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Aflatoxins, fumonisins, citrinin, and zearalenone contents and health risk estimates in bee products in Turkey

Eda Keskin [a,*], Ozan Emre Eyupoglu [b]

[a] Department of Nutrition and Dietetics, Faculty of Health Sciences, Istanbul Medipol University, 34810, Istanbul, Turkey
[b] Department of Biochemistry, School of Pharmacy, Istanbul Medipol University, 34810, Istanbul, Turkey

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ABSTRACT

Food contamination is an important global food safety issue. This study aimed to detect aflatoxins (AFB1, AFB2, AFG1, AFG2), fumonisins (FB1, FB2), citrinin (CIT), and zearalenone (ZEA) in four bee product groups (honey, royal jelly, bee pollen, propolis) in Turkey. Additionally, exposure and risk assessments were made for mycotoxins detected in these products. Mycotoxin analyzes in bee products were carried out by high-performance liquid chromatography-ultraviolet (HPLC-UV) and liquid chromatography-tandem mass spectrometry (LC/MS-MS) methods. The most frequently detected mycotoxins in all bee products were ZEA (37.5%) and AFB2 (36.6%). The analysis showed that the average concentrations of FB2 (2.091 μg/kg), AFB1 (0.595 μg/kg), and ZEA (0.009 μg/kg) in all bee products were above the detection limit levels, while the levels of AFB2, AFG1, AFG2, FB1, and CIT were below the limit of detection. When comparing bee products, it was found that honey samples were more exposed to AFB2 and FB1, bee pollen to FB1, propolis to AFB2, and royal jelly to ZEA contamination. AFG1 and CIT were not detected in propolis and bee pollen, respectively. All mycotoxins analyzed in the study pose no health risks when bee products are consumed daily.

1. Introduction

Bees, particularly honeybees, produce and store a variety of bee products in their hives that may have potential health benefits for humans (Coppock, 2021). Bee products include honey, royal jelly, bee bread, bee pollen, beeswax, propolis, and bee venom. For many years, however, studies have focused on bee pollen, royal jelly, honey, and propolis (Wang et al., 2021).

Numerous studies conducted in the last two decades have demonstrated that bee products may lower the risk of cancer, diabetes, hepatological, cardiovascular, and neurodegenerative diseases. Additionally, they have treatment-supporting effects on existing diseases (Cornara et al., 2017; Hossen et al., 2017). The biological activities of bee products have been analyzed and found to include antioxidant, anti-inflammatory, anticarcinogenic, antibacterial, neuroprotective, immunomodulatory activities that can regulate body functions (Martinello & Mutinelli, 2021; Mărgăoan et al., 2019). It has been revealed that the presence of polyphenols, such as phenolic acids and flavonoids, in bee products' composition may be responsible for the health effects mentioned (Cheng et al., 2013; Huang et al., 2017; Pashte et al., 2020).

Contamination by mycotoxins is a persistent global food safety concern in the food, agriculture, and livestock sectors (Kharayat & Singh, 2018; Marin et al., 2013). These toxic compounds frequently contaminate the foods we consume daily (Bajkacz & Kycia-Slocka, 2020). Mycotoxins are compounds produced during secondary metabolism mainly by the *Aspergillus*, *Penicillium* and *Fusarium* fungi (Marin et al., 2013). Aflatoxin, fumonisin, ochratoxin A, deoxynivalenol, T-2 toxin, zearalenone, patulin and citrinin are among the important mycotoxins known for their high prevalence in foods and advers effects on both human and animal health (Alshannaq & Yu, 2017; Silva et al., 2020).

Aflatoxins (AFs) are compounds produced by various species of *Aspergillus*. *A. flavus* and *A. parasiticus* are the two main specific species producing AFs. These toxins can be found in agricultural products such as oilseeds, corn, grain, spices, and animal feed (Wacoo et al., 2014; WHO, 2023). Liver cancer is mainly linked with dietary intake of aflatoxins. However, these mycotoxins have also been associated with the development of cancer in other organs, such as the pancreas, bladder, bones, and internal organs (Benkerroum, 2020). The most potent hepatocarcinogen is aflatoxin B1 (AFB1), followed by aflatoxin G1 (AFG1), aflatoxin B2 (AFB2), and aflatoxin G2 (AFG2) (IARC, 2012). The International Agency for Research on Cancer (IARC) and the Scientific

* Corresponding author. Kavacik South Campus, Goztepe Dist. Ataturk Str. No.40, 34810, Beykoz, Istanbul, Turkey.
E-mail addresses: eda.keskin@medipol.edu.tr (E. Keskin), oeeyupoglu@medipol.edu.tr (O.E. Eyupoglu).

