

Toxicological assessment of honey from conventional and organic production and risk assessment for public health

Toksikološka procena meda iz konvencionalne i organske proizvodnje i procena rizika za javno zdravlje stanovništva

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ABSTRACT

Honey has become a focal point of concern due to the potential presence of pesticide residues originating from agricultural practices. This study undertakes a comprehensive toxicological assessment of pesticide residues in conventional and organic honey, as well as a risk assessment employing estimated daily intakes (EDIs) in the evaluation of the potential risks of these agrochemicals to public health. A total of 200 honey samples originating from conventional and organic producers were collected and analyzed by a Quick Easy Cheap Effective Rugged Safe method (QuEChERS). Analysis of organic honey didn't detect any residue of investigated pesticides. The most detected pesticides in conventional honey samples were boscalid (0.01 µg/kg), and coumaphos ranging between 0.012 µg/kg to 0.016 µg/kg, respectively. Detected pesticides such as acetamiprid, pyraclostrobin, thiacloprid, and azoxystrobin were under the reporting level (RL). Results obtained by EDI indicated that all investigated honey samples are safe for human consumption. In conclusion, this research contributes to the understanding of the toxicological implications of pesticide residues in both conventional and organic honey consumption. By delineating the potential health hazards associated with each type, this study aims to provide consumers, regulators, and beekeeping industries with valuable insights to make informed decisions that safeguard human health while promoting sustainable agricultural practices.

Keywords: honey, pesticides, toxins, bees, public health, risk assessment

APSTRAKT

Med je postao fokus zabrinutosti zbog potencijalne prisutnosti ostataka pesticida. Cilj ovog rada je toksikološka procena ostataka pesticida u konvencionalnom i organskom medu, kao i procena rizika primenom procenjenih dnevnih unosa (EDI) po javno zdravlje stanovništva. Ukupno 200 uzoraka meda poreklom od konvencionalnih i organskih proizvođača prikupljeno je i analizirano pomoću Quick Easy Cheap Effective Rugged Safe (QuEChERS) metode. Analiza organskog meda nije zabeležila ostatke ispitivanih pesticida. Najviše detektovani pesticidi u uzorcima konvencionalnog meda su bili boskalid (0,01 µg/kg) i kumafos, u rasponu od 0,012 µg/kg do 0,016 µg/kg. Pesticidi poput acetamiprida, piraklostrobina, tiakloprida i azoksistrobina bili su ispod nivoa izveštavanja (<RL). Rezultati dobijeni putem EDI ukazuju da su svi ispitivani uzorci meda bezbedni za konzumaciju ljudi. Na osnovu dobijenih rezultata može se zaključiti, da ovo istraživanje doprinosi razumevanju toksikoloških posledica ostataka pesticida u konzumaciji kako konvencionalnog, tako i organskog meda. Proučavanjem potencijalnih zdravstvenih opasnosti, ovo istraživanje ima za cilj da pruži potrošačima, regulatornim telima, kao i pčelarima značajne informacije za donošenje odluka koje čuvaju ljudsko zdravlje promovišući istovremeno održivu poljoprivrednu proizvodnju.

Ključne reči: med, pesticidi, toksini, pčele, javno zdravlje, procena rizika

INTRODUCTION

Honey has recently come under scrutiny due to concerns about pesticide residues (Kaila et al., 2022; Sharma et al., 2023; Xiao et al., 2022). The two main categories of honey, conventional and organic, differ significantly in their approach to farming practices and pesticide use (Panseri et al., 2020; Castro et al., 2023). Understanding the hazards associated with pesticide residues in both types of honey is crucial for consumers making informed choices about their dietary habits and health (van Dijk et al., 2008). Honey has been a staple in human diets for centuries, revered not only for its sweet taste but also for its potential health benefits (Momtaz et al., 2023). The debate between conventional and organic honey has gained prominence in recent years, with consumers increasingly seeking not just a sweetener but a product that aligns with their health and environmental values (Vapa-Tankosić et al., 2020). Both conventional and organic honey have their merits, and understanding their importance can aid individuals in making informed choices based on their preferences and health goals (Popa and Dabija, 2019).

Conventional honey is the product of beekeeping practices that may involve the use of synthetic pesticides and antibiotics to protect bee colonies from diseases and pests (Kushwaha et al., 2023). The bees may also forage in areas where conventional farming methods, including

the use of chemical fertilizers, are prevalent (Wakgari and Yigezu, 2021). While these practices might raise concerns about potential pesticide residues in honey, it's essential to acknowledge the benefits that conventional honey brings to the table. Conventional honey tends to be more widely available and affordable, making it accessible to a broader population (García, 2018). The global demand for honey is met, thanks to the efficiency and scalability of conventional beekeeping practices (Danieli et al., 2023). Moreover, the flavor and nutritional profile of conventional honey can be diverse, reflecting the varied floral sources the bees visit (Soares et al., 2017). One primary hazard associated with conventional honey consumption is the potential presence of pesticide residues (Juan-Borrás et al., 2016; Yaqub et al., 2020). Residual traces of these chemicals can accumulate in honey, posing health risks to consumers. Pesticides such as neonicotinoids and organophosphates, commonly used in conventional agriculture, have been linked to various health issues, including disruptions in the endocrine system, neurotoxicity, and carcinogenic effects (Thompson et al., 2020). Additionally, long-term exposure to low levels of pesticides, even within accepted safety limits, has raised concerns about cumulative health effects. Certain pesticides may persist in the environment and bioaccumulate in the food chain, leading to a potential build-up of these substances in the human body over time (Kim et al., 2017).

Organic honey, on the other hand, is produced following stringent organic farming and beekeeping standards. Bees that produce organic honey forage in areas free from synthetic pesticides and fertilizers. The colonies are managed without the use of antibiotics, and the overall emphasis is on sustainable and environmentally friendly practices (Hermanns et al., 2020). One of the key advantages of organic honey is its reduced risk of pesticide contamination. For health-conscious consumers, this makes organic honey an appealing choice, as it aligns with the desire for a more natural and chemical-free product (Alleva et al., 2016; Ferenczi et al., 2023). Additionally, organic honey production supports the health of bee colonies and promotes biodiversity, contributing to the overall well-being of ecosystems (Papa et al., 2022). Organic farming relies on natural pesticides, which, while considered safer than synthetic alternatives, can still leave residues in honey (Benbrook et al., 2021). Copper-based fungicides and botanical extracts used in organic agriculture may result in detectable traces in honey. However, it's important to note that organic standards typically set lower limits for pesticide residues, aiming to reduce overall exposure compared to conventional practices (Koch et al., 2017; Tsadila et al., 2023).

