






## Pesticide contamination in apicultural products: An updated and comprehensive review of analytical methods, occurrence, and safety concerns

Adrián Fuente-Ballesteros<sup>a,\*</sup> , Maj Smerkol<sup>b</sup>, Anton Gradišek<sup>b</sup>, Artur Sarmento<sup>c</sup>, Julie Fourrier<sup>d</sup>, Mila Arapcheska<sup>e</sup>, Zehra Hajrulai-Musliu<sup>f</sup>, Filip Franeta<sup>g</sup>, Željko Milovac<sup>g</sup>, Sonja Gvozdencac<sup>g</sup>, Nesrin İçli<sup>h</sup>, Harun Kurtagić<sup>i</sup>, Ina Pasho<sup>j</sup>, Elena Zioga<sup>k</sup>, Beatriz I. Vazquez<sup>l</sup>, Damir Pavliček<sup>m</sup>, Rosa Busquets<sup>n</sup>, Jelena Ciric<sup>o</sup>, Nurinisa Esenbuga<sup>p</sup>, Miriam Cavaco<sup>q,r</sup>, Helena Rodrigues<sup>q,s,t</sup>, Rosa Ferreira<sup>q,s,t</sup>, Marta Leite<sup>q,t</sup>, Zane Lace<sup>u</sup>, Ana M. Ares<sup>a</sup>, José Bernal<sup>a,\*</sup> , Iveta Pugajeva<sup>u,\*</sup> 

<sup>a</sup> Analytical Chemistry Group (TESEA), I. U. CINQUIMA, Faculty of Sciences, University of Valladolid, Valladolid 47011, Spain

<sup>b</sup> Jožef Stefan Institute, Ljubljana, Slovenia

<sup>c</sup> Centre for Functional Ecology, Associate Laboratory TERRA, Department of Life Sciences, University of Coimbra, Portugal

<sup>d</sup> ITSAF-Institut de L'abeille, Avignon, France

<sup>e</sup> University "St. Kliment Ohridski" – Bitola, Faculty of Biotechnical Sciences – Bitola, North Macedonia

<sup>f</sup> University "Ss. Cyril and Methodius" - Skopje, Faculty of Veterinary Medicine – Skopje, North Macedonia

<sup>g</sup> Institute of Field and Vegetable Crops, Novi Sad, Serbia

<sup>h</sup> Kastamonu University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Kastamonu, Türkiye

<sup>i</sup> Federal Institute of Agriculture Sarajevo, Butmirska cesta 40, 71 210 Ilidža, Bosnia & Herzegovina

<sup>j</sup> Faculty of Natural Sciences, University of Tirana, Blv. "Zogu i Parë", Tirana, Albania

<sup>k</sup> School of Natural Sciences, Trinity College Dublin, Ireland

<sup>l</sup> LHICA, Science and Veterinary Science Faculties, Santiago de Compostela University, Spain

<sup>m</sup> Croatian Veterinary Institute, Veterinary Institute Krizevci, Krizevci, Croatia

<sup>n</sup> School of Life Sciences, Pharmacy and Chemistry, Kingston University, Kingston upon Thames, KT1 2EE, UK

<sup>o</sup> Institute of Meat Hygiene and Technology, Kačanskog 13, Belgrade, Serbia

<sup>p</sup> Department of Animal Science, Faculty of Agriculture, Ataturk University, Erzurum, Türkiye

<sup>q</sup> National Institute for Agrarian and Veterinary Research, I.P. (INIAV), Av. da república, Quinta do Marquês, Oeiras 2780-157, Portugal

<sup>r</sup> Centro de Estudos Florestais, Instituto Superior de Agronomia, Universidade de Lisboa, Lisboa 1349-017, Portugal

<sup>s</sup> Faculty of Pharmacy of University of Porto, Porto, Portugal

<sup>t</sup> Associated Laboratory for Green Chemistry (LAQV) of the Network of Chemistry and Technology (REQUIMTE), Praça Coronel Pacheco, 15, 6° Andar, Porto 4050-453, Portugal

<sup>u</sup> Institute of Food Safety, Animal Health and Environment "BIOR", Riga, Latvia

### ARTICLE INFO

#### Keywords:

Apicultural products

Honey

Pesticide residues

Analytical methods

Mass spectrometry

Food safety

Environmental monitoring

Plant Protection Products

### ABSTRACT

Honeybees and their products integrate landscape-level chemical exposure, making apicultural matrices valuable bioindicators for both food safety and environmental monitoring. This review summarizes current knowledge on pesticide residues in honey, pollen, beebread, beeswax, royal jelly, and propolis from 2019 to 2024, with an overview of analytical methodologies used in their determination. Multi-residue methods remain dominated by Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) extraction combined with liquid and gas chromatography coupled to tandem mass spectrometry, while high-resolution MS enables broader screening. Highly polar pesticides, particularly glyphosate and its metabolites, require specialised single-residue approaches, such as the Quick Polar Pesticides (QuPPE) method and ion chromatography–high-resolution mass spectrometry (IC-HRMS). Co-occurrence patterns frequently involve mixtures of neonicotinoids, acaricides, and fungicides, reflecting combined agricultural and in-hive treatments. Regarding matrices, honey typically shows insecticide and acaricide residues, pollen concentrates fungicides and insecticides as the main exposure route, and beeswax

\* Corresponding authors.

E-mail addresses: [adrian.fuente.ballesteros@uva.es](mailto:adrian.fuente.ballesteros@uva.es) (A. Fuente-Ballesteros), [jose.bernal@uva.es](mailto:jose.bernal@uva.es) (J. Bernal), [iveta.pugajeva@bior.lv](mailto:iveta.pugajeva@bior.lv) (I. Pugajeva).

<https://doi.org/10.1016/j.teac.2026.e00300>

Received 16 November 2025; Received in revised form 6 February 2026; Accepted 9 February 2026

Available online 10 February 2026

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acts as a long-term sink for lipophilic compounds; royal jelly generally exhibits the lowest contamination levels. Although exceedances of Maximum Residue Limits in honey remain uncommon in European monitoring programs, the presence of pesticide mixtures and limited residue data for bee-related products beyond honey raise concern. Future research should prioritize harmonized residue limits for all beekeeping matrices, standardized quality control and reporting practices, targeted mixture-toxicity assessment under realistic co-exposure scenarios, and the broader adoption of green, miniaturized, and matrix-tailored sample preparation strategies to enhance sensitivity, sustainability, and comparability across studies.

## 1. Introduction

Honeybees (*Apis mellifera*) are indispensable to both agricultural systems and natural ecosystems due to their critical role as pollinators. Most flowering plants rely on pollinators for reproduction, making the recent decline in pollinator populations a major global concern. Among the stressors contributing to this decline, pesticide exposure stands out as one of the most debated and impactful. In particular, the phenomenon known as Colony Collapse Disorder (CCD), where adult bees abruptly abandon the hive, has been linked to multiple interacting factors, including pesticide exposure, parasitic infestations, and environmental degradation [1].

Pesticides comprise a broad class of chemical compounds, including insecticides, herbicides, fungicides, and acaricides, which are widely used to protect crops and increase agricultural productivity. In modern large-scale intensive farming systems, particularly in monocultures, their use has become indispensable, as comparable crop yields could hardly be achieved without them [2]. However, due to their persistence and long-range transport, these substances can accumulate in environmental compartments far from their original point of application. Bees are especially vulnerable to such pollutants, as they forage over large areas and are in constant contact with pollen, nectar, water, and plant resins. Their limited enzymatic detoxification systems make them more susceptible to the toxic effects of these compounds, even at sublethal concentrations. Pesticide exposure can impair bees' memory, learning ability, immune function, and orientation, leading to colony decline [1]. Contamination does not stop at the organismal level. Apicultural products such as honey, pollen, bee bread, royal jelly, and propolis, valued for their nutritional, medicinal, and cosmetic applications, are also prone to pesticide accumulation [3]. The presence of pesticides in these matrices not only compromises their quality and therapeutic value but also raises legitimate concerns about food safety. While the European Union has established Maximum Residue Limits (MRLs) for pesticides in honey, many other bee products remain insufficiently regulated, and global harmonisation of residue limits is still lacking. Moreover, bees and their products serve as effective bioindicators of environmental pollution [4]. Beyond documenting the quality and safety of bee products, monitoring pesticide residues offers valuable insights into the health of agroecosystems [1]. This dual role, as food products and environmental sentinels, underscores the importance of regular and accurate pesticide residue analysis in apicultural matrices.

This review aims to provide a detailed and up-to-date overview of pesticide contamination in apicultural products, with a particular focus on the period from 2019 to 2024. The objectives are threefold: (i) to assess the occurrence and levels of pesticide residues in various bee-related matrices, (ii) to evaluate the analytical techniques employed for their detection, and (iii) to explore the implications of such contamination for food safety, environmental monitoring, and bee health. By doing so, this work seeks to support future regulatory efforts, guide methodological innovation, and promote more sustainable practices in both apiculture and agriculture.

## 2. Methodology for literature review and statistical analysis

This review provides a comprehensive overview of the occurrence of pesticide residues in bee-related products, based on literature published

between 2019 and 2024. The scientific databases Scopus, Web of Science, and PubMed were searched using specific keyword combinations related to bee matrices ("bee pollen", "honey", "royal jelly", "propolis", "beebread" and "beeswax") and pesticide classes (e.g., "neonicotinoids", "fungicides", "herbicides", "acaricides", "insecticides", "inert ingredients", "glyphosate"). In case of inert ingredients, additional keywords were used: "adjuvant", "co-formulant", "inert ingredient", "surfactant", "emulsifier", "dispersant", "stabilizer", "solvent", "carrier", "penetrant", "wetting agent", "spreader"). Only primary research articles published in English were considered. Selected studies had to meet the following criteria: (i) be conducted in European countries or Turkey; (ii) include analysis of more than 15 samples; (iii) analyse one or more bee-related products (honey, pollen, beebread, beeswax, royal jelly, or propolis); and (iv) use validated analytical methods. The review focused on seven major pesticide families and aimed to identify trends, analytical approaches, and potential risks associated with their presence in bee-derived matrices.

