations (Khalil et al. 2022).

Nowadays, a variety of pollutants are present in the environment in which bee products are produced. When pesticides are applied to crops, they can negatively affect soil (Wołejko et al. 2020), air (Zaller et al. 2022), and water (Agarski et al. 2023) as well as the flowers that bees collect nectar from to make honey. The food chain may be contaminated with these toxic chemicals, which may affect human health. Several studies have demonstrated that organochlorines accumulate in the aerial and root tissues of plants and organisms from contaminated soil (Singh and Singh 2017). Bioconcentration of fat-soluble pesticides by these organisms is 10–1000 times higher than their concentration in the surrounding environment. A monitoring program is required to determine the proper assessment of human exposure to pesticides due to the presence of pesticide residues in honey. Therefore, when making policy decisions, health hazards should be taken into account as part of the decision-making process.

It is also possible for hives to be contaminated directly or indirectly. The first case may have been caused by pesticide residues caused by acaricides used to control *Varroa destructor* (Higes et al. 2020). The second case involves bees being exposed to pesticides when foraging within up to a few kilometres away from the hive (Beekman and Ratnieks 2000). It is possible that pesticides may suppress the beneficial properties of honey (Berenbaum 2016) and, when present in significant amounts, may pose a serious threat to human health, therefore, determining them in honey and other bee products has become a growing concern in recent years (Milone and Tarpy 2021). Pesticide residue monitoring in honey helps determine whether this product poses a risk to consumer health and provides information on pesticide treatments used on nearby fields. Bees and their products may be useful indicators of pollution in their areas based on research by several authors (Simon-Delso et al. 2017).

The monitoring of bee products is primarily intended to protect consumer health, increase international competition, and improve the product quality. Pesticide residue limits (MRLs) in honey are determined by different national regulations, but lack of homogeneity creates problems in international trade and marketing. It is important to examine the possible health effects of honey residues before evaluating their potential risks (El-Nahhal 2020).

To assess the potential health risks associated with honey contamination, this study examined pesticide residues in different types of honey.

Materials and Methods

The pesticide mix standards (dissolved in acetonitrile) were purchased from LabStandard (Castellana Grotte, Italy). The concentration of all pesticides in standards was 100 µg/ml. The concentration of the working mix standard solutions in acetonitrile was 1 µg/ml. As an internal standard, 10 g/ml of carbofuran-D3 was used. J.T. Baker (Gliwice, Poland), was the supplier of acetonitrile and methanol. High-performance liquid chromatography (HPLC) ultra gradient grade organic solvents were used in the experiment. Analytically graded formic acid was supplied by Fisher Scientific (Loughborough, United Kingdom). For extraction and clean-up, the Hillium QuEChERS extraction pouch 550 ml (P/N QEHLL0510P) and the Hillium QuEChERS dispersive kit 15 ml (P/N QDHLL15032) (Heidenrod, Germany) were used.

The analyses comprised 88 honey samples (9 samples of pine, 23 samples of multifloral, 24 samples of sunflower, 17 samples of acacia, 8 samples of linden, and 7 samples of canola honey) collected from different local markets of Vojvodina (Serbia) produced and obtained in the year 2022. Before storing the obtained samples, H NMR analysis of organic extracts of honey was performed to confirm its botanical origin. Following, samples were stored in plastic containers in a refrigerator (4 °C), until further analysis. The sampling was performed following SANTE/11312/2021.

The steps of using the QuEChERS method for pesticide extraction from honey samples are described in Fig. 1 (Plate XI).

Pesticides were detected using an HPLC Agilent 1290 Infinity II chromatograph (Santa Clara, USA) coupled to an Agilent 6470 TSQ mass spectrometer with AJS ESI (Jet Stream Technology Ion Source). For the chromatographic separation,a Zorbax Eclipse Plus C18 column Rapid Resolution HD (50 × 2.1mm, 1.8 µm particle size) was used. An injection volume of 2 µl for the LC system was used, with the mobile phase flow rate at 0.3 ml/min, with the temperature of the column kept constant at 35 °C. In a gradient mode, pesticides were separated by chromatographic separation using water (A) and acetonitrile (B) in a mobile phase containing formic acid (0.1%, v/v). The mobile phase flow rate was 0 min 5% B; 1 min 5% B; 2 min 15% B; 2.5 min 30% B; 6 min 45% B and 12 min 95% B. This study was conducted using an ESI source set to 200 °C for the drying gas, 16 l/min for the drying gas flow rate, 40 psi for the nebulizer pressure, 350 °C for the sheath gas temperature, 12 l/min for sheath gas flow and 3,000 V for the capillary voltage. Dynamic multiple reaction monitoring was used for detection. Optimization and quantification were performed using Agilent MassHunter (version B.10.1 SR1 Agilent Technologies, 2006–2019).