Regardless of whether it's conventional or organic, honey offers several health benefits. Honey is a rich source of antioxidants, which help combat oxidative stress in the body. It also possesses anti-inflammatory properties and has been used in traditional medicine for its potential to soothe sore throats and coughs (Pasupuleti et al., 2020). The antimicrobial properties of honey, attributed to compounds like hydrogen peroxide, make it a natural preservative and a healing agent for wounds (Dalugodage and Tennakoon, 2022). Furthermore, honey serves as a natural energy booster due to its blend of sugars, providing a quick source of energy. It also contains trace amounts of vitamins and minerals, contributing to overall nutritional intake (Guiné et al., 2022).

The hazards associated with pesticide residues in honey are not limited to a specific type; both conventional and organic honey may carry some level of risk. Chronic

exposure to certain pesticides has been linked to adverse health effects, including developmental issues in children, reproductive disorders, and compromised immune function (Ashraf et al., 2023). Regulatory bodies, such as the U.S. Environmental Protection Agency (EPA) and the European Food Safety Authority (EFSA), establish maximum residue limits (MRLs) for pesticides in food, including honey. However, concerns persist about the adequacy of these limits and the potential for cumulative effects, especially given the variety of pesticides used in agriculture.

In the debate between conventional and organic honey, it's important to recognize that both types have their place in the market and offer distinct advantages. Conventional honey provides affordability and availability, meeting the needs of a larger consumer base. On the other hand, organic honey appeals to those who prioritize environmental sustainability and want to minimize exposure to synthetic chemicals (Canwat and Onakuse, 2022). Ultimately, the choice between conventional and organic honey depends on individual preferences, values, and health considerations (Vapa-Tankosić et al., 2020). Whichever type one chooses, incorporating honey into a balanced diet can contribute to a range of health benefits, making it a versatile and valuable addition to the modern pantry. As consumers continue to prioritize health and sustainability, the honey industry is likely to evolve, offering more options and transparency to meet the diverse needs of a conscientious market.

Pesticides, while instrumental in enhancing agricultural productivity, have raised concerns due to their inadvertent impact on non-target organisms, particularly pollinators like honey bees (Sponsler et al., 2019). The transfer of pesticide residues from plants to nectar and ultimately into honey has become a focal point of research and regulatory scrutiny (Ledoux et al., 2020). This risk assessment aims to explore the presence and potential consequences of pesticide residues in honey, addressing the dual concern for public health and the well-being of honey bee colonies. As pollinators, honey bees play a pivotal role in maintaining biodiversity and

ensuring food security (van der Sluijs and Vaage, 2016; Ali et al., 2023). Their foraging activities expose them to various environmental stressors, including pesticides applied to crops. Consequently, the honey produced by these industrious insects may carry residues of these chemicals, posing potential risks to both consumers and the pollinators themselves (Kędzierska-Matysek et al., 2022). Understanding the extent of these risks is crucial for developing informed policies, safeguarding public health, and implementing sustainable agricultural practices.

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 QEHL0510P) and the Hillium QuEChERS dispersive kit 15 mL (P/N QDHLL15032) (Heidenrod, Germany) were used (Table 1).

The analyses comprised 200 honey samples (100 samples from conventional-produced honey, and 100 samples from organic-produced honey) collected from different local producers in Vojvodina (Serbia) in the year 2023. 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.

Table 1. Steps of using the QuEChERS method for pesticide extraction from honey samples

Steps	Procedure
Step 1	Sample 5 g of honey Add internal standard (IS) 100 µL Add MeCN 10 mL (acidified with 1% formic acid) Shake for 10 min at 2000 rpm
Step 2	Add QuEChERS extract salts Shake for 10 min at 2000 rpm Centrifugate 6 min at 6000 rpm at 5 °C
Step 3	Add primary secondary amine (PSA), C18, MgSO ₄
Step 4	Shake for 10 min at 2000 rpm Centrifugate 6 min at 6000 rpm at 5 °C
Step 5	Extract filtration over a nylon filter of 45 µm

Pesticides were detected using an HPLC Agilent 1290 Infinity II chromatograph 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 3000 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 reporting limit (RL) was set at 0.01 mg/kg. Internal standard calibration was used to check linearity from 10 to 100 µg/kg. Analyzing 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 2, along with an average recovery rate and R², 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.

Adults' daily average consumption of honey 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 – the average concentration of pesticides in honey (µg/kg);

K – average consumption rate (kg of honey/day);

BW – average human body weight (kg).

Table 2. Multiple reaction monitoring mode transitions, collision energies, retention time, recovery, and correlation coefficient for detected pesticides

Pesticide	Precursor ion (m/z)	Product ions (m/z)	CE (V)	Rt (min)	Recovery (%) ± RSD	R ²																																								
Acetamiprid	223.1	125.8	20	12.28	89.6±12.71	0.9987																																								
		55.7	15				Thiacloprid	253.0	186.0	10	13.40	96.7±18.12	0.9914	126.0	20	Coumaphos	362.0	221.0	28	22.70	92.3±9.12	0.9937	334.0	16	Boscalid	343.0	307.1	15	17.30	93.9±7.88	0.9992	271.0	35	Azoxistrobin	404.1	372.1	9	16.80	91.8±10.43	0.9990	344.1	25	Pyraclostrobin	388.1	194.0	10
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		126.0	20				Coumaphos	362.0	221.0	28	22.70	92.3±9.12	0.9937	334.0	16	Boscalid	343.0	307.1	15	17.30	93.9±7.88	0.9992	271.0	35	Azoxistrobin	404.1	372.1	9	16.80	91.8±10.43	0.9990	344.1	25	Pyraclostrobin	388.1	194.0	10	18.60	82.6±9.71	0.9914	163.0	10				
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MRM - multiple reaction monitoring mode; Rt - retention time, R² - correlation coefficient; CE - collision energy; RSD - relative standard deviation

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

RESULTS AND DISCUSSION

After adhering to SANTE/11312/2021 guidelines, the approved Liquid Chromatography coupled with tandem

mass spectrometry (LC-MS/MS) technique demonstrated favorable linearity coefficients within the 10 to 100 µg/kg range for the studied pesticides, achieving R^2 values exceeding 0.99. The impact of honey on pesticides was established through analysis of matrix and solvent calibration graph slopes. To counteract matrix effects (ME), a matrix match calibration approach was employed, particularly for samples spiked with 10 µg/kg of honey Figure 1.