The literature search and applied inclusion criteria yielded 52 research articles addressing pesticide occurrence in bee products, of which 10 focused specifically on glyphosate. In contrast, only two studies on inert ingredients met the initial publication-period criteria; therefore, the timeframe for these was extended to 10 years, resulting in a total of nine relevant articles.

Occurrence data was collected in Excel spreadsheets with several sheets (data about samples collected in studies, measurement values, metadata, etc.). Statistical analyses were conducted in Python, while relational co-occurrence analyses across the selected studies were implemented using a custom-developed Python script. Visualisation of graphs was performed in Python using the libraries NetworkX, Matplotlib, Geopandas, and Pandas.

## 3. Relational analysis

To move beyond compound-by-compound reporting, a relational co-occurrence analysis was conducted. Network graphs quantify how active substances cluster within and across pesticide classes, highlighting recurrent pairs that mirror agronomic and in-hive practices (see Fig. 1). Each node represents an individual pesticide, colour-coded according to its substance group (e.g., insecticide, acaricide, etc.). Node size indicates the total number of references (studies) in which the pesticide was detected, and edge width corresponds to the number of studies reporting both pesticides simultaneously. To minimise visual clutter and emphasise the most relevant associations, only pairs of pesticides co-detected in at least six studies were included. The resulting network highlights common co-occurrence patterns among active substances frequently found together in bee-related matrices.

Insecticides appeared most often together, with neonicotinoids such as imidacloprid, thiacloprid, acetamiprid, thiamethoxam, and clothianidin frequently detected in combination. These repeated co-detections are consistent with the widespread and often concurrent use of neonicotinoids. Other insecticides, including chlorpyrifos and dimethoate, also appeared repeatedly with neonicotinoids, further highlighting the prominence of this group. The acaricides showed notable cross-group associations. Tau-fluvalinate was often detected in conjunction with insecticides (e.g., acetamiprid, imidacloprid), fungicides (e.g., fluopyram, tebuconazole), and other acaricides (e.g., coumaphos).

Coumaphos was also frequently found in combination with insecticides (e.g., chlorpyrifos, chlorfenvinphos) and acaricides (e.g., amitraz). The fungicides formed a moderately cohesive cluster, with boscalid being the most frequently detected. It was commonly found in combination with fluopyram and tebuconazole, both of which also showed repeated co-occurrence with tau-fluvalinate, suggesting an overlap between fungicide and acaricide applications. At the group level, insecticides were most often detected in combination with other insecticides, but significant cross-group co-detections were also observed, particularly between acaricides and insecticides, and to a lesser extent between acaricides and fungicides. These findings highlight that pesticide exposure rarely involves single compounds, but rather recurring combinations shaped by agricultural and apicultural practices. Such scenarios are consistent with contexts where different treatments converge, including agricultural fields with sequential or combined applications, stored products, or hive matrices where veterinary acaricides overlap with agricultural residues [1,5]. From a monitoring perspective, the repeated detection of specific pairs, such as imidacloprid-thiacloprid or tau-fluvalinate-fluopyram, points to realistic co-exposures that should be prioritised for toxicological evaluation, given the potential for additive, synergistic, or antagonistic effects, as mixture interactions can be highly variable.

Moreover, the resulting co-detections were then projected onto hive matrices to reveal matrix-specific accumulation pathways and realistic mixture exposures (see Fig. 2). Honey was the most frequently studied matrix and showed consistent associations with insecticides and acaricides, reflecting both nectar transfer and in-hive treatments. Pollen, while analysed slightly less frequently, exhibited the strongest single connection, particularly with fungicides, followed by insecticides, confirming its role as a major exposure route for foragers and larvae.

Beeswax displayed its strongest associations with acaricides, consistent with the persistence of lipophilic compounds that accumulate over time. Royal jelly was the least associated matrix, with only minor links to pesticide groups, which may reflect both fewer studies and its more protected production pathway inside the hive. The integration of co-occurrence and matrix analyses highlights distinct exposure scenarios. Honey emerges as the main consumer matrix, pollen as the primary pollinator exposure route, and beeswax as a long-term reservoir of acaricides, while royal jelly remains comparatively less affected. Together, these findings indicate that pesticide mixtures appear to accumulate in matrix-specific patterns, potentially leading to different risk scenarios for pollinators, hive products, and consumers. However, it should be noted that non-detection does not necessarily imply absence, as some substance groups may fall outside the analytical scope or below detection limits.

#### 4. Analytical methodologies

Three distinct sections are summarised to accurately reflect the diversity of analytical methods used for determining pesticide and formulant residues in apicultural products. The first section highlights multi-residue methods designed primarily to identify non-polar or semi-polar compounds across a wide range of pesticide classes, which exhibit varying physicochemical properties. The second section focuses on polar pesticides, particularly glyphosate, which require specialised analytical techniques due to their high polarity, low volatility, and poor recoverability with conventional multi-residue workflows. Finally, the third section examines inert ingredients. Although these substances are usually not categorised as active substance, they can still present

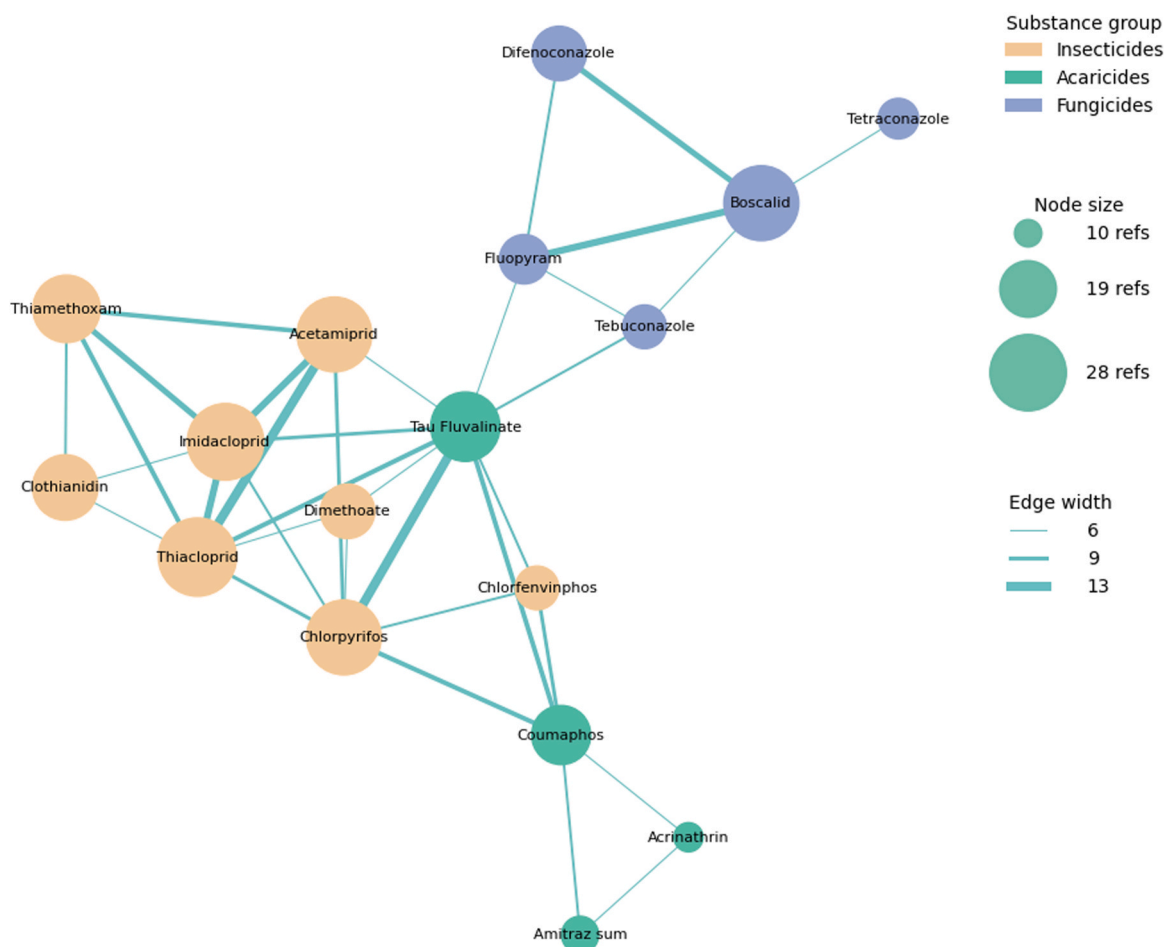


Fig. 1. Pesticide co-occurrence map. Node size indicates number of references analysing the substance, and edge width the number of co-detections.