Using the chromatogram of the sample spiking at the lowest concentration level, the limits of detection (LOD) were calculated using a signal-to-noise ratio of 5.0. The limit of quantification (LOQ) was set at 0.01 mg/kg. Internal standard calibration was used to check linearity from 10 to 100 µg/kg. Analysing honey samples spiked at 10 grams and 50 µg/kg were used for accuracy (recovery) and precision (repeatability, % RSDr).

An analysis of pesticides was conducted by LC-MS/MS in positive electrospray ionization (ESI+) and fragmentation of the H^+ molecular ion is shown in Table 1, along with an average recovery rate and R^2, respectively. A selected reaction monitoring mode (SRM) for each pesticide detection was performed to obtain the highest sensitivity, whereas two transitions of the SRM were used for pesticide confirmation, taking into account the retention time (Rt) as it relates to each pesticide detection.

The average daily consumption of honey in adults is used to calculate their pesticide exposure. Using the European Commission's maximum residue limit (MRL), chronic effects on public health are evaluated. FAO and WHO recommended acceptable daily intakes (ADIs) as percentages of estimated daily intakes (EDIs), while ADIs were calculated based on a mice model for carcinogenicity: NOAEL = 10 mg/kg of body weight/day. To calculate the EDIs of the pesticide residues, the following equation was used (Puvača et al. 2023):

$$EDI = (C \times K)/BW$$

where:
EDI – estimated daily intake (µg/kg of body weight/day);
C – average concentration of pesticides in honey (µg/kg);
K – average consumption rate (kg of honey/day);
BW – average human body weight (kg).

Approximately 0.828 kg of honey per person is consumed annually by the European adult populations. A mean body weight of 70.8 kg was set as the normal distribution for European adults aged 20 years and older, respectively.

Various types of honey were assessed for pesticide residue risk using the Hazard Quotient (HQ). The HQ was determined for each pesticide found in honey, in addition to dietary exposure to pesticides. To compute the HQs, the following formula was used:

$$HQ = EDI/ADI$$

As long as the HQ is ≤ 1, no adverse effects are likely to occur (health-protecting). In the case of HQ > 1, chronic effect occurrence is of high concern. Chronic toxic effects are more likely to occur at higher HQs, emphasizing the need for immediate risk management.

Results

Following SANTE/11312/2021, the validated Liquid Chromatography with tandem mass spectrometry (LC-MS/MS) method obtained good linearity coefficients in the range of 10 to 100 µg/kg for investigated pesticides, with R^2 above 0.99. It was determined that honey has a strong influence on pesticides based on the matrix and solvent calibration graph slopes. Matrix match calibration was used to compensate for matrix effects (ME). Samples that were spiked with 10 µg/kg of honey.

The LOQ was determined experimentally for each pesticide at 0.01 mg/kg as the lowest quantified value. Two levels of recovery studies were conducted with blank honey samples spiked at 10 and 500 µg/kg. Among the others, bifenazate, bupirimate, and cyprodinil showed an average recovery of 69.1, 69.5, and 69.8%, while bendiocarb showed an average recovery of 71.3 and cyproconazole showed an average recovery of 98.3%. As measured by a relative standard deviation (RSD), the repeatability ranged from 2.58 to 10.48%.

Table 1. Multiple reaction monitoring transitions (MRM), fragmentation energy (Frag), collision energies (CE), retention time (Rt), recovery (Re), and correlation coefficients (R^2).