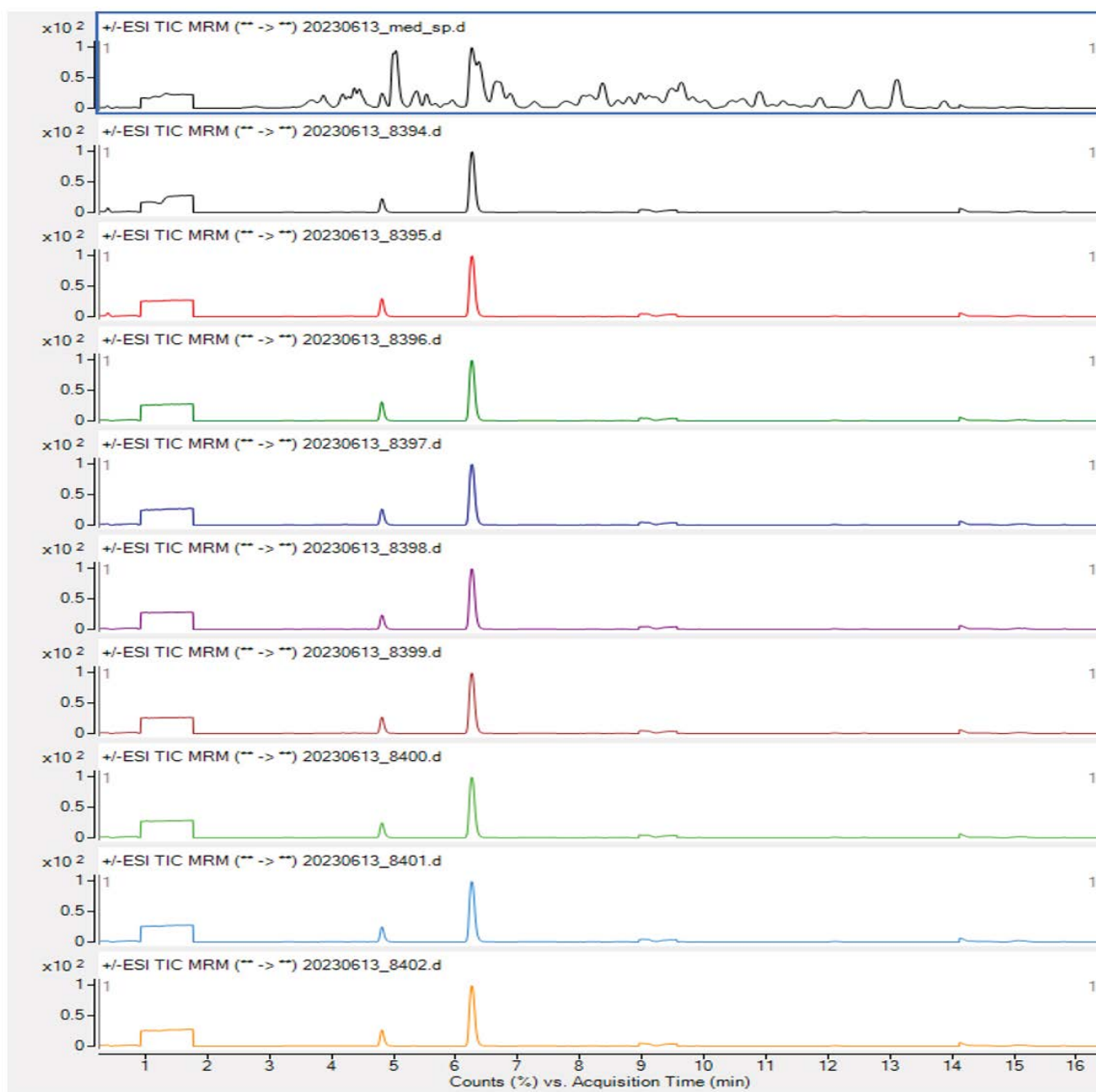


Figure 1. Spiking honey sample at the concentration level of 10 µg/kg

The experimental determination set the reporting level (RL) at 0.01 mg/kg for each pesticide, representing the minimum quantifiable value. Recovery studies involved two tiers, wherein blank honey samples were artificially enriched with pesticides at concentrations of 10 and 500 µg/kg. Thiachloprid, boscalid, coumaphos, and azoxystrobin showed an average recovery of 96.7, 93.9, 92.3, and 91.8%, while acetamiprid showed an average

recovery of 89.6 and pyraclostrobin showed an average recovery of 82.6%. As measured by a relative standard deviation (%RSD), the repeatability ranged from 7.88 to 18.12%. We employed a highly sensitive and selective LC-MS/MS method to examine honey samples, with Figure 2 depicting the total ion chromatograms (TIC) of the analyzed honey samples.