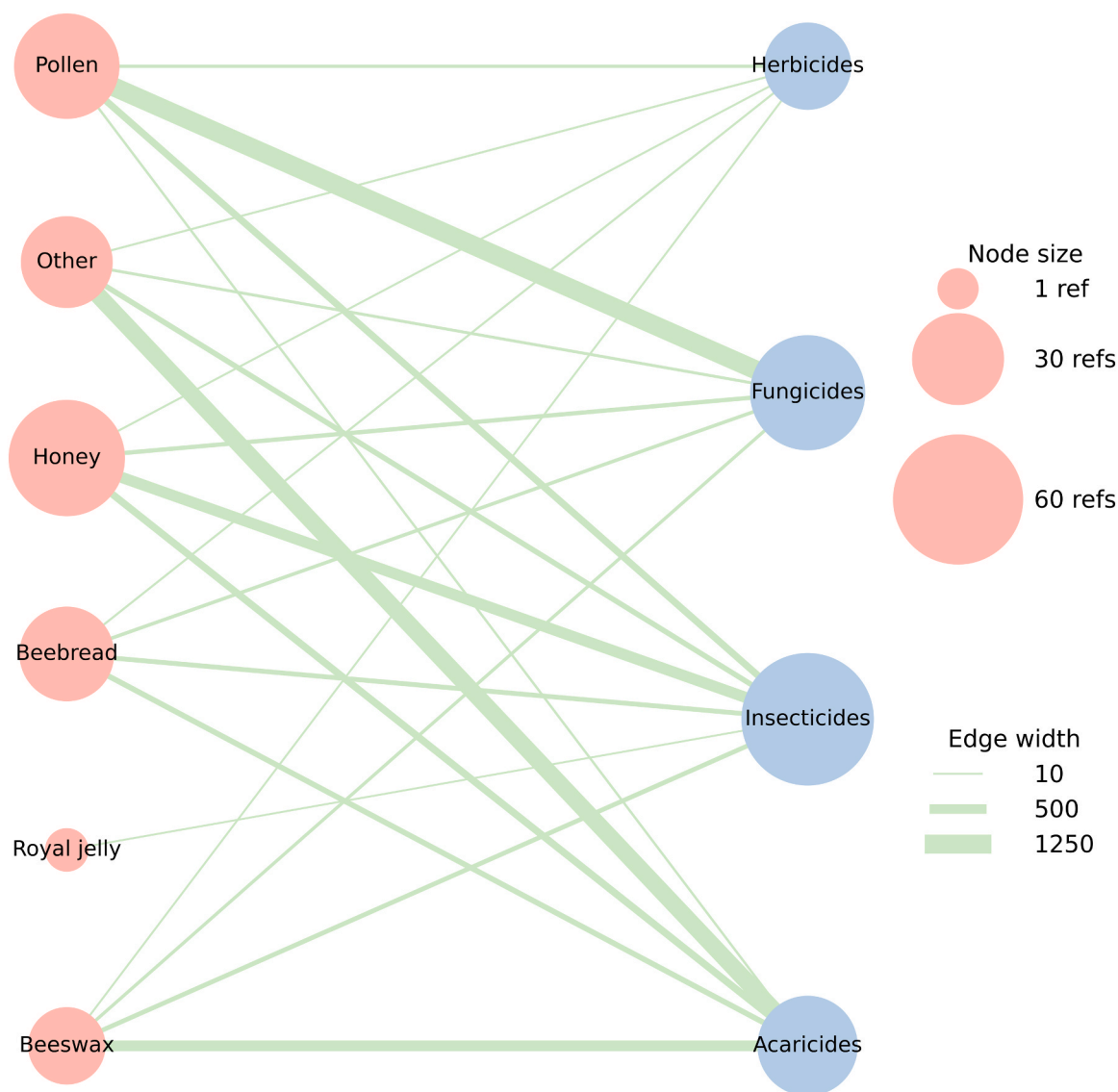


Fig. 2. Associations between matrices and pesticide groups. Node size indicates the number of references analysing the matrix type and substance group; connection thickness indicates the number of positively detected substances in a matrix.

toxicological concerns. Their structural diversity and limited characterisation in regulatory monitoring make them analytically challenging.

#### 4.1. Non-polar and semi-polar pesticides

Most of the studies included in the review focused on the analysis of honey, but a wide variety of apicultural matrices, including pollen, beebread, beeswax, and royal jelly, were also investigated. While some methods were developed for specific matrices, others provided multi-matrix workflows applicable to various bee products. Table S1 presents the list and the main features of the reviewed studies on pesticide residue methods, including matrices, extraction procedures, cleanup, detection techniques, liquid and gas chromatography, columns used, and the number of analytes included in each method. The scope of targeted pesticides varied considerably: some studies investigated only a few analytes (< 50) from a single pesticide class (e.g., bio-insecticide [6], neonicotinoids [7], pyrethroid [8] or acaricides [3]), whereas others covered broad-spectrum multi-residue methods encompassing up to 399 or more compounds [9], including different pesticide families.

The accurate determination of pesticide residues in apicultural matrices depends critically on the effectiveness of both extraction and

cleanup steps. Bee products, such as honey, pollen, beebread, wax, and honeybee tissue, exhibit markedly different physicochemical properties, ranging from hydrophilic, sugar-dominated matrices like honey to highly lipophilic materials, including beeswax and protein-rich tissues like whole bees, thereby necessitating matrix-specific extraction and cleanup strategies [10]. QuEChERS, which stands for Quick, Easy, Cheap, Effective, Rugged, and Safe, based extraction procedures were used in nearly 70 % of the reviewed studies, known for its simplicity and efficiency. These protocols typically involved acetonitrile extraction with salting-out partitioning ( $\text{MgSO}_4$ , NaCl). In 20 % of methods, cleanup using freezing-out or matrix-specific protocol adaptations were used, such as for pollen, wax, or beebread [5,11,12]. Alternative extraction techniques included solid-phase extraction (SPE) [13,14], Soxhlet extraction [4], liquid-liquid partitioning using organic solvents such as cyclohexane, ethyl acetate, acetone, petroleum ether, or dichloromethane [3,15], or the dilute-and-shoot approach [10]. Clean-up was frequently performed (around 70 % of protocols) using dispersive solid-phase extraction (d-SPE) with sorbents adapted to the matrix: primary secondary amine (PSA, ethylenediamine-N-propyl functionalized silica) for removing sugars, acids, and polar interferences in honey and pollen [16], octadecylsilane ( $\text{C}_{18}$ ) for

eliminating lipids and nonpolar compounds across the matrices [5] and Z-Sep® (zirconia-based sorbent) applied for pigment and phospholipid removal in complex matrices like beebread, pollen or nectar [17]. Gel permeation chromatography was also employed as a cleanup procedure for the detection of pesticides by GC-MS/MS [18]. Evaporation of the extract prior to analysis was performed in about 50 % of the studies, predominantly in those involving GC-MS/MS [19]. In a few cases, evaporation was also incorporated into LC-MS/MS workflows to enhance analyte concentration or facilitate solvent exchange [7]. LC-MS/MS was used in over 90 % of the reviewed studies, often with triple quadrupole (QQQ) detectors operated in Multiple Reaction Monitoring (MRM) mode. This setup offers high sensitivity for polar, thermolabile compounds, such as insecticides, fungicides, and herbicides [12]. Gas chromatography (GC-MS or GC-MS/MS) was applied in methods targeting volatile and thermally stable compounds, such as acaricides, organochlorines, and pyrethroids [15,20,21]. Additional mass spectrometric platforms, such as ion trap MS/MS or linear ion trap systems [21], and HRMS platforms, including Q-TOF for untargeted screening [6], were used. Other detection modes included HPLC-UV-Vis [22] for the analysis of neonicotinoids, and GC-electron capture detector (ECD) for the analysis of captan, thiacloprid, penthiopyrad, and  $\lambda$ -cyhalothrin [23]. LC separations were typically achieved using C<sub>18</sub> reversed-phase columns with various configurations. GC separations commonly use non-polar columns from different manufacturers, particularly DB-5MS, which is 30 m in length, has an internal diameter of 0.25 mm, and a film thickness of 0.25  $\mu$ m (see Table S1).

In this review, only studies that reported validated analytical methods or provided sufficient methodological detail were included; consequently, nearly half of the publications within the selected time frame were excluded due to insufficient information. Explicit validation data are typically reported in studies primarily focused on method development [6,20]. In contrast, when analytical methodology is used as a supporting tool rather than the primary objective, essential performance parameters, such as limits of quantification, recovery rates, and matrix effects, are often omitted. Lack of this performance data makes it difficult to compare different matrices effectively and reduces the reliability of these data when used in exposure and risk assessment frameworks.

When validation was reported, there were some patterns across matrices. In honey, multi-residue methods were typically validated at 0.01 mg kg<sup>-1</sup> (10  $\mu$ g kg<sup>-1</sup>) and achieved recoveries within 70–120 % [9]. Targeted methods could be even more sensitive, for example, spinetoram analysis achieved LODs of 0.1–0.3  $\mu$ g kg<sup>-1</sup> and LOQs of 0.3–1.2  $\mu$ g kg<sup>-1</sup> with recoveries of 82–95 % [6]. Beeswax analyses were generally less sensitive: a dedicated flumethrin method reported an LOD of 1–2  $\mu$ g kg<sup>-1</sup> and an LOQ of 20  $\mu$ g kg<sup>-1</sup> with 95 % recovery [8], while a broad screen of 294 compounds had LOQs around 10  $\mu$ g kg<sup>-1</sup> and recoveries between 70 % and 120 % [24]. Bee-bread methods varied widely, from a miniaturised QuEChERS assay capable of quantifying most analytes at 0.005–0.5 mg kg<sup>-1</sup> with recoveries near 98 % [13] to studies with LOQ 0.5–100  $\mu$ g kg<sup>-1</sup> and recoveries 75–125 % [12]. Methods for royal jelly and propolis achieved LODs of 0.1–2.8  $\mu$ g kg<sup>-1</sup> and recoveries of 93–118 % [15].