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Committee on Food (SCF) have classified AFs as 'group 1 human carcinogens' and 'genotoxic carcinogens', respectively. Additionally, the European Food Safety Authority (EFSA) has concluded that even minimal exposure to AFs (1 ng/kg/day) can elevate the risk of developing liver cancer (EFSA, 2007).

Fumonisins are a group of mycotoxins primarily produced by the fungi *F. verticillioides* and *F. proliferatum*. They are commonly found in grains, corn and corn-based products (Braun & Wink, 2018). The literature reports the most common forms of Fumonisins as B1, B2, B3 and B4, with Fumonisin B1 (FB1) and Fumonisin B2 (FB2) classified as possible carcinogens. Exposure to fumonisins has been associated with various toxic effects, with the liver and kidneys being the most sensitive target organs (JECFA, 2017). To ensure safety, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has established a Provisional Maximum Tolerable Daily Intake (PMTDI) of 2 μg/kg body weight (bw) for all fumonisins, while EFSA has set the Tolerable Daily Intake (TDI) for FB1 and FB2 at 1 μg/kg bw (JECFA, 2017; EFSA, 2018). The IARC has classified fumonisins, particularly FB1, as potentially carcinogenic (Group 2B) to humans (IARC, 2002).

Zearalenone (ZEA) is a toxin synthesized by several *Fusarium* species (mainly *F. graminearum* and *F. culmorum*). It is commonly found in grains (wheat, corn, rye, barley, and oats) and grain-based foods (EFSA, 2017). ZEA is structurally similar to endogenous estrogen and acts as an estrogen antagonist by competing with 17-β estradiol to bind to estrogen receptors. Therefore, ZEA may inhibit the activities of natural estrogens, alter their synthesis and metabolism, or interfere with the synthesis of estrogenic receptors (Wu et al., 2021). ZEA has been associated with reproductive disorders and hyperestrogenism in both human and animal studies due to its estrogenic activity and binding affinity to estrogen receptors (Wang et al., 2020). Furthermore, epidemiological studies have suggested a role for ZEA in the etiology of breast cancer and adenocarcinoma (Claeys et al., 2020). However, due to conflicting data, the carcinogenic effect of ZEA cannot be definitively determined and it is currently categorized as a group three carcinogen by IARC (Ostry et al., 2017). EFSA has established a TDI value of 0.25 μg/kg bw for this mycotoxin (EFSA, 2011).

Citrinin (CIT) is a mycotoxin produced by various species within the genera *Aspergillus*, *Penicillium* and *Monascus* species (Ostry et al., 2013). This toxin is commonly found in oilseeds, grains, grain-based products, and spices (EFSA, 2012a). Nephrotoxicity is the most well-known toxic effect of CIT. This mycotoxin has high genotoxic effects. The mycotoxin has been demonstrated to be non-mutagenic, but it can cause other types of DNA damage that indicate clastogenic effects (Ali & Degen, 2019). However, due to limited evidence of carcinogenicity in animals and humans, CIT has been classified as a third group carcinogen by the IARC (Ostry et al., 2017). To protect against the negative health effects, TDI for CIT has been determined by EFSA to be 0.2 μg/kg body weight (López Sánchez et al., 2017).