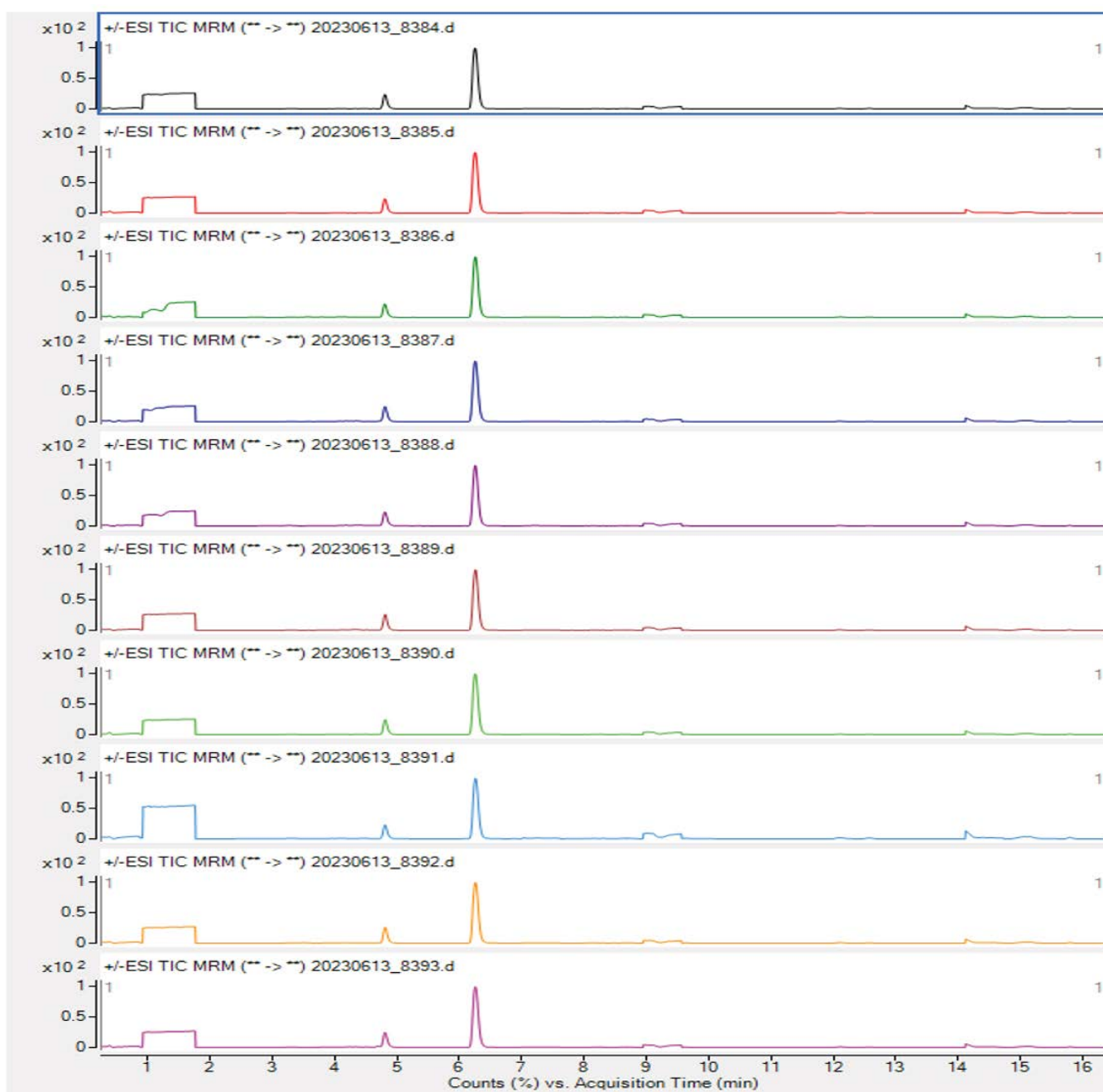


Figure 2. The total ion chromatogram (TIC) chromatograms of honey samples

Based on the obtained results it can be noticed that analysis of organic honey didn't detect any residue of investigated pesticides. Residues of pesticides were detected in conventional honey samples ranging between 0.12 and 0.16 µg/kg for coumaphos and 0.01 µg/kg for boscalid (Table 3). Of all covered pesticides (81), only six of them were detected, pyraclostrobin, and boscalid in the honey samples from conventional production,

while other pesticides were <RL and <LOD, mainly in honey samples from organic production, and some of them in conventionally produced honey, respectively. In the research of Lazarus et al. (2021), a total of 61 honey samples with identified botanical origins were gathered between 2018 and 2019 from both registered organic and conventional beekeepers in Croatia. The aim was to examine potential variations in contaminant

Table 3. Results of pesticide detections in 200 investigated honey samples from conventional and organic production (µg/kg)

Honey types	Pesticides					
	Acetamiprid	Thiacloprid	Coumaphos	Boscalid	Azoxystrobin	Pyraclostrobin
<i>Conventional honey production</i>						
Acacia (n=10)	*	*	*	*	*	*
Linden (n=10)	*	*	*	*	*	*
Wildflower (n=10)	*	*	<RL	<RL	<RL	*
Sage (n=10)	*	*	*	*	*	*
Chestnut (n=10)	*	*	*	*	*	*
Forest (n=10)	<RL	*	*	*	*	*
Apple blossom (n=10)	*	*	*	*	*	0.016
Thyme (n=10)	*	*	*	*	*	*
Raspberry (n=10)	*	*	*	*	*	0.014
Sunflower (n=10)	*	*	*	0.010	*	0.012
<i>Organic honey production</i>						
Acacia (n=10)	*	*	*	*	*	*
Linden (n=10)	*	*	*	*	*	*
Wildflower (n=10)	*	*	*	*	*	*
Sage (n=10)	*	*	*	*	*	*
Chestnut (n=10)	*	*	*	<RL	*	*
Forest (n=10)	*	*	*	*	*	*
Apple blossom (n=10)	<RL	*	<RL	*	*	*
Thyme (n=10)	*	*	*	*	*	*
Raspberry (n=10)	*	*	*	*	*	*
Sunflower (n=10)	*	<RL	*	*	*	*

<RL - values are under the reporting level; * - values are under the limit of detection (<LOD)

residues between the two types of honey by analyzing 121 pesticides (LC-MS/MS, GC-MS/MS) associated with the environment and beekeeping practices. All honey samples were found to have contaminant levels below the legally defined maximum thresholds, indicating their safety for consumers. However, the analysis revealed that 2 out of 16 organic kinds of honey and 34 out of 45 conventional kinds of honey contained one or two synthetic acaricides, with coumaphos being the most prevalent. Moreover, organic honey exhibited lower average levels of coumaphos, amitraz, and the amitraz metabolite N-(2,4-dimethylphenyl) formamide compared to conventional honey. The study highlighted beehive disease control treatments as a significant source of pesticide residues, with some reduction observed in organic kinds of honey (Lazarus et al., 2021). In research by Panseri et al. (2014) in the neighboring country Slovenia, were analyzed 72 honey samples for the presence of 28 pesticides, chosen to represent various contamination sources. The analysis employed methods involving SPE clean-up and GC-MS/MS detection, with a specific focus on pesticides commonly used in intensive apple orchards. The objective was to understand the connection between honey contamination and potential contamination sources. Residues of numerous pesticides were identified in a majority of the samples. Even though the concentrations were below their maximum residue limits (MRLs), 94% of the honey samples contained at least one pesticide. DDT, DDD, and DDE were the most frequently identified compounds in honey samples from industrialized areas, while chlorpyrifos and quinoxifen were commonly found in samples from apple orchard regions. No residues were detected in honey from mountain areas dedicated to organic production, following the results obtained in our research regarding organic honey, respectively. The study demonstrates that the contamination of honey with pesticides is closely linked to the contamination source, indicating specific environmental pollution. The findings confirm honey bee and beehive matrices as effective indicators for monitoring environmental contamination. This information could serve as a valuable tool for beekeepers, assisting them