#### 4.2. Polar pesticides – glyphosate and its metabolites

Determining glyphosate and its metabolites in honey and hive products presents challenges due to their small size and unique physicochemical properties, which differ from those of other pesticides. Glyphosate is characterised by high polarity, high water solubility, and low volatility. These properties contribute to difficulties in its selective extraction, leading to significant matrix effects that adversely affect the selectivity and sensitivity of analytical methods. As a result, single-residue or specialised methods for polar pesticides are frequently employed to detect glyphosate and other polar pesticide residues [25]. The group of polar pesticides typically includes glyphosate metabolites

such as aminomethylphosphonic acid (AMPA), N-acetyl-AMPA, and N-acetyl-glyphosate, as well as other pesticides like glufosinate and its metabolites, ethephon, foseyl aluminium, and its degradation product, phosphonic acid [26]. The EU Reference Laboratories (EURL) for Residues of Pesticides have published a method for analysing highly polar pesticides in food, which includes honey (QuPPe-PO-Method). It involves extraction with acidified methanol, where glyphosate is directly analysed by LC-MS/MS or by IC-HRMS [27]. IC-HRMS has recently been one of the most widely published techniques for determining polar pesticides [26,28,29]. It is worth noting that all studies involving IC-HRMS utilised equipment manufactured by Thermo Fisher Scientific (USA). The most used system was Dionex ICS 5000 + coupled with Q-Exactive Focus or Orbitrap Exploris 120™. For chromatographic separation, a Dionex IonPac AS19–4  $\mu$ m (Thermo Scientific, USA) column with a guard column Dionex IonPac AS19–4  $\mu$ m (Thermo Scientific, USA) was used in all studies using this technique. A notably broad selection of chromatography columns is available for the separation of polar analytes by HPLC. For example, HILIC Torrus DEA (Waters, USA), APP (anionic polar pesticide column) (Waters, USA), Acclaim Trinity Q1 (Thermo Scientific, USA), Obelisc N (SIELC Technologies, USA), Raptor Polar X (Restek, USA), Luna Polar Pesticides (Phenomenex, USA) [30]. Several methods using reverse-phase columns have also been adapted for glyphosate determination, typically through compound derivatisation. Most used columns are Luna® C<sub>18</sub> column (Phenomenex, USA), Acquity UPLC BEH C<sub>18</sub> column (Waters, USA), XBridge UPLC BEH column (Waters, USA), Hypercarb (Thermo Scientific, USA) and others. HPLC systems coupled with tandem mass spectrometry are a common choice for analysing polar pesticide residues [31–33]. In sample preparation, honey samples were typically extracted using a mixture of acidic water and methanol. For honey samples, no additional clean-up procedure was recommended in any of the summarised studies [32–34]. To tackle issues related to low sensitivity and chromatographic challenges, polar compound derivatisation has been successfully implemented and reported by several authors [35–37].

#### 4.3. Inert ingredients

In addition to the active pesticide substance, apicultural products may be contaminated with various inert ingredients (e.g., adjuvants, co-formulants, etc.) that are often added to pesticide formulations to improve efficacy, stability, or application properties. Although these substances are not classified as active substances, many of them can exhibit biological activity or may break down into toxic by-products. Detecting and characterising these compounds in bee-related materials presents analytical challenges due to their diverse chemical structures, the lack of reference standards, and their limited inclusion in regulatory monitoring programs. Analytical methods for adjuvants and surfactants trisiloxane surfactants – (TSSs), nonylphenol ethoxylate (NP(EO)<sub>n</sub>) and octylphenol ethoxylate (OP(EO)<sub>n</sub>), the solvent (N-methyl-2-pyrrolidone (NMP)) are based on LC-MS [38,39]. NMR spectroscopy was employed for the structure confirmation of the three purified TSSs compounds [40]. In the metabolism study of Fine and Mullin [41], Q-TOF-MS was also employed for the identification of NMP and its metabolites. Additionally, for the identification and quantification of the solvent piperonyl butoxide (PBO) and TSS residues, an analytical method based on the use of LC-MS/MS was employed [42,43]. A QuEChERS method was applied for the extraction of NP(EO)<sub>n</sub> and OP(EO)<sub>n</sub> oligomers, which were extracted from beehive samples (honey, pollen and wax) [38] and TSSs (R = H, CH<sub>3</sub>, and C(O)CH<sub>3</sub>) from almond flowers, pollen, beeswax and honey [38,43]. For the extraction of NMP from honey matrices, a QuEChERS method with ethanol was used [39].

### 5. Pesticide occurrence in apiculture

A general overview of the studies included in this review reveals spatial patterns in research activity across Europe and Turkey, which

helps to contextualise the subsequent discussion of individual pesticide families. The geographic distribution of the reviewed works published between 2019 and 2024 (see Fig. S1) shows the number of unique references per country according to the origin of the analysed bee product samples. This mapping highlights regional differences in monitoring intensity and scientific productivity, providing insight into how research efforts on pesticide residues in apicultural products are distributed across the continent. The results reveal an evident imbalance, with Southern and Western Europe, particularly Spain and Italy, accounting for the majority of published studies. These countries combine longstanding apicultural traditions with strong analytical infrastructures and active monitoring programmes. Moderate contributions are observed in Central Europe, such as Poland and the Czech Republic, while Northern and Eastern Europe remain underrepresented. This uneven landscape may reflect differences in honey production volume, research funding, and national pesticide monitoring priorities. Consequently, available data on pesticide residues in apicultural products are geographically concentrated, limiting the comparability of results among regions. Expanding surveillance programmes and harmonising analytical methodologies would contribute to a more balanced and comprehensive understanding of pesticide occurrence.

Similarly, a variety of bee product matrices analysed in the reviewed studies reveal differences in research focus among matrices. Honey is the most frequently studied product (27 %), reflecting its economic importance and the existence of harmonised regulatory limits. Bee pollen (24 %) and bee bread (17 %) follow as the next most common matrices, highlighting their relevance as both nutritional products and key exposure routes for foraging bees. These matrices are particularly valuable for assessing direct pesticide intake and its potential impact on colony health. Beeswax (11.5 %) also appears regularly in the literature, primarily due to its ability to retain lipophilic compounds over time and its utility in assessing the persistence of contaminants within hives. In contrast, royal jelly (0.5 %) is rarely investigated. This underrepresentation may be attributed to its limited production, high commercial value, and the analytical challenges associated with its protein-rich composition. Other, less common matrices (20 %) include propolis and hive debris, which are generally considered complementary indicators of environmental exposure. The variety suggests that research efforts remain concentrated on matrices with established analytical protocols and market relevance, while certain bee products still lack systematic residue monitoring. Expanding the analytical focus toward underexplored matrices would provide a more complete understanding of pesticide dynamics within the hive ecosystem.

The compilation of recent studies highlights the pesticide compounds that have attracted the greatest attention in apicultural research (see Fig. S2). Imidacloprid clearly stands at the forefront, often serving as a model compound due to its extensive agricultural use and well-documented toxicity to honeybees. Other neonicotinoid insecticides, such as thiacloprid, acetamiprid, thiamethoxam, and clothianidin, also appear frequently, consistent with their former widespread use before European restrictions. Among fungicides, tebuconazole, boscalid, and propiconazole were among the most examined, while tau-fluvalinate, coumaphos, and amitraz dominate among acaricides because they are commonly used within hives to control *Varroa destructor*. The focus of research has remained on compounds known for their persistence, systemic action, and potential sublethal effects. By contrast, herbicides like glyphosate and certain co-formulants have been studied far less, despite being regularly found in environmental samples. This uneven attention likely results from analytical challenges that necessitate an additional single-residue method, including a diverse range of materials and techniques for glyphosate analysis, while the co-formulants lack policy priorities, as active substances tend to be monitored more closely.

Lastly, pesticide residues exceeding the regulatory MRL values were considered (see Fig. S3). Fluzinam and chlorfenvinphos were most often found above the established limits, with disulfoton and coumaphos appearing next in frequency. These findings are not unexpected, as all

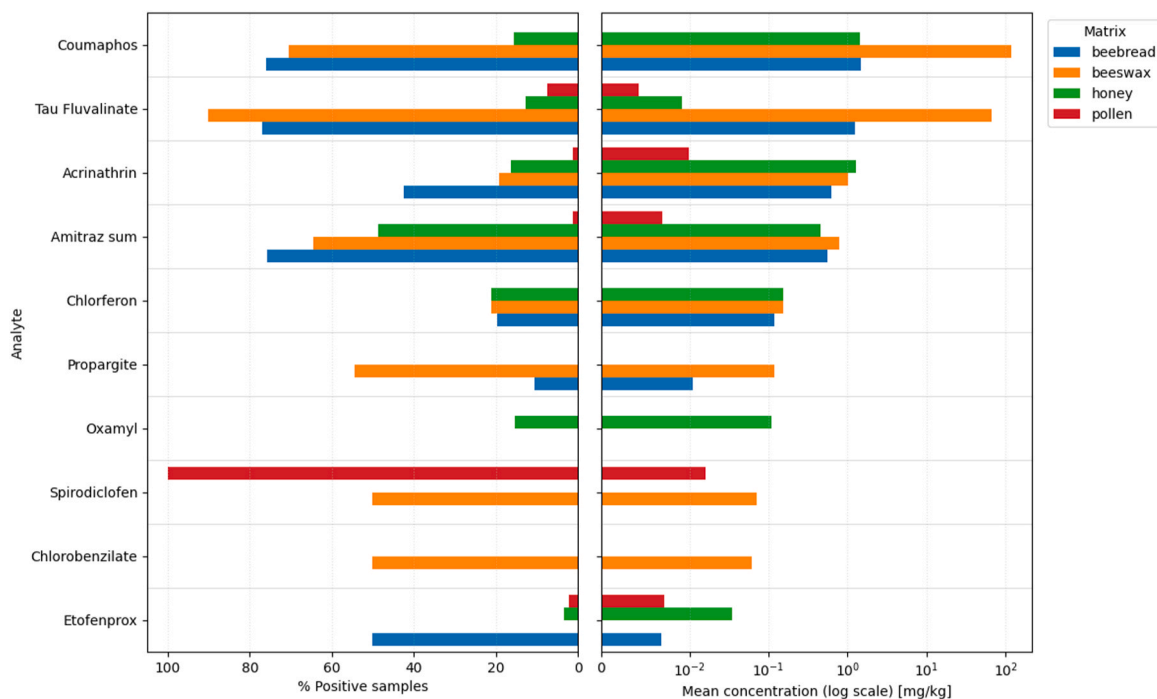
four are characterised by their long persistence and strong tendency to accumulate in wax or other lipid-rich materials. Coumaphos continues to be detected even where its use has been discontinued, likely due to residues remaining in old hive structures and recycled wax. Other substances, including propargite, acrinathrin, and dodine, also exceeded legal limits in a smaller number of samples. Glyphosate, chlorpyrifos, and dimethoate occasionally went beyond the MRLs, showing that contamination is not limited to pesticide compounds applied within hives but also involves agricultural inputs from the surrounding environment. The colour scale in the figure (see Fig. S3) highlights that exceedances are mostly associated with analytes measured in a high number of samples, suggesting that well-monitored compounds are also those with the longest environmental footprint. These trends suggest that the main concern lies not with newly introduced pesticides but with older, persistent ones that continue to circulate within the hive ecosystem. Their endurance over time makes them particularly relevant for food safety and environmental monitoring, emphasising the importance of long-term residue control and the proper management of contaminated beekeeping materials.