Pesticide	Precursor ion (m/z)	Product ions (m/z)	Frag (V)	CE (V)	Rt (min)	Re (%) ± RSD	R^2
Bendiocarb	224.1	167.1	120	12	5.30	71.3 ± 8.25	0.9984
		109.1		25			
Bitertanol	338.2	296.2	120	5	7.21	78.3 ± 4.45	0.9989
		99.1		10			
Bifenazate	301.1	198.2	120	20	10.56	69.1 ± 3.56	0.9938
		170.1		16			
Benzoximate	364.0	105.0	120	15	7.98	89.5 ± 3.21	0.9899
		99.1		15			
Bupirimate	317.2	166.1	120	33	6.13	69.5 ± 9.43	0.9911
		108.1		35			
Buprofezin	306.2	201.1	120	5	8.54	81.2 ± 8.21	0.9845
		116.1		10			
Carbaryl	202.1	145.1	120	4	7.73	90.3 ± 2.58	0.9999
		127.1		28			
Chlorpyrifos	349.93	198.0	120	20	12.9	76.3 ± 7.76	0.9961
		97.0		41			
Clothianidin	250.0	169.0	120	8	5.00	78.2 ± 9.15	0.9903
		131.9		8			
Cyproconazole	292.1	125.0	120	32	6.41	98.3 ± 3.20	0.9918
		70.0		16			
Cyprodinil	226.1	76.9	140	50	6.38	69.8 ± 7.21	0.9932
		65.1		56			
Cyazofamid	325.0	261.0	120	4	9.92	87.2 ± 10.48	0.9918
		108.0		8			
Dimethoate	230	198.8	120	0	4.78	83.3 ± 4.12	0.9987
		125		16			
Omethoate	214	125	120	16	1.65	92.4 ± 5.42	0.9981
		109		24			
Thiacloprid	253	186	120	10	5.34	86.8 ± 6.11	0.9992
		126		20			
Thiamethoxam	292	211	120	211	4.26	74.9 ± 4.93	0.9991
		181		181			
Carbofuran-D3	225.1	165	120	10	7.83		
		123		22			

RSD - Relative standard deviation

The highly sensitive and selective LC-MS/MS technique was used to analyse the 88 honey samples; the total ion chromatograms (TIC) of analysed honey samples are illustrated by Fig. 2 (Plate XI).

Based on the obtained results it can be noticed that analysis of pine honey did not detect any residue of investigated pesticides. Residues of pesticides were detected in multifloral honey samples ranging between 3.3 µg/kg (bendiocarb) and 22.3 µg/kg (chlorpyrifos). The presence of buprofezin was detected in sunflower (5.0 µg/kg) and canola honey (4.6 µg/kg). The most detected pesticides in honey samples were chlorpyrifos ranging between 15.1 µg/kg (linden honey) to 22.3 µg/kg (multifloral honey), clothianidin ranging between 12.0 µg/kg (acacia honey) to 22.0 µg/kg (canola honey), dimethoate ranging between

Table 2. Pesticide residues in 88 honey samples of different types (mean concentration and detection frequency).

Pesticide	Type of honey									
	Pine (n = 9)		Multifloral (n = 23)		Sunflower (n = 24)		Acacia (n = 17)		Linden (n = 8)		Canola (n = 7)	
	µg/kg ± SD	DF	µg/kg ± SD	DF	µg/kg ± SD	DF	µg/kg ± SD	DF	µg/kg ± SD	DF	µg/kg ± SD	DF
Bendiocarb	nd	-	3.3 ± 0.2	3 (13%)	nd	-	nd	-	nd	-	nd	-
Bitertanol	nd	-	nd	-	nd	-	nd	-	nd	-	nd	-
Bifenazate	nd	-	nd	-	nd	-	nd	-	nd	-	nd	-
Benzoximate	nd	-	nd	-	nd	-	nd	-	nd	-	nd	-
Bupirimate	nd	-	nd	-	nd	-	nd	-	nd	-	nd	-
Buprofezin	nd	-	nd	-	5.0 ± 0.1	4 (17%)	nd	-	nd	-	4.6 ± 0.1	1 (14%)
Carbaryl	nd	-	nd	-	nd	-	nd	-	nd	-	nd	-
Chlorpyrifos	nd	-	22.3 ± 0.1	5 (22%)	21.0 ± 0.2	7 (29%)	nd	-	15.1 ± 0.1	1 (13%)	21.7 ± 0.2	2 (29%)
Clothianidin	nd	-	14.0 ± 0.3	6 (26%)	16.5 ± 0.1	5 (21%)	12.0 ± 0.2	5 (29%)	16.5 ± 0.3	2 (25%)	22.0 ± 0.1	2 (29%)
Cyproconazole	nd	-	4.1 ± 0.2	2 (9%)	nd	-	nd	-	nd	-	nd	-
Cyprodinil	nd	-	nd	-	nd	-	nd	-	nd	-	nd	-
Cyazofamid	nd	-	nd	-	nd	-	17.0 ± 0.1	4 (24%)	nd	-	nd	-
Dimethoate	nd	-	8.9 ± 0.1	6 (26%)	10.0 ± 0.1	3 (13%)	11.0 ± 0.1	5 (29%)	17.3 ± 0.2	2 (25%)	18.9 ± 0.2	3 (43%)
Omethoate	nd	-	nd	-	nd	-	nd	-	nd	-	nd	-
Thiacloprid	nd	-	nd	-	nd	-	nd	-	3.9 ± 0.1	1 (13%)	nd	-
Thiamethoxam	nd	-	5.3 ± 0.1	2 (9%)	5.8 ± 0.1	3 (13%)	nd	-	4.2 ± 0.1	3 (38%)	15.6 ± 0.1	2 (29%)