in selecting suitable production areas, particularly for organic honey production (Panseri et al., 2014). Kumar et al. (2018) validated and applied the QuEChERS method followed by chromatographic analysis using GC- μ ECD/FTD and GC-MS to identify 24 pesticides in 100 raw honey samples from different floral sources in Northern India. Matrix-matched calibrations demonstrated the method's selectivity and linearity ($r^2 > 0.99$), with a detection limit of < 9.1 ng/g for all studied pesticides except monocrotophos (21.3 ng/g). Across various fortification levels, average recoveries ranged from 86.0 to 107.7%, with a relative standard deviation of $< 20\%$. Pesticide residues were found in 19.0% of the samples, with the most prevalent compounds being dichlorvos in 6.0% of the samples, followed by monocrotophos (5.0%), profenofos (5.0%), permethrin (4.0%), ethion (3.0%), and lindane (3.0%). Honey samples originating from cotton, sunflower, and mustard crops (33.3%) that tested positive for pesticide residues were significantly higher ($p < 0.05$) than honey from natural and fruity vegetation (13.5%). The study of Kumar et al. (2018) suggests that, due to the extensive use of pesticides in the area and their potential transfer to honey by bees, honey can serve as an indicator of environmental pollution. In some cases, pesticide residues in honey can express their potential reproductive toxicity (El-Nahhal, 2020). The findings of the previous author revealed the presence of 92 pesticide residues in honey samples collected from 27 different countries. The computed hazard indices (HIs) indicate a significant health risk associated with the consumption of honey. El-Nahhal (2020) concluded the ingestion of honey, among various food items containing pesticide residues, may lead to reproductive toxicity in both male and female consumers. An investigation conducted in the year 2002 analyzed fifty honey samples obtained from local markets in Portugal and Spain for the presence of 42 pesticide residues, including organochlorines, carbamates, and organophosphorus compounds. A testing method involving solid-phase extraction with octadecyl sorbent, followed by gas chromatography-mass spectrometry (GC-MS) for organochlorines, and liquid chromatography-atmospheric pressure

chemical ionization-mass spectrometry (LC-APCI-MS) for organophosphorus and carbamates, was developed (Blasco et al., 2003). The predominant pesticides detected in honey were organochlorines. Among the studied carbamates, methiocarb and carbofuran were present in 10% of the samples, pirimicarb in 4%, and carbaryl in 2%. The only organophosphorus pesticides detected were heptenophos in 16%, methidathion in 4%, and parathion methyl in 2% of the honey samples. Blasco et al. (2003) suggest that Portuguese kinds of honey exhibited higher contamination levels compared to Spanish ones.

However, consumers in both countries need not be concerned about the levels of pesticide residues in the kinds of honey available on the market. In the Western Serbia region, conventional honey production is exceptionally prevalent. Despite the advantages offered by organic honey production, beekeepers are hesitant to take that step, regardless of the challenges in marketing to foreign markets. Besides the undisputed nutritional values, honey produced conventionally, with the use of agrotechnical measures and a wide range of products for treating bee diseases (Puvača et al., 2022), often contains residues of hazardous chemical compounds, pesticides, and antibiotics (Lika et al., 2021; Puvača, 2018; Vapa Tankosić et al., 2022). The research was conducted in the Western Serbia region, involving five honey producers—two practicing organic production and three following conventional methods. Following the results of Ivanović et al. (2021) it was determined that one sample from conventional production is unsafe for human consumption, as it contains residues of the pesticide amitraz. On the other hand, the results obtained in the research of Bursić et al. (2021) offer insights into pesticide residues found in organic apples. However, these findings alone should not undermine the organic classification of apples as a produced commodity according to the authors. While the number of samples analyzed is limited, results underscore the importance of ongoing monitoring for both organic and conventionally produced products.

One can assess the toxicological significance of human exposure to pesticide residues by comparing the

estimated honey intake. The results of human health risk assessment for various types of honey are presented in Table 3. Based on the findings from our study, it is evident that the estimated daily intake of pesticide residues in various types of honey such as acacia, linden, wildflower, sage, chestnut, forest, and thyme was below the limit of detection (<LOD). The lowest recorded concentration of boscalid (0.000116 µg/kg of bw/day) was found in sunflower honey from conventional production, while the highest concentration of pyraclostrobin (0.000187 µg/kg of bw/day) was found in apple blossom honey.

Concentrations of the same pesticide were also detected in sunflower honey (0.000140 µg/kg of bw/day), and raspberry honey (0.000163 µg/kg of bw/day) from conventional production, while pesticide residues in organic honey samples were not detected. Utilizing the acceptable daily intakes (ADI) established by FAO/WHO, Table 4 and Figure 3 compare the calculated honey contribution to these intake levels. ADI represents the daily amount of a pesticide that can be ingested without significant health risks. Our study reveals that honey consumption minimally contributes to toxicological risk, as the daily pesticide intake is considerably lower than the established ADI.

Wang et al. (2022) in their study examined pesticide residues in both beebread and honey. They assessed the risk of these detected residues to honey bees using the hazard quotient (HQ) and BeeREX. Additionally, they evaluated the chronic and acute risks to humans through dietary exposure. Their findings indicate the detection ratio of pesticide residues (25.4 for beebread and 2.8% for honey). Further risk assessments suggest that the levels of pesticide residues in the tested honey do not present a risk to human consumers which is in accordance with the findings of our study. Although the overall risks seem to be minimal, analysis of Sanchez-Bayo and Goka (2014) indicates that the presence of residues from pyrethroid and neonicotinoid insecticides poses the greatest risk through contact exposure of bees to contaminated pollen. Notably, the combination of ergosterol-inhibiting fungicides with these two insecticide

Table 4. Human health risk assessment for different honey samples

Pesticide	ADI	EDI of pesticide residues of different honey types									
		Acacia	Linden	Wildflower	Sage	Chestnut	Forest	Apple blossom	Thyme	Raspberry	Sunflower
Acetamiprid	100	-	-	-	-	-	-	-	-	-	-
Thiacloprid	10	-	-	-	-	-	-	-	-	-	-
Coumaphos	0.5	-	-	-	-	-	-	-	-	-	-
Boscalid	60	-	-	-	-	-	-	-	-	-	0.000116
Azoxystrobin	100	-	-	-	-	-	-	-	-	-	-
Pyraclostrobin	30	-	-	-	-	-	-	0.000187	-	0.000163	0.000140
Σ of pesticides	300.5	-	-	-	-	-	-	0.000187	-	0.000163	0.000256