### 5.1. Acaricides

Among the wide range of pesticides, acaricides are chemical agents specifically designed to control mites, particularly those that parasitise honeybee colonies. In apiculture, their use is indispensable due to the devastating impact of *Varroa destructor*, a parasitic mite that has become a global threat to honeybee health [3]. These mites feed on the haemolymph of adult and larval bees, causing physical weakening and serving as vectors for several pathogenic viruses, such as the deformed wing virus and the acute bee paralysis virus. The adoption of acaricides in apiculture is primarily driven by their efficacy in rapidly reducing mite populations. Despite the development of alternative management strategies, such as breeding mite-resistant bee strains and employing biotechnological tools, acaricides remain the cornerstone of integrated pest management frameworks in apiculture [20].

Some differences were observed in the presence of acaricides across the studied bee matrices (see Fig. 3). Beeswax exhibited the highest frequency and concentration of residues, confirming its role as a long-term reservoir for lipophilic compounds such as coumaphos and tau-fluvalinate. These substances persist for extended periods and can accumulate after successive treatments, reaching concentrations several orders of magnitude higher than those found in other matrices. Honey and bee bread presented intermediate levels, reflecting both environmental exposure and in-hive contamination, while pollen occasionally contained traces of certain acaricides, likely due to indirect transfer during foraging. Among all analytes, coumaphos and tau-fluvalinate were detected most consistently, followed by amitraz and its metabolites. In contrast, compounds such as oxamyl and chlorobenzilate present lower occurrence rates.

Coumaphos, an organophosphate acaricide, inhibits acetylcholinesterase activity, causing mite paralysis. It is directly applied to the beehives and, hence, is not expected to be detected at high concentrations in corbicular pollen samples. Tau-fluvalinate, a pyrethroid compound, functions by targeting the sodium channels in mites' nervous systems. While effective, its extensive use has led to resistance in several *Varroa destructor* populations. Both coumaphos and tau-fluvalinate are non-polar compounds that tend to accumulate in wax for long periods, so they are very frequently found in bee products even if the beekeepers did not apply them [5]. Amitraz acts by disrupting the nervous system of mites, leading to paralysis and death. Amitraz and its metabolites 2, 4-dimethylformamide (DMF) and N-(2,4-dimethylphenyl)-N-methylformamide (DMPF) are polar biocides that migrate easily to honey. Although coumaphos, tau-fluvalinate, and amitraz remain widely used by most beekeepers and have been highlighted in recent studies, there is an increasing shift in apiculture toward substituting these traditional miticides with "green" and nature alternatives, such as



**Fig. 3.** Frequency of acaricide detection (top 10) and average concentration detected for pesticides in apicultural products. Bars on the left indicate the percentage of positive samples for each analyte, while bars on the right represent the mean concentrations (log scale, mg/kg) measured in bee matrices (beebread, beeswax, honey, and pollen).

oxalic acid, formic acid, and thymol, among others. It is important to note that the classification of pesticides into compound families is somewhat relative, as many of these substances belong to multiple classes due to their diverse modes of action. For instance, fenpyroximate, acrinathrin, hexythiazox, pyridaben, fipronil, and bromopropylate are primarily acaricides but also exhibit potential insecticidal properties [44].

Numerous commercially available acaricides and their active substance have been shown to negatively impact honeybee health, affecting brood development, queen and drone reproductive health, learning abilities, longevity, and overall colony strength. Acaricides can lead to increased mortality and behavioural changes in adult worker bees, disrupt food distribution, and cause physiological stress. In queen bees, these chemicals reduce longevity, impair reproductive output, and increase susceptibility to toxic effects, while drones experience reduced lifespan and reproductive capabilities [20].

## 5.2. Insecticides

A wide variety of insecticides were used for pest management, as pest infestations account for approximately 45 % of annual food production losses. Over the past three decades, insecticide application increased by about 48 % in absolute terms, but its share of total pesticide use fell from around 26 % to roughly 22 % between 1990 and 2022, reflecting changes in the composition of agrochemical use globally [45]. The main classes of insecticides in use include organochlorines, organophosphates, carbamates, pyrethroids, neonicotinoids, as well as botanical and biologically derived products and other synthetic insecticidal compounds. With the introduction of organophosphates, methyl carbamates, organochlorines, and pyrethroids from the 1940s to the 1970s, synthetic organic compounds largely replaced inorganic and botanical ones. However, with each new chemical class, resistant strains quickly emerged, reducing their effectiveness. Many deficiencies in pest control capabilities were addressed in the 1990s with the commercialization of neonicotinoids. In the period from 1991 to 2002, all seven main neonicotinoid representatives were registered – imidacloprid, nitenpyram,

acetamiprid, thiamethoxam, thiacloprid, clothianidin and dinotefuran [46]. In 2018, neonicotinoids were the most widely used insecticides globally, accounting for 25 % of the world's insecticide market. This remarkable success can be explained by their unique chemical and biological properties, such as a broad spectrum of efficacy, systemic and translaminar action, pronounced residual activity, a unique mode of action and the higher toxicity and persistence compared to other insecticide groups, such as organophosphates and carbamates, as well as increasing insect resistance to existing insecticides and the use of insecticides through seed coating. Neonicotinoids act as agonists of the nicotinic acetylcholine receptors (nAChRs), with structural differences between insect and mammalian receptors greatly influencing selective toxicity to insects [7].

There has been a significant increase in awareness among regulatory bodies and the public concerning the adverse effects of insecticides in recent years. This growing awareness has culminated in the prohibition of certain substances, alongside the implementation of stricter regulations and initiatives aimed at promoting sustainable agricultural practices. In Europe, several insecticides have been banned due to their negative environmental impacts, particularly on pollinators such as bees. Among other pesticide groups, it is worth mentioning that insecticides are the most significant and widely used group, which have been banned or restricted in many countries. For example, fipronil was restricted by the European Commission in July 2013 under Regulation (EU) No. 781/2013 following the EFSA's identification of a high acute risk to bees. Subsequently, outdoor uses of the neonicotinoids imidacloprid, clothianidin, and thiamethoxam were prohibited across the EU in 2018 in response to EFSA's 2018 risk assessments, which also confirmed their harmful effects on bees. In addition, since 2018, several other insecticides, including dimethoate, ethoprophos, indoxacarb, and phosmet, have also been banned [47]. Finally, chlorpyrifos, one of the most widely used insecticides worldwide, was banned in the European Union in 2020 following concerns regarding its potential neurodevelopmental toxicity, in accordance with Commission Implementing Regulation (EU) 2020/17.

In relation to the occurrence of insecticides in apicultural products,

beeswax consistently presented the highest detection frequencies and concentrations, confirming its strong affinity for nonpolar compounds such as chlorpyrifos and chlorfenvinphos, which were among the most frequently reported individual compounds across the reviewed studies. These residues often persist long after field applications, as lipophilic insecticides tend to accumulate and remain trapped in the wax structure. Honey and beebread exhibited intermediate levels, mainly reflecting contamination from agricultural sources rather than in-hive treatments. Pollen occasionally contained measurable traces, likely transferred during foraging in treated crops. In parallel, neonicotinoid residues were consistently detected in honey, bee pollen, beebread, and beeswax, making them one of the most prevalent insecticide classes across all examined matrices. However, their distribution varied depending on the bee matrix (see Fig. 4). Beebread and honey contained the largest number of detections, which is consistent with their direct contact with nectar and pollen gathered from crops treated with these insecticides. For example, dinotefuran appeared in almost all pollen samples. In beeswax, concentrations of thiacloprid and clothianidin were common, likely due to their ability to remain embedded within the wax structure for long periods. Pollen contained irregular amounts that fluctuated across studies and regions. Even though several of these compounds have been banned, their persistence in different hive materials demonstrates how long their residues can linger in the environment. The simultaneous detection of multiple neonicotinoids in honey, beebread, and wax points to continuous low-level exposure, both for bees and for consumers. The relatively high concentrations observed in beeswax, together with the recurrent detection of organophosphates and pyrethroids, underline the persistence of older insecticides within the hive ecosystem despite their regulatory restrictions in Europe.