It is a well-established fact that foods may contain both healthy natural compounds and toxic compounds, including mycotoxins, heavy metals, pesticides, and metalloids (Thakali & MacRae, 2021). Bee products, defined as natural products rich in both nutritional value and bioactive components, have been known for their health-improving and supportive effects since ancient times (Durazzo et al., 2020; Eteraf-Oskouei & Najafi, 2013). Mycotoxin formation in bee products is typically observed during the harvesting and storage stages of these products. The presence of high moisture content in the air during the harvesting and/or storage periods and infrequency of harvesting increase mold growth and cause mycotoxin formation. Furthermore, these mycotoxins are not only caused by this natural occurrence in the harvesting and storage processes. They are also formed as a consequence of cross-contamination of the beehive and/or harvested bee products by humans and insects (Kačániová et al., 2011; Végh et al., 2021). Upon examining the literature, it was found that the analysis of toxic compounds in these foods was insufficient. No studies on mycotoxin analysis in bee products other than bee pollen have been found in international publications. Only deoxynivalenol, T2 toxin, HT2 toxin, and ochratoxin A mycotoxins have been investigated in bee products in Turkey (Keskin & Eyupoglu, 2023). In this study, we aimed to determine the levels of AFs, FUMs, CIT, and ZEA in bee products (propolis, bee pollen, honey, and royal jelly) from various regions and provinces of Turkey. In addition, our study evaluated dietary exposure and potential health risks to these mycotoxins taken into the body through bee products for Turkish consumers.

2. Materials and methods

2.1. Samples

A total of 112 bee products, categorized into four principal product groups: fresh bee pollen, raw propolis, honey, and royal jelly (n = 28/group), were analyzed. The samples were procured from local beekeepers in 28 distinct provinces, encompassing 7 distinct regions of Turkey during the summer/fall of 2021. Four provinces were selected from each region (Fig. 1). The provinces from which bee products were sourced were selected based on data from the Turkish Beekeeping 2021 Statistics report (Turkish Statistical Institute, 2021). The samples were stored in their original packaging in appropriate conditions: honey was stored at 23–25 °C, bee pollen was stored at +4 °C, and propolis and royal jelly were stored at −18 °C.

2.2. Mycotoxins and chemicals

The certified mix standard for AFs (AFB1+AFB2+AFG1+AFG2, ~50 μl/mL in acetonitrile, each of AFs) and FUMs (FB1+FB2, ~50 μl/mL in acetonitrile, each of FB1 and FB2) were obtained from Supelco (Bellefonte, Pennsylvania, USA). CIT and ZEA (a purity of ≥98%) were obtained from Sigma Aldrich Co. (St. Louis, MO, USA). The solvents utilised in the analytical procedure were of high purity (HPLC-grade). These included acetonitrile from Carlo Elba (Val-de-Reuil, France), methanol and acetic acid from Sigma Aldrich Co. (St. Louis, MO, USA), and formic acid from Honeywell (Riedel-de Haën™, USA). Ultra-pure water used in all solution preparation and analysis steps was employed with the PURELAB flex-3-Ultra Purified Water Machine (ELGA LabWater NA, USA).

2.3. Preparation of mycotoxin standards

The stock AFs standard dissolved in acetonitrile to make a 10 μg/mL stock solution. For calibration, AFs concentrations of (0.125, 0.25, 0.50, 1 μg/mL) were prepared via serial dilution with methanol. FUM stock standard (10 μg/mL) was obtained from a certified mixed FUM standard with acetonitrile:ultra-pure water (50:50 v/v). Working standard solutions for calibration of FUMs were obtained from the stock standard in range of 0.125, 0.25, 0.50, 1 μg/mL. The CIT stock standard solution was obtained at a concentration of 5 mg/mL in methanol. This stock solution was diluted to five distinct concentrations (0.625, 1.25, 2.5, 5, 10 μg/mL) with a methanol. The stock ZEA solution was prepared at a 2 mg/mL in acetonitrile. This stock solution were diluted to five separate concentrations (0.5, 1, 2, 4, 8 μg/mL) using a acetonitrile. All solutions were stored in amber glass bottles coated with parafilm at a temperature of −20 °C until analysis.

2.4. Sample extraction procedure

Solid raw propolis and fresh bee pollen samples were weighed as 250 ± 0.1 mg on an electronic precision balance (AND A&D Company, Tokyo, Japan) and placed in glass beakers. To remove any possible moisture, the fresh pollen and raw propolis samples were dried in a drying oven at 60 °C for 2 h. After drying, the samples were dissolved in 50 mL of methanol for 2 h on a magnetic stirrer (DLAB Scientific Inc., USA) at 1500 rpm/min without heat. The royal jelly and honey samples

Fig. 1. Provinces where the bee products analyzed in the study were purchased.