ADI - acceptable daily intake ($\mu\text{g}/\text{kg}$ of bw/day); EDI - estimated daily intake ($\mu\text{g}/\text{kg}$ of bw/day); bw - body weight; - values are under the limit of quantification ($< \text{LOQ}$)

classes leads to significantly higher risks, despite the relatively low occurrence of their combined residues. Concerns arise regarding the risks associated with the ingestion of contaminated pollen and honey, especially in the case of systemic insecticides such as imidacloprid, thiamethoxam, chlorpyrifos, and mixtures of cyhalothrin and ergosterol-inhibiting fungicides. It is crucial to focus attention on specific combinations of residues that may result in synergistic toxicity to bees (Sanchez-Bayo and Goka, 2014). Kumar et al. (2018) employed the QuEChERS method as in our research, but in conjunction with gas chromatography coupled to selective detectors (ECD/FTD/MS) to analyze 24 pesticide residues and their metabolites in 150 honey samples obtained from Northern Indian markets. Pesticide residues were identified in 12% of the samples, with organophosphate residues being predominant. Evaluation of human health risks indicates that the contaminated honey, at its current levels, poses a minimal contribution to toxicological risks, which is in accordance with our results. Mahdavi et al. (2022) computed hazard index (HI) for adults (0.18) and children (0.57), both below 1, indicating that there are no apparent health risks for individuals consuming honey with recorded residues of pesticides.

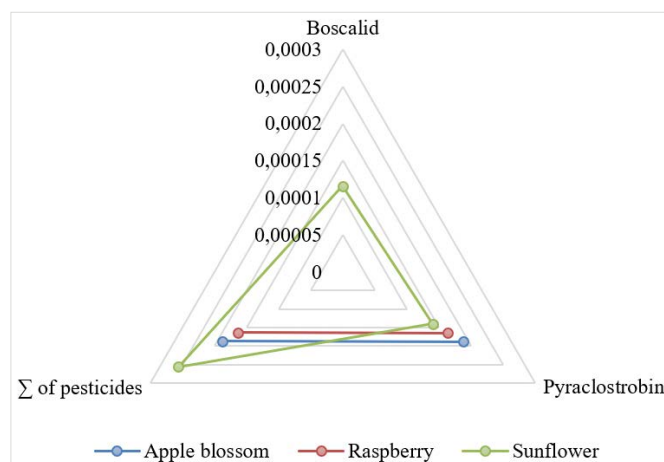


Figure 3. Radar of human health risk assessment for boscalid and pyraclostrobin detected in apple blossom, raspberry, and sunflower honey samples ($\mu\text{g}/\text{kg}$ of bw/day)

CONCLUSIONS

To minimize the hazards associated with pesticide residues in honey, consumers can take proactive measures. Washing fruits and vegetables thoroughly, choosing honey from reputable sources, and opting for organic honey can be steps toward reducing pesticide exposure. Additionally, supporting sustainable and eco-friendly agricultural practices helps promote a healthier environment and food system. Consumer awareness plays a crucial role in driving change in farming practices and influencing food supply chains. Educating the public

about the potential hazards of pesticide residues in honey empowers individuals to make choices that align with their health and environmental values. In conclusion, while honey remains a natural and nutritious sweetener, the hazards associated with pesticide residues underscore the importance of informed consumer choices. Whether opting for conventional or organic honey, understanding the potential risks and advocating for sustainable and transparent agricultural practices can contribute to a safer and more resilient food supply.