In agriculture, horticulture and forestry, neonicotinoids can be applied in many ways, such as seed coating (peeling), soil drenching, foliar spraying and chemigation. Since they are systemic insecticides, they are absorbed through the roots upon application and then transported to other parts of the plants, such as leaves, flowers, and pollen. This makes them suitable for seed coatings and controlling sap-sucking

pest insects. Given their global commercialisation, mechanism of action, systemic properties, and environmental persistence, it was crucial to examine the toxicological profile of these substances and their metabolites, as well as the potential risks that neonicotinoids pose to the health of non-target organisms, such as honeybees and other pollinators. Today, neonicotinoids are often linked with the observed decline in honeybee populations worldwide. It has been observed that in bees and other pollinating insects, neonicotinoids, even at very low concentrations, can cause disorientation, reduced normal food yield, significantly weaker development of bee brood and community vitality, and at higher concentrations, even death [48]. Currently, only acetamiprid remains approved for use in the European Union among the main neonicotinoid representatives (imidacloprid, nitenpyram, thiamethoxam, thiacloprid, clothianidin, and dinotefuran), with its approval renewed until 28 February 2033 under Commission Implementing Regulation (EU) 2023/2668 of 29 November 2023.

### 5.3. Herbicides

Weeds are perennial pests that possess effective seed dispersal mechanisms and can thrive everywhere due to their efficient reproduction and seeding abilities. They are considered the biggest obstacle to modern farming systems, as they reduce crop yield and quality, resulting in billions of dollars in crop losses annually. In agricultural lands, weeds compete with crop plants for space, moisture, light and nutrients, leading to a 20–50 % decrease in crop yield production, depending on their abundance and life span in the agricultural land [49]. Chemical weed control began with inorganic substances, such as copper sulfate, first documented in 1896 in France. It advanced with the 1941 synthesis of 2,4-D, which was developed and commercialised during World War II. Herbicides target specific plant species through different mechanisms of action, offering effective weed control at low costs. Despite their benefits, herbicides can harm non-target organisms through processes like spray drift, runoff, and leaching, which spread residues into the environment. Herbicide exposure affects bees directly and indirectly.

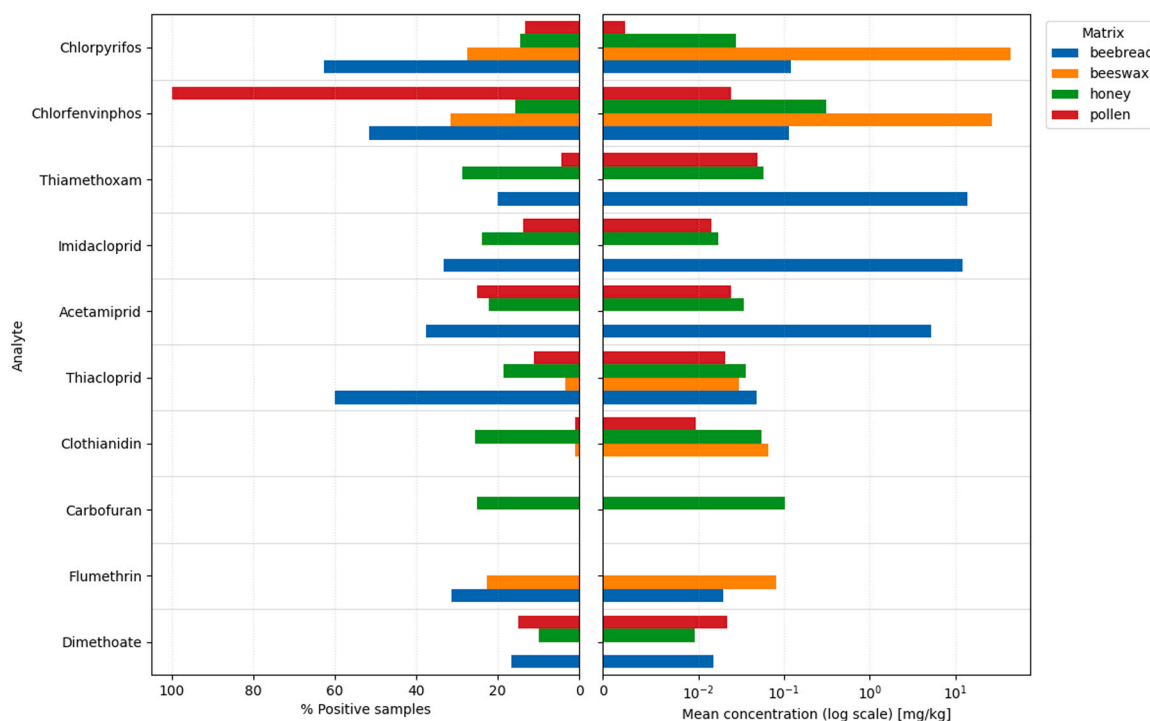


Fig. 4. Frequency of insecticide detection (top 10) and average concentration detected for pesticides in apicultural products. Bars on the left indicate the percentage of positive samples for each analyte, while bars on the right represent the mean concentrations (log scale, mg/kg) measured in bee matrices (beebread, beeswax, honey, and pollen).



Contact with herbicide-contaminated surfaces or ingestion of polluted water during foraging allows herbicides to accumulate in bees and their hives [50].

Although herbicides are less frequently studied in apicultural matrices compared to insecticides or acaricides, their residues have also been identified in several bee-derived products (see Fig. 5). Beebread consistently appeared as the most affected matrix, followed by honey and beeswax, which occasionally contained traces of these compounds. Glyphosate and its primary degradation product, AMPA, were the most frequently detected analytes across all matrices, with variable concentrations. Other herbicides, such as 2,4-D, propoxycarbazone, and fluzifop-butyl, occurred more sporadically but were still present in a notable proportion of samples. The widespread presence of glyphosate residues reflects its extensive use in agriculture and environmental mobility, as this compound can easily reach hives through contaminated water or dust. Although herbicides are not directly aimed at insects, their indirect effects on bee nutrition, microbiota balance, and habitat quality deserve closer attention.

Glyphosate is a broad-spectrum herbicide that has been used in agriculture since 1974 and is primarily used for weed control, typically before and shortly after sowing the crop. It hinders plant growth by inhibiting the synthesis of arylated amino acids. This disruption affects protein metabolism within plant tissues, ultimately resulting in plant death. The advancement of genetically modified organisms (GMO) plant technology, along with the expansion of GMO agriculture in the U.S. and other regions, has driven the widespread use of glyphosate [51]. Recent studies specifically focusing on glyphosate occurrence have shown that it is one of the most frequently detected analytes in honey across several countries, including Italy [29,52], Belgium [36], Estonia [11] and Latvia [53]. In addition, research from these regions has confirmed the presence of glyphosate in beebread, wax, nurse bees, and even larvae, although its detection is not consistent across all matrices. Overall, evidence indicates that glyphosate is a widespread and persistent contaminant within hive environments, with honey serving as a particularly reliable indicator of environmental glyphosate pollution.

However, its monitoring remains limited, as glyphosate cannot be determined using conventional multi-residue pesticide methods due to its unique chemical and physical properties. This necessitates the development and application of dedicated single-residue analytical methods, which substantially increase the cost and complexity of routine analysis.

#### 5.4. Fungicides

Fungi are significant contributors to numerous plant diseases, leading to substantial losses in global agricultural production. Fungal spores that reach the surface of plants germinate and can penetrate inside the plant and grow, or they remain on the surface and, as part of their secondary metabolism, can synthesise mycotoxins that also affect the plant and pose serious health risks to humans. They can cause root rot, seedling diseases, vascular wilt, leaf blight, rust, downy mildew, and facilitate the attack of viruses and bacteria on flowers and fruits. Either way, the fungus will render the plant's production useless by disease or even death. Fungicides are widely used, as most of them are inexpensive and easily disposed of (e.g., copper sulphate), and they are not only used in agriculture but also in protecting materials such as textiles, wood, and coatings from fungal deterioration [54]. Since there are currently a large number of active substances with antifungal capacity, fungicides can be classified according to various approaches. Succinate dehydrogenase inhibitor (SDHI) such as boscalid or fluopyram, and sterol biosynthesis inhibitors of the demethylation inhibitor class (DMI), a subgroup of ergosterol biosynthesis inhibitors (EBIs), mainly azole fungicides (e.g. tebuconazole and prothioconazole), are among the most widely used fungicides in agriculture in Europe to control a broad range of fungal diseases in arable crops, vineyards, vegetables and fruit orchards. Azole fungicides were first introduced in agriculture in the late 1970s. More than 25 azoles have been developed for plant protection products (PPPs). They have represented 20–25 % of the global fungicide market since the 1990s [55]. Even though fungicides are the most widely sold and used pesticides, especially in Europe, few studies have investigated