n – number of samples; SD – standard deviation; DF – detection frequency; nd – not detected.

8.9 µg/kg (multifloral honey) to 18.9 µg/kg (canola honey), and thiamethoxam ranging between 4.2 µg/kg (linden honey) to 15.6 µg/kg (canola honey), respectively. Cyazofamid was detected in acacia honey (17.0 µg/kg), and thiacloprid in linden honey (3.9 µg/kg), whereas bitertanol, bifenazate, benzoximate, bupirimate, carbaryl, cyprodinil, and omethoate were not detected in any of the investigated honey samples (Table 2).

It is apparent from Fig. 3 (Plate XII) that the pesticides present the most were related to the sunflower, canola, and multifloral honey samples.

Comparison of the honey intake estimated to contribute to human exposure to pesticide residues can be made to determine the toxicological significance of human exposure. Table 3 shows the results of human health risk assessment for different honey types. The results obtained in our study show that the estimated daily intake of pesticide residues of different honey types was 0 µg/kg of body weight per day for pine honey. The lowest recorded EDI of 128 × 10^{-3} µg/kg of body weight per day was in acacia honey; the highest EDI of 265 × 10^{-3} µg/kg

Table 3. Human health risk assessment for different honey samples.

Pesticide	ADI (μg/kg of bw/day)	EDI of pesticide residues of different honey types (μg/kg of bw/day)					
		Pine	Multifloral	Sunflower	Acacia	Linden	Canola
1. Bendiocarb	4	-	11×10^{-4}	-	-	-	-
2. Bitertanol	3	-	-	-	-	-	-
3. Bifenazate	10	-	-	-	-	-	-
4. Benzoximate	150	-	-	-	-	-	-
5. Bupirimate	50	-	-	-	-	-	-
6. Buprofezin	9	-	-	16×0^{-4}	-	-	15×10^{-4}
7. Carbaryl	8	-	-	-	-	-	-
8. Chlorpyrifos	10	-	71×10^{-4}	67×10^{-4}	-	48×10^{-4}	70×10^{-4}
9. Clothianidin	100	-	45×10^{-4}	53×10^{-4}	38×10^{-4}	53×10^{-4}	70×10^{-4}
10. Cyproconazole	20	-	13×10^{-4}	-	-	-	-
11. Cyprodinil	30	-	-	-	-	-	-
12. Cyazofamid	170	-	-	-	54×10^{-4}	-	-
13. Dimethoate	2	-	29×10^{-4}	32×10^{-4}	35×10^{-4}	55×10^{-4}	61×10^{-4}
14. Omethoate	2	-	-	-	-	-	-
15. Thiacloprid	10	-	-	-	-	12×10^{-4}	-
16. Thiamethoxam	2	-	17×10^{-4}	19×10^{-4}	-	13×10^{-4}	50×10^{-4}
∑ of pesticides	580	-	186×10^{-3}	187×10^{-3}	128×10^{-3}	183×10^{-3}	265×10^{-3}

ADI - acceptable daily intake; EDI - estimated daily intake; bw – body weight; - detection < LOQ

of body weight per day was in canola honey. Similar values of EDI were recorded for multifloral, sunflower, and linden honey (186×10^{-3}, 187×10^{-3}, and 183×10^{-3}, respectively).