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REFERENCES

- Ali, M. A., Abdellah, I. M., Eletmany, M. R. (2023) Towards sustainable management of insect pests: Protecting food security through ecological intensification. *International Journal of Chemical and Biochemical Sciences*, 24 (4), 386-394.
- Alleva, R., Manzella, N., Gaetani, S., Ciarapica, V., Bracci, M., Caboni, M. F., Pasini, F., Monaco, F., Amati, M., Borghi, B., Tomasetti, M. (2016) Organic honey supplementation reverses pesticide-induced genotoxicity by modulating DNA damage response. *Molecular Nutrition and Food Research*, 60 (10), 2243–2255. DOI: <https://doi.org/10.1002/mnfr.201600005>
- Ashraf, S. A., Mahmood, D., Elkhalfá, A. E. O., Siddiqui, A. J., Khan, M. I., Ashfaq, F., Patel, M., Snoussi, M., Kieliszek, M., Adnan, M. (2023) Exposure to pesticide residues in honey and its potential cancer risk assessment. *Food and Chemical Toxicology*, 180, 114014. DOI: <https://doi.org/10.1016/j.fct.2023.114014>
- Benbrook, C., Kegley, S., Baker, B. (2021) Organic farming lessens reliance on pesticides and promotes public health by lowering dietary risks. *Agronomy*, 11 (7), 1–7. DOI: <https://doi.org/10.3390/agronomy11071266>
- Blasco, C., Fernández, M., Pena, A., Lino, C., Silveira, M. I., Font, G., Picó, Y. (2003) Assessment of pesticide residues in honey samples from Portugal and Spain. *Journal of Agricultural and Food Chemistry*, 51 (27), 8132–8138. DOI: <https://doi.org/10.1021/jf034870m>
- Bursić, V., Vuković, G., Cara, M., Kostić, M., Stojanović, T., Petrović, A., Puvača, N., Marinković, D., Konstantinović, B. (2021) Plant protection products residues assessment in the organic and conventional agricultural production. *Sustainability*, 13 (3), 1075. DOI: <https://doi.org/10.3390/su13031075>
- Canwat, V., Onakuse, S. (2022) Organic agriculture: A fountain of alternative innovations for social, economic, and environmental challenges of conventional agriculture in a developing country context. *Cleaner and Circular Bioeconomy*, 3, 100025. DOI: <https://doi.org/10.1016/j.clcb.2022.100025>
- Castro, E., Quicazán, M., Mojica, A., Zuluaga-Domínguez, C. (2023) Bioactive and physicochemical profile of honey collected from Colombian organic and conventional coffee growing areas. *Journal of Apicultural Research*, 62 (3), 518-529.
- Dalugodage, S. S., Tennakoon, I. (2022) Antibacterial activity of honey in combination with cinnamon. *Sri Lankan Journal of Health Sciences*, 1 (1), 43–50. DOI: <https://doi.org/10.4038/sljhs.v1i1.29>
- Danieli, P. P., Addeo, N. F., Lazzari, F., Manganello, F., Bovera, F. (2023) Precision beekeeping systems: State of the art, pros and cons, and their application as tools for advancing the beekeeping sector. *Animals*, 14 (1), 70.
- El-Nahal, Y. (2020) Pesticide residues in honey and their potential reproductive toxicity. *Science of The Total Environment*, 741, 139953. DOI: <https://doi.org/10.1016/j.scitotenv.2020.139953>
- Ferenczi, A. F., Szűcs, I., Gáthy, A. B. (2023) Evaluation of the pollination ecosystem service of the honey bee (*Apis mellifera*) based on a beekeeping model in Hungary. *Sustainability*, 15 (13), 9906.
- García, N. L. (2018) The current situation on the international honey market. *Bee World*, 95 (3), 89–94. DOI: <https://doi.org/10.1080/0005772X.2018.1483814>
- Guiné, R. P. F., Florença, S. G., Correia, P. M. R., Anjos, O., Coelho, C., Costa, C. A. (2022) Honey bee (*Apis mellifera* L.) broods: Composition, technology and gastronomic applicability. *Foods*, 11 (18), 2750. DOI: <https://doi.org/10.3390/foods11182750>
- Hermanns, R., Mateescu, C., Thrasylvoulou, A., Tananaki, C., Wagener, F. A. D. T. G., Cremers, N. A. J. (2020) Defining the standards for medical grade honey. *Journal of Apicultural Research*, 59 (2), 125–135. DOI: <https://doi.org/10.1080/00218839.2019.1693713>
- Ivanović, M., Galonja-Coghil, T., Janković, S., Rajković, M., Stanković, V., Paunović, D., Zlatanović, S. (2021) Sample analysis of organically produced honey and conventionally produced honey in Western Serbia. *Zaštita Materijala*, 62 (1), 22–33. DOI: <https://doi.org/10.5937/zasmat21010221>
- Juan-Borrás, M., Domenech, E., Escriche, I. (2016) Mixture-risk-assessment of pesticide residues in retail polyfloral honey. *Food Control*, 67, 127–134. DOI: <https://doi.org/10.1016/j.foodcont.2016.02.051>
- Kaila, L., Ketola, J., Toivonen, M., Loukola, O., Hakala, K., Raiskio, S., Hurme, T., Jalli, M. (2022) Pesticide residues in honeybee-collected pollen: Does the EU regulation protect honeybees from pesticides? *Environmental Science and Pollution Research*, 29 (12), 18225–18244. DOI: <https://doi.org/10.1007/s11356-021-16947-z>
- Kędzierska-Matyszek, M., Teter, A., Skatecki, P., Topyła, B., Domaradzki, P., Poleszak, E., Florek, M. (2022) Residues of Pesticides and Heavy Metals in Polish Varietal Honey. *Foods*, 11 (15), 2362. DOI: <https://doi.org/10.3390/foods11152362>
- Kim, K.-H., Kabir, E., Jahan, S. A. (2017) Exposure to pesticides and the associated human health effects. *Science of The Total Environment*, 575, 525–535. DOI: <https://doi.org/10.1016/j.scitotenv.2016.09.009>
- Koch, S., Epp, A., Lohmann, M., Böhl, G.-F. (2017) Pesticide residues in food: Attitudes, Beliefs, and misconceptions among conventional and organic consumers. *Journal of Food Protection*, 80 (12), 2083–2089. DOI: <https://doi.org/10.4315/0362-028X.JFP-17-104>
- Kumar, A., Gill, J. P. S., Bedi, J. S. (2018) Multiresidue determination of pesticides in market honey from northern India using QuEChERS approach and assessment of potential risks to consumers. *Current Science*, 115 (2), 283–291.
- Kumar, A., Gill, J. P. S., Bedi, J. S., Kumar, A. (2018) Pesticide residues in Indian raw honeys, an indicator of environmental pollution. *Environmental Science and Pollution Research*, 25 (34), 34005–34016. DOI: <https://doi.org/10.1007/s11356-018-3312-4>
- Kushwaha, D., Teja, K. S. S., Kumar, N., Aman, A. S., Kumar, A., Jaiswal, S., Khan, A. (2023) Diseases and pests harmful to honeybees (*Apis* spp.) and their management tactics: A review. *International Journal of Environment and Climate Change*, 13 (11), 95–109.