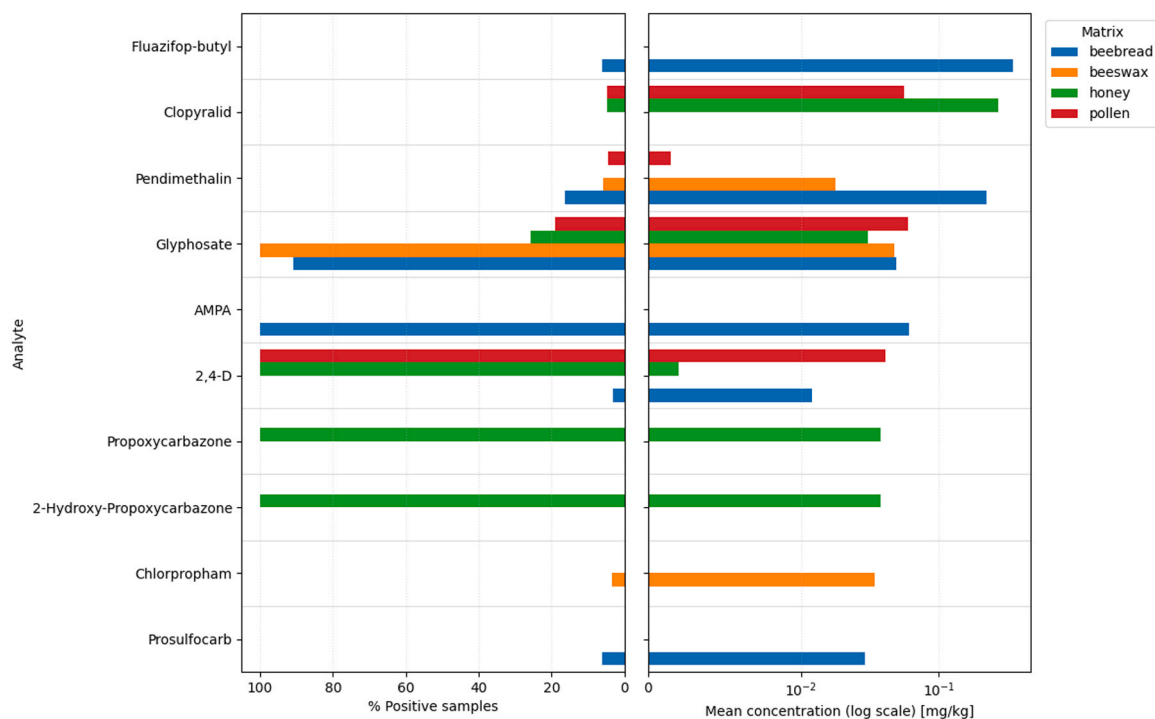


Fig. 5. Frequency of herbicide detection (top 10) and average concentration detected for pesticides in apicultural products. Bars on the left indicate the percentage of positive samples for each analyte, while bars on the right represent the mean concentrations (log scale, mg/kg) measured in bee matrices (beebread, beeswax, honey, and pollen).

their potential side effects on bees [56]. Like many other fungicides, SDHI or azole fungicides are generally considered to be at low or no risk to bees based on standard acute short-term toxicity studies, which have been conducted after a single oral or contact exposure in individual bees [55]. Azole fungicides are also well known to have strong synergistic negative effects on honeybees when combined with insecticides, such as pyrethroids or neonicotinoids [56], as they inhibit detoxification mechanisms and increase the toxicity of these insecticides.

Residues of fungicides were detected in four bee-related products – beebread, beeswax, honey and pollen, revealing the persistence and widespread nature of these compounds within the hive environment (see Fig. 6). Beebread and beeswax appeared to be the most exposed matrices, likely due to their strong capacity to trap lipophilic molecules. Honey and pollen also contained traces of several fungicides, though generally at lower concentrations. Penthiopyrad, boscalid, and tebuconazole were the most frequently occurring compounds, reflecting their popularity in crop protection. Other molecules, such as prothioconazole or fluxapyroxad, emerged occasionally but sometimes reached remarkable levels, especially in wax or stored pollen. These results indicate a slow and continuous transfer of residues from treated crops to bee colonies. Even if most fungicides are not directly lethal to bees, their coexistence with insecticides and acaricides creates a chemical landscape that could undermine detoxification processes and immune responses.

### 5.5. Inert ingredients

‘Inert’ ingredients used in PPPs are any substance (or group of similar substances) other than an active substance that is intentionally included in a PPP and often comprise safeners, synergists, co-formulants and adjuvants, as described in Article 2 of Reg. (EC) No 1107/2009. These chemicals are often incorporated into pesticide formulations to enhance the safety, effectiveness, and efficiency of the active substance [38,42]. Recent studies have highlighted the significant impact of co-formulants, substances added to pesticide products alongside active substances on bee health. Traditionally considered ‘inert’, these co-formulants can

independently cause harm to bees [57]. Our literature review identified only five inert ingredients that have been studied for residues in honeybees or their related products. Specifically, these were all pesticide co-formulants; the adjuvants and surfactants trisiloxane surfactants (TSSs), nonylphenol ethoxylate (NP(EO)<sub>n</sub>) and octylphenol ethoxylate (OP(EO)<sub>n</sub>), the solvent *N*-methyl-2-pyrrolidone (NMP) and piperonyl butoxide (PBO), which is a common synergist. TSSs act as penetrants and spreaders. By decreasing surface tension, they can enhance the diffusion of the pesticide active substance across and through plant and animal tissues, thereby increasing the efficiency of the product and ultimately reducing the amount of plant protection product required [42, 43]. NP(EO)<sub>n</sub> and OP(EO)<sub>n</sub> are widely used nonionic surfactants, which, like TSS, also act by decreasing surface tension and increasing the spread of the active substance on waxy surfaces [38]. NMP is often used to enhance the movement of other chemicals across biological membranes [39,57], used in pesticide formulations to enhance the efficacy of active substances, particularly pyrethroids. TSSs, alkylphenol ethoxylates (APEs; e.g., NP(EO)<sub>n</sub>) and NMP have been proven to be toxic to honeybees. TSSs adjuvants have been shown to have deleterious effects on important aspects of honeybee health, such as immunity, oviposition, learning, and inclusively, in combination with viral pathogens, on survival [42,43]. Exposure to NPEs has been shown to impair learning and nest recognition behaviours in bees. A study found that the nonylphenol polyethoxylate adjuvant N-90, when applied at field rates, adversely affected the nest recognition behaviour of two managed solitary bee species [30]. When combined with an active pesticide substance, co-formulants can enhance the overall toxicity of the product, leading to greater harm than the active substance alone [58].

### 5.6. Global research overview

Although research on pesticide residues in bee products has been predominantly conducted in European studies, evidence synthesized in recent comprehensive reviews indicates that contamination of apicultural matrices is an issue of global concern. The most recent comprehensive review by Véghe et al. [59] includes analytical data from Asia

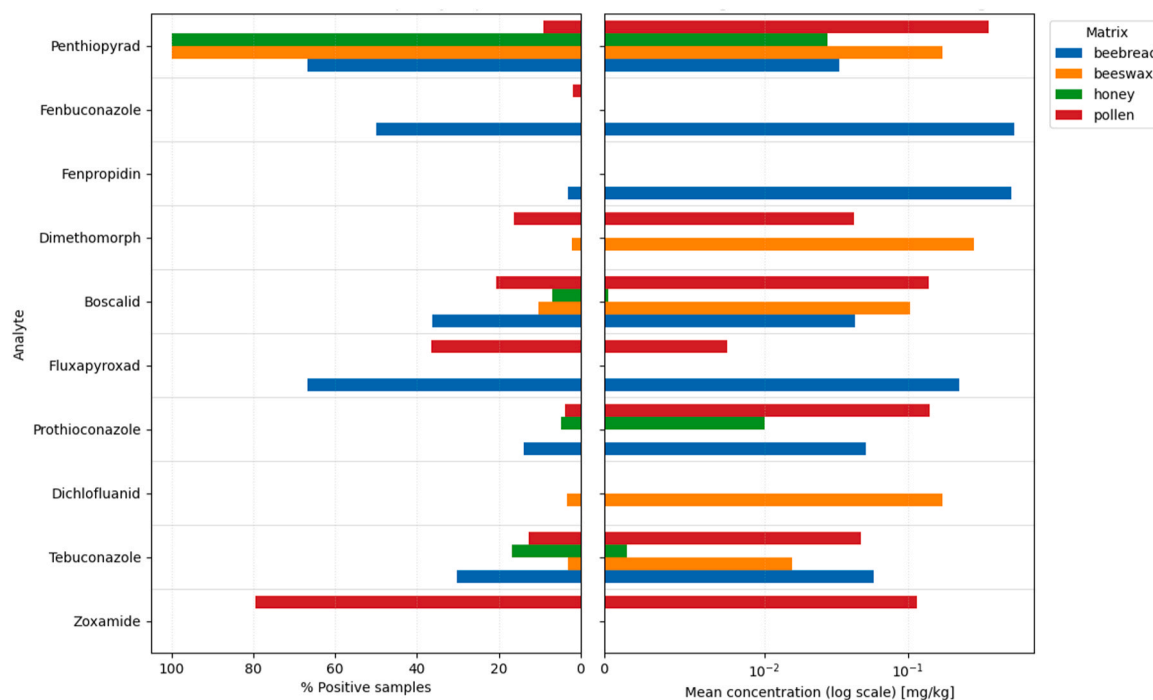


Fig. 6. Frequency of fungicide detection (top 10) and average concentration detected for pesticides in apicultural products. Bars on the left indicate the percentage of positive samples for each analyte, while bars on the right represent the mean concentrations (log scale, mg/kg) measured in bee matrices (beebread, beeswax, honey, and pollen).

(China, Jordan, India), Africa (Egypt, Ethiopia), the Americas (United States, Canada, Brazil, Uruguay, Argentina, Chile, Mexico) and Oceania (Australia). Studies from China and Australia reported pesticide residues in bee bread, including carbendazim, chlorpyrifos, coumaphos, tau-fluvalinate, atrazine and trifluralin, in some cases reaching concentrations above 1000 µg/kg. Propolis samples from South America (Brazil, Uruguay, Argentina, Chile) and Asia (China, Turkey) showed frequent contamination with organophosphates and fungicides, notably chlorpyrifos, coumaphos and triadimefon, with mean concentrations up to several thousand µg/kg. Beeswax particularly from the United States, Canada, Brazil, Egypt and Uruguay, exhibited the highest residue burdens, dominated by in-hive acaricides such as tau-fluvalinate, coumaphos and amitraz, often at ppm-level concentrations, reflecting strong accumulation of lipophilic compounds. In contrast, royal jelly samples generally contained no or only trace levels of pesticide residues, supporting the hypothesis of biological filtering by nurse bees.