Using FAO/WHO's acceptable daily intakes (ADI), Table 3 compares the estimated contribution of honey to these consumptions. The ADI refer to an amount of pesticide that can be consumed daily by a person without posing an appreciable health risk. In this study, we found honey consumption to have only a minimal contribution to toxicological risk since the daily pesticide intake was much lower than the ADI.

As part of health risk assessment, Table 4 provides the hazard quotient (HQ) values for each pesticide. As a result of our investigation, no active substances were identified for which the health risk assessment would show alarming results. An HQ value below the threshold was obtained when mean pesticide concentrations in honey were included in endpoint calculations.

The lowest value for HQ was recorded in acacia honey (18×10^{-4}), followed by multifloral and sunflower honey (34×10^{-4}), linden honey (41×10^{-4}), and canola honey with the highest

Table 4. Human health risk hazard quotient.

Pesticide	Hazard quotient (HQ)					
	Pine	Multifloral	Sunflower	Acacia	Linden	Canola
1. Bendiocarb	-	3×10^{-5}	-	-	-	-
2. Bitertanol	-	-	-	-	-	-
3. Bifenazate	-	-	-	-	-	-
4. Benzoximate	-	-	-	-	-	-
5. Bupirimate	-	-	-	-	-	-
6. Buprofezin	-	-	2×10^{-5}	-	-	2×10^{-5}
7. Carbaryl	-	-	-	-	-	-
8. Chlorpyrifos	-	7×10^{-5}	7×10^{-5}	-	5×10^{-5}	7×10^{-5}
9. Clothianidin	-	-	1×10^{-5}	-	1×10^{-5}	1×10^{-5}
10. Cyproconazole	-	1×10^{-5}	-	-	-	-
11. Cyprodinil	-	-	-	-	-	-
12. Cyazofamid	-	-	-	-	-	-
13. Dimethoate	-	14×10^{-4}	16×10^{-4}	18×10^{-4}	28×10^{-4}	30×10^{-4}
14. Omethoate	-	-	-	-	-	-
15. Thiacloprid	-	-	-	-	1×10^{-5}	-
16. Thiamethoxam	-	8×10^{-5}	9×10^{-5}	-	7×10^{-5}	25×10^{-4}
∑ of pesticides	-	-	34×10^{-4}	18×10^{-4}	41×10^{-4}	65×10^{-4}

HQ - hazard quotient; when HQ is ≤1 - no adverse effect is likely to occur (health-protective); when HQ is >1 - high level of concern for chronic effect occurrence; - detection < LOQ

recorded HQ value (65×10^{-4}), respectively. The HQ value for pine honey was 0 indicating that this honey is the safest for consumption, but other types of honey investigated in this study posed no risk to humans after potential consumption.

Discussion

Approximately 80% of wild plants depend on insect pollination, where bees play a pivotal role (Ben Mukiibi et al. 2021). Honeybees readily fly up to a 4 km radius from their apiary, covering an area of about 50 km^2, which makes them excellent bioindicators of environmental contamination (Malhat et al. 2015). Previously, there have been few studies that studied pesticide residue levels in honey (El-Nahhal 2020; Xiao et al. 2022). The studies also only evaluated one class of pesticides or tested residues from *Varroa* control programs (Herrera López et al. 2016; Kiljanek et al. 2016). There is little information available on pesticide residues in organic honey samples, including residues introduced into hives by contaminated wax or bees, and in contaminated wax introduced into hives by contaminated bees.

Research has shown that organochlorine pesticides have been detected in honey samples produced in industrialized areas, even though they have been banned for quite some time (Günes et al. 2021). However, because of their high persistence in the environment, organochlorine pesticides are present in the environment (Rani et al. 2017; Pelić et al. 2023).

Compared to other research, our results showed lower levels and frequencies of pesticide residues in honey (Xiao et al. 2022). Over 35% of honey samples of Western honey bees around the world contain pesticide residues, according to previous studies (Giroud et al. 2013; Mitchell et al. 2017). In order to control pests or diseases in crops, many pesticides are used which can remain in the nectar, pollen, water, and soil that bees are exposed to (Botías et al. 2015).