- Lazarus, M., Tariba Lovaković, B., Orct, T., Sekovanić, A., Bilandžić, N., Đokić, M., Solomun Kolanović, B., Varenina, I., Jurić, A., Denžić Lugomer, M., Bubalo, D. (2021) Difference in pesticides, trace metal(loid)s and drug residues between certified organic and conventional honeys from Croatia. *Chemosphere*, 266, 128954. DOI: <https://doi.org/10.1016/j.chemosphere.2020.128954>
- Ledoux, M. L., Hettiarachchy, N., Yu, X., Howard, L., Lee, S.-O. (2020) Penetration of glyphosate into the food supply and the incidental impact on the honey supply and bees. *Food Control*, 109, 106859. DOI: <https://doi.org/10.1016/j.foodcont.2019.106859>
- Lika, E., Kostić, M., Vještica, S., Milojević, I., Puvača, N. (2021) Honeybee and plant products as natural antimicrobials in enhancement of poultry health and production. *Sustainability*, 13 (15), 8467. DOI: <https://doi.org/10.3390/su13158467>
- Mahdavi, V., Eslami, Z., Omidvari, Z., Rezadoost, H., Thai, V. N., Fakhri, Y. (2022) Carcinogenic and non-carcinogenic risk assessment induced by pesticide residues in honey of Iran based on Monte Carlo simulation. *Journal of Food Composition and Analysis*, 109, 104521. DOI: <https://doi.org/10.1016/j.jfca.2022.104521>
- Momtaz, M., Bublji, S. Y., Khan, M. S. (2023) Mechanisms and health aspects of food adulteration: A comprehensive review. *Foods*, 12 (1), 199.
- Panseri, S., Bonerba, E., Nobile, M., Di Cesare, F., Mosconi, G., Cecati, F., Arioli, F., Tantillo, G., Chiesa, L. (2020) Pesticides and environmental contaminants in organic honeys according to their different productive areas toward food safety protection. *Foods*, 9 (12), 1863. DOI: <https://doi.org/10.3390/foods9121863>
- Panseri, S., Catalano, A., Giorgi, A., Arioli, F., Procopio, A., Britti, D., Chiesa, L. M. (2014) Occurrence of pesticide residues in Italian honey from different areas in relation to its potential contamination sources. *Food Control*, 38, 150–156. DOI: <https://doi.org/10.1016/j.foodcont.2013.10.024>
- Papa, G., Maier, R., Durazzo, A., Lucarini, M., Karabagias, I. K., Plutino, M., Bianchetto, E., Aromolo, R., Pignatti, G., Ambrogio, A., Pellicchia, M., Negri, I. (2022) The honey bee *Apis mellifera*: An insect at the interface between human and ecosystem health. *Biology*, 11 (2), 233. DOI: <https://doi.org/10.3390/biology11020233>
- Pasupuleti, V. R., Arigela, C. S., Gan, S. H., Salam, S. K. N., Krishnan, K. T., Rahman, N. A., Jeffree, M. S. (2020) A review on oxidative stress, diabetic complications, and the roles of honey polyphenols. *Oxidative Medicine and Cellular Longevity*, 2020, 1–16. DOI: <https://doi.org/10.1155/2020/8878172>
- Popa, I. D., Dabija, D.-C. (2019) Developing the Romanian organic market: A producer's perspective. *Sustainability*, 11 (2), 467. DOI: <https://doi.org/10.3390/su11020467>
- Puvača, N. (2018) Honeybee and medicinal plants products in poultry postantibiotic era production. *Journal of Agronomy, Technology and Engineering Management*, 1 (1), 8–17.
- Puvača, N., Halfawi, R., Čosić, M., Prodanović, R., Soleša, D., Vladislavjević, R., Lekić, S., Aćimović, M. (2022) Influence of lavender essential oil (*Lavandula angustifolia*) and oxytetracycline in nutrition of honey bees, prevention of American foulbrood and overall welfare. *Journal of the Hellenic Veterinary Medical Society*, 73 (1), 3773–3782. DOI: <https://doi.org/10.12681/jhvms.25747>
- Puvača, N., Ljubojević Pelić, D., Pelić, M., Bursić, V., Tufarelli, V., Piemontese, L., Vuković, G. (2023) Microbial resistance to antibiotics and biofilm formation of bacterial isolates from different carp species and risk assessment for public health. *Antibiotics*, 12 (1), 143. DOI: <https://doi.org/10.3390/antibiotics12010143>
- Sanchez-Bayo, F., Goka, K. (2014) Pesticide residues and bees – A risk assessment. *Plos One*, 9 (4), e94482. DOI: <https://doi.org/10.1371/journal.pone.0094482>
- Sharma, A., Pant, K., Brar, D. S., Thakur, A., Nanda, V. (2023) A review on Api-products: Current scenario of potential contaminants and their food safety concerns. *Food Control*, 145, 109499. DOI: <https://doi.org/10.1016/j.foodcont.2022.109499>
- Soares, S., Amaral, J. S., Oliveira, M. B. P. P., Mafra, I. (2017) A comprehensive review on the main honey authentication issues: Production and origin. *Comprehensive Reviews in Food Science and Food Safety*, 16 (5), 1072–1100. DOI: <https://doi.org/10.1111/1541-4337.12278>
- Sponsler, D. B., Grozinger, C. M., Hitaj, C., Rundlöf, M., Botías, C., Code, A., Lonsdorf, E. V., Melathopoulos, A. P., Smith, D. J., Suryanarayanan, S., Thogmartin, W. E., Williams, N. M., Zhang, M., Douglas, M. R. (2019) Pesticides and pollinators: A socioecological synthesis. *Science of The Total Environment*, 662, 1012–1027. DOI: <https://doi.org/10.1016/j.scitotenv.2019.01.016>
- Thompson, D. A., Lehmler, H.-J., Kolpin, D. W., Hladik, M. L., Vargo, J. D., Schilling, K. E., LeFevre, G. H., Peeples, T. L., Poch, M. C., LaDuca, L. E., Cwiertny, D. M., Field, R. W. (2020) A critical review on the potential impacts of neonicotinoid insecticide use: Current knowledge of environmental fate, toxicity, and implications for human health. *Environmental Science: Processes and Impacts*, 22 (6), 1315–1346. DOI: <https://doi.org/10.1039/C9EM00586B>
- Tsadila, C., Amoroso, C., Mossialos, D. (2023) Microbial diversity in bee species and bee products: Pseudomonads contribution to bee well-being and the biological activity exerted by honey bee products: A narrative review. *Diversity*, 15 (10), 1088.
- van der Sluijs, J. P., Vaage, N. S. (2016) Pollinators and global food security: The need for holistic global stewardship. *Food Ethics*, 1 (1), 75–91. DOI: <https://doi.org/10.1007/s41055-016-0003-z>
- van Dijk, H., Houghton, J., van Kleef, E., van der Lans, I., Rowe, G., Frewer, L. (2008) Consumer responses to communication about food risk management. *Appetite*, 50 (2), 340–352. DOI: <https://doi.org/10.1016/j.appet.2007.08.011>
- Vapa Tankosić, J., Puvača, N., Giannenas, I., Tufarelli, V., Ignjatijević, S. (2022) Food safety policy in the European Union. *Journal of Agronomy, Technology and Engineering Management*, 5 (2), 712–717. DOI: <https://doi.org/10.55817/EMRK6646>
- Vapa-Tankosić, J., Ignjatijević, S., Kiurski, J., Milenković, J., Milojević, I. (2020) Analysis of consumers' willingness to pay for organic and local honey in Serbia. *Sustainability*, 12 (11), 4686. DOI: <https://doi.org/10.3390/su12114686>
- Wakgari, M., Yigezu, G. (2021) Honeybee keeping constraints and future prospects. *Cogent Food and Agriculture*, 7 (1), 1872192. DOI: <https://doi.org/10.1080/23311932.2021.1872192>
- Wang, F., Wang, Y., Li, Y., Zhang, S., Shi, P., Li-Byarlay, H., Luo, S. (2022) Pesticide residues in beebread and honey in *Apis cerana cerana* and their hazards to honey bees and human. *Ecotoxicology and Environmental Safety*, 238, 113574. DOI: <https://doi.org/10.1016/j.ecoenv.2022.113574>
- Xiao, J., He, Q., Liu, Q., Wang, Z., Yin, F., Chai, Y., Yang, Q., Jiang, X., Liao, M., Yu, L., Jiang, W., Cao, H. (2022) Analysis of honey bee exposure to multiple pesticide residues in the hive environment. *Science of The Total Environment*, 805, 150292. DOI: <https://doi.org/10.1016/j.scitotenv.2021.150292>
- Yaqub, G., Khalid, M., Ikram, A., Sohail, A. (2020) Monitoring and risk assessment due to presence of metals and pesticides residues in honey samples from the major honey producing forest belts and different brands. *Food Science and Technology*, 40, 331–335. DOI: <https://doi.org/10.1590/fst.01919>