## 6. Consumer health concerns and product safety

In addition to being an essential food source for bees, honey has been used as a primary sweetener by humans for thousands of years. Today, it remains valued as a clean and healthy dietary supplement, especially recommended for children, the elderly, and those with health issues. Its use is particularly prevalent in developing countries, where it is considered a nutritional resource of great importance. Consumer health concerns regarding pesticide residues in food products are a particularly sensitive topic as they include issues of food safety, public health, and environmental sustainability. The potential for chronic exposure to pesticide residues has raised alarms about the long-term health effects, including endocrine disruption and carcinogenic risks, particularly among children. Consequently, there is an increasing demand for transparent labelling, rigorous monitoring, and stringent regulatory oversight to ensure that agricultural practices prioritise consumer safety while maintaining ecological integrity. Since 2020, maximum residue levels (MRLs) for honey have been established based on experimental residue trials and dietary risk assessment data, in line with the approach applied to other food commodities. These assessments take into account the daily consumption of honey by the European population, as defined in the European Union technical guidelines (SANTE/11956/2016, rev. 9) for determining the magnitude of pesticide residues in honey and setting MRLs. As a whole, the low number of pesticide residues and levels quantified in honey, with only occasional MRL exceedances, is reassuring for consumers' safety and beekeepers. In an EFSA report [60], 3.6 % of the total honey samples evaluated in 2022 ( $n = 1272$  samples) exceeded MRLs, but for nearly 85 % of them, no quantifiable levels of residues were reported. However, attention must be paid to the co-occurrence of multiple chemical residues in honey, including not only pesticides but also emerging contaminants such as plasticizers, UV filters, and other industrial chemicals, as combined exposure may result in additive or synergistic toxic effects. MRLs for honey are not applicable to other apiculture products, such as pollen, propolis, royal jelly, and wax, due to their distinct chemical characteristics. According to SANTE/11956/2016, the relevance of the consumption and daily intake of these products is considered negligible compared to honey, which would limit the risk for human health.

## 7. Perspectives

The future of pesticide residue research in apicultural products will depend on combining analytical innovation with a broader understanding of environmental and biological processes. HRMS and hybrid chromatographic systems, such as LC-QTOF and ion chromatography coupled to Orbitrap detectors, will continue to improve the detection of pesticide residues.

Future monitoring of pesticide residues in apicultural products will increasingly benefit from the complementary use of targeted and non-

target screening approaches. While targeted LC-MS/MS methods remain essential for regulatory control and quantification of known compounds, HRMS enables non-target and suspect screening of a broader range of substances, including transformation products and previously unmonitored contaminants. As analytical instrumentation, databases, and data-processing tools continue to advance, non-target screening is expected to become a powerful supporting tool for comprehensive exposure assessment in apiculture.

These tools will allow researchers to study pesticide compounds that are currently underexplored, such as inert ingredients, metabolites, and other degradation products. The growing application of miniaturised and greener sample preparation techniques will reduce solvent consumption and laboratory waste while maintaining analytical performance. At the same time, international efforts to harmonise validation procedures and share analytical data through open-access repositories will be crucial to increasing comparability among studies and strengthening the basis for future regulations.

Regarding matrices, royal jelly and propolis were included in the search strategy; however, only a very limited number of eligible studies reported pesticide residue data for these matrices. In the references, royal jelly was either not analyzed or reported residues below quantification levels, preventing the extraction of meaningful concentration ranges. Propolis was likewise scarcely assessed despite its relevance as a nutraceutical product. This paucity of information does not demonstrate the absence of contamination but rather a knowledge gap that currently precludes robust comparisons across matrices and any data-driven considerations regarding MRL setting. Additional occurrence studies, coupled with consumption data and dietary exposure assessments, particularly for specific user groups, are needed to enable a more comprehensive risk evaluation for these two products.

Although research on so-called inert ingredients (co-formulants and adjuvants) remains limited, growing evidence suggests that these substances may contribute to toxic effects, mixture interactions, and altered residue behavior in apicultural products. Their monitoring is therefore increasingly important, particularly given their widespread use in pesticide formulations and the lack of transparency regarding formulation composition. Future research should prioritize the development of standardized, harmonized analytical approaches, supported by both targeted and HRMS-based screening, to enable reliable detection, comparison of data across studies, and improved risk assessment for bees and consumers.

A second important direction concerns toxicological assessment, which should move toward a more realistic view of how bees and hive products are exposed to multiple pesticides simultaneously. Recent studies have shown that honeybees rarely encounter single substances, but rather mixtures of insecticides, fungicides, and acaricides that may interact in complex ways. Future research should give greater attention to mixture toxicity, additive and synergistic effects, and the subtle behavioural and physiological changes caused by low concentrations of contaminants. The study of long-term accumulation in beeswax and other lipophilic matrices will also provide valuable insights into how residues persist within hives and may affect subsequent generations of bees. In addition, several matrices remain underrepresented in current research, which limits our understanding of how residues move and transform within the hive environment. Strengthening analytical studies on royal jelly, propolis, and bee brood would help clarify the transfer pathways between hive components and provide a more complete picture of the interplay of interactions inside the beehive.

In a broader context, the protection of pollinators and the quality of apicultural products will depend on regulatory, technological, and social changes that promote more sustainable practices. Although honey is currently the only apicultural matrix with established MRLs, several studies have demonstrated that pollen and beebread tend to accumulate higher levels of pesticide residues. However, the establishment of MRLs for these products would require robust data on consumption patterns and dietary exposure, which are still limited. Therefore, rather than

suggesting an immediate extension of MRLs, these matrices could be prioritized as bioindicators within environmental monitoring programs to identify contamination trends and guide policy actions. Collaboration between scientists, beekeepers, and authorities will be essential to develop realistic and coordinated strategies. Integrating the principles of green analytical chemistry with data management and education initiatives will contribute to a more transparent and responsible approach to pesticide monitoring, ensuring both consumer safety and environmental protection.

Looking ahead, the next decade is expected to bring a more integrated vision of apicultural research, connecting analytical chemistry, toxicology, ecology, and data science. Artificial intelligence and big data tools will likely play a prominent role in predicting contamination risks and modelling the environmental fate of pesticides. Automated sampling systems and biosensors may enable real-time monitoring of hive contamination, transforming how data are collected and interpreted. These innovations, combined with increased awareness of the importance of pollinators, could transform how societies perceive and manage the use of pesticides. The goal should be to establish a mutually beneficial relationship between agriculture and apiculture, where both production and protection coexist in balance, guided by scientific evidence and environmental responsibility.

## 8. Conclusions

Pesticide contamination in apicultural products reflects the close connection between agricultural practices and environmental health. The reviewed studies demonstrate that bees and their products are continuously exposed to diverse pesticide mixtures, originating both from agricultural applications and in-hive treatments. Analytical progress has been significant, with LC-MS/MS and HRMS methods offering high sensitivity and selectivity. In parallel, GC-MS/MS remains an essential complementary technique for the determination of volatile, semi-volatile, and non-polar pesticides. The combined use of LC- and GC-based platforms enables a more comprehensive coverage of pesticide residues in apicultural matrices. However, challenges remain for polar compounds and co-formulants, and as a result, compounds requiring single-residue analytical methods are still frequently overlooked. The results highlight matrix-specific accumulation patterns, with honey and pollen serving as the primary exposure routes and beeswax acting as a persistent reservoir. Although most residues in honey remain below regulatory limits, unregulated matrices such as pollen, beebread, propolis, and royal jelly and mixture effects still pose concerns for bee health. Strengthening harmonised residue monitoring, improving analytical validation, and promoting green and miniaturised methods will be essential steps toward more sustainable apiculture. Ultimately, pesticide surveillance in bee products should safeguard consumers while serving as a reliable indicator of environmental integrity.

## CRedit authorship contribution statement

**Adrián Fuente-Ballesteros:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation, Formal analysis, Data curation. **Maj Smerkol:** Visualization, Data curation. **Anton Gradišek:** Visualization, Data curation. **Artur Sarmento:** Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Fourrier Joulie:** Writing – original draft, Investigation, Data curation. **Mila Arapcheska:** Writing – original draft, Investigation, Data curation. **Zehra Hajrulai-Musliu:** Writing – original draft, Investigation, Data curation. **Filip Franeta:** Writing – original draft, Investigation, Data curation. **Željko Milovac:** Writing – original draft, Investigation, Data curation. **Sonja Gvozdenac:** Writing – original draft, Investigation, Data curation. **Nesrin İÇLİ:** Writing – original draft, Investigation, Data curation. **Harun Kurtagić:** Writing – original draft, Investigation, Data curation. **Ina Pasho:** Writing – original draft, Investigation, Data curation. **Elena Zioga:** Writing – original draft,

Investigation, Data curation. **Vázquez Beatriz I:** Writing – original draft, Investigation, Data curation. **Damir Pavliček:** Writing – original draft, Investigation, Data curation. **Rosa Busquets:** Writing – original draft, Investigation, Data curation. **Jelena Ćiric:** Writing – original draft, Investigation, Data curation. **Nurinisa Esenbuga:** Writing – original draft, Investigation, Data curation. **Miriam Cavaco:** Writing – original draft, Investigation, Data curation. **Helena Rodrigues:** Writing – original draft, Investigation, Data curation. **Rosa Ferreira:** Writing – original draft, Investigation, Data curation. **Marta Leite:** Writing – original draft, Investigation, Data curation. **Zane Lace:** Writing – original draft, Investigation, Data curation. **Ana M. Ares:** Visualization, Supervision, Investigation, Data curation. **José Bernal:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Iveta Pugajeva:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgement

This article is based upon work from COST Action CA22105 - BEEkeeping products valorization and biomonitoring for the SAFETY of BEEs and HONEY (BeSafeBeeHoney), supported by COST (European Cooperation in Science and Technology), and all authors would like to express their gratitude for its support. Anton Gradišek and Maj Smerkol acknowledge the funding from the Slovenian Research and Innovation Agency, grant number J7-50040.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.teac.2026.e00300](https://doi.org/10.1016/j.teac.2026.e00300).

## Data availability

Data will be made available on request.

## References

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