Although an integrated pest management system is used during the growing season to control pests in intensively cultivated agricultural fields, pesticides are often used extensively to control the majority of pests (Meissle et al. 2010). This could explain why there is a greater number of pesticides present in these honey varieties in the present research. Honey and pollen samples in Poland were positive for residues following the application of fungicides on cherry trees, including captan, iprodione, and difenconazole (Al-Waili et al. 2012). A Swiss investigation has detected penconazol and dithianon residues in honey that were applied to fruit trees (Lambert et al. 2013). In a study of honey samples from Italy that was conducted by Saitta et al. (2017), the presence of 4.4'-DDD (1.15 μg/kg) and endosulphan (1.42 μg/kg) was detected. In Argentina, Villalba et al. (2020), recorded the highest concentration of chlorpyrifos in almost all honey samples from a soybean field. These results revealed that land uses and seasonal variations directly impact levels of agrochemicals. In studies conducted by Woodcock et al. (2018), clothianidin was the most frequently detected neonicotinoid in honey samples from the UK in a very low concentration (<2.0 ng/g), whereas the concentrations of pesticide residues in Greek honey and pollen studied by Kasiotis et al. (2023) ranged from 1.3 ng/g to 785 ng/g. In studies by Ponce-Vejar et al. (2022), the pesticides most frequently found at higher concentrations were neonicotinoids, followed by organophosphates, herbicides, and fungicides. Beekeeping practices could lead to direct pollution of honey via acaricides, especially when attempting to control the *Varroa* mite disease. Raw materials or bee products contaminated with pesticides are not only hazardous to public health but are also degraded in quality (Tudi et al. 2021).

It is important not to ignore the current findings when assessing risks to human health. In the last two decades, raw, unprocessed food has become increasingly popular, especially organic food with proven health benefits, and this trend involves apiculture products as well (Kieliszek et al. 2018; Puvača 2018). In addition, the scientific reviews on the benefits of apiculture products on human and animal health further strengthen the inclination toward these products (Mărgăoan et al. 2019). Therefore, honey consumption is expected to increase among adults as well as children (Vapa-Tankosić et al. 2020).

Different types of honey were examined for pesticide residues using the optimized analytical method. This method requires few samples, minimizing the amount of solvent used, and is simple and rapid. Apart from providing quantitative information, MS/MS detection is also useful for confirming pesticide residue in honey. To ensure that humans do not consume excessive amounts of contaminants, especially in diet, pesticide residues need to be determined in the environment and foods. According to the obtained results, honey contributed significantly less to dietary intakes than ADIs when estimated daily intakes were calculated. Honey investigated in this study posed no risk to humans after potential consumption, but further investigation into monitoring contamination in the environment is highly necessary.

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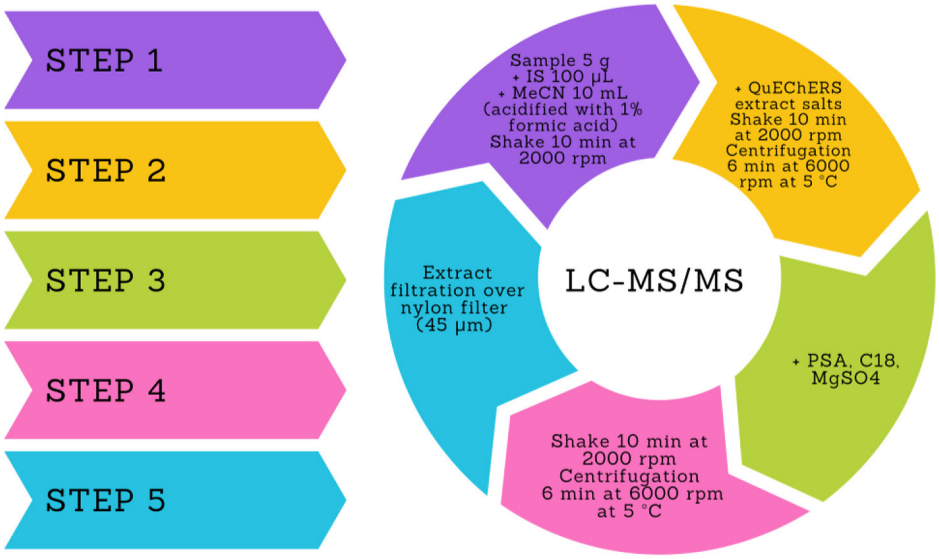

Fig. 1. Schematic display of QuEChERS extraction.
PSA - Primary secondary amine; LC-MS/MS - liquid chromatography with tandem mass spectrometry

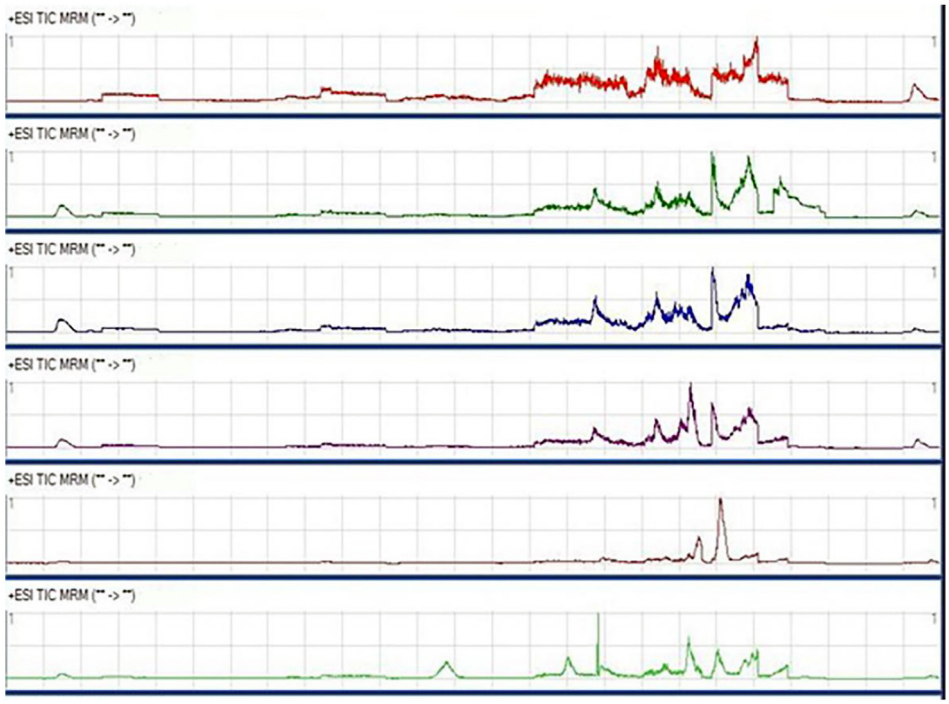

Fig. 2. The total ion chromatograms (TIC) of honey samples

Plate XII

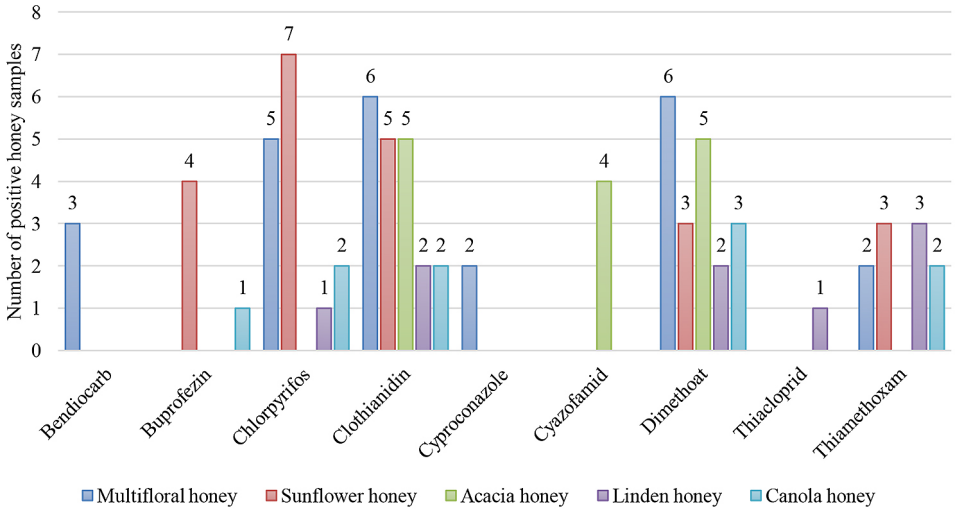

Fig. 3. Pesticide detection frequency in honey samples according to their type