(250 ± 0.1 mg) were dissolved in 50 mL of methanol for 2 h on a magnetic stirrer (DLAB Scientific Inc., CA, USA) (without heat, 1500 rpm/min) without pre-drying. Following the extraction process, all samples were subjected to filtration by Whatman No. 4 (Sigma Aldrich Co., St. Louis, MO, USA) to eliminate potential residues from the solutions. The all filtered bee product samples were evaporated in a fume hood (TOLKIM, Turkey) (3 h, 60 °C). Following evaporation, 2 mg of each bee product extract was weighed and transferred to Eppendorf tubes. The dried residues (2 mg) were mixed again with 2 mL of methanol:ultra pure water (20:80 v/v). Then filtered into vials using a syringe filter (0.22 μm, PVDF) (ISOLAB Laborgerâte GmbH, Germany).

2.5. Mycotoxins detection by HPLC-UV

Samples in vials were injected into the HPLC system with an injection volume of 100 μL per sample. The qualitative detection of AFs, FUMs, ZEA, and CIT was performed by HPLC-UV analysis. The HPLC-UV analysis was performed with an Agilent 1100 series HPLC system (Agilent, USA). An encapped purospher star reverse phase- C_{18} column with a guard column (4.6 × 250 mm, 5 μm) (Merck, Germany) was used as HPLC column. The working conditions for mycotoxins analysis are shown in Table 1. Throughout the analytical procedure, a constant temperature of 25 °C (±1) was maintained using a column oven. Measurements were performed in triplicate to ensure reliability.

Calibration graphs were generated by analyzing dilute standards of AFs, FUMs, ZEA, and CIT at different concentrations using HPLC-UV for 15 min.

The contents of AFs, FUMs, ZEA, and CIT detected were confirmed through additional analysis using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Table 1
Working conditions for mycotoxins analysis.

Mycotoxins	Mobile phase	Injection volume (μL)	Flow rate (mL/min)	UV wavelength
AFs	MeOH: ultra-pure water: AA (97:2:1 v/v/v)	100	1	358
FUMs	Ultra-pure water: ACN: FA (60:40:0.3 v/v/v)	100	1	335
CIT	ACN: ultra-pure water: FA (10:90:0.1 v/v/v)	100	1	325
ZEA	MeOH: ultra-pure water: AA (10:89:1 v/v/v)	100	0.7	236

AA: Acetic acid, ACN: Acetonitrile, AFs: Aflatoxins, CIT: Citrinin, FA: Formic acid, FUMs: Fumonisins, MeOH: Methanol, ZEA: Zearalenone.

2.6. Confirmation with LC-MS/MS

Samples with toxin(s) identified through HPLC-UV were subsequently subjected to confirmation of toxins via an LC-MS/MS system. The LC-MS/MS analysis was conducted using a DIONEX UltiMate 3000 UHPLC system, which was interfaced with a triple quadrupole mass spectrometer (TSQ Quantum Dıscovery Max) (Thermo Scientific™, USA). The method of Keskin and Eyupoglu (2023) was used and LC-MS/MS procedure was described in detail.

2.7. Method validation

The method was validated based on linearity, recovery, correlation coefficient (R), limits of quantification (LOQ), and limits of detection (LOD) (Table 2).

To create a calibration curve, the areas of the peaks formed by the mycotoxin standards in the calibration solutions prepared at different concentrations and injected into the HPLC device were calculated.

Calibration curves were constructed using standard solutions to assess linearity. The resulting calibration curves were found to be linear.

The areas of all peaks from both standards and samples were recorded for quantitative analysis. The concentrations of AFs, FUMs, ZEA, and CIT in bee products were calculated using the linear equation obtained from the calibration graph. Correlation coefficient was determined through the use of the least-square approach. Correlation coefficient values ranged from 0.947 to 0.999, confirming the linearity of the analytical range.

LOD (3.3 times) and LOQ (10 times) values for AFs, FUMs, CIT, and ZEA in all samples were defined as multiples of the signal-to-noise ratio.

To determine recovery, different concentrations of AFs, FUMs, CIT, and ZEA were incorporated into toxin-free bee product samples, after which the peak areas of these bee product samples were compared. The recovery values observed ranged from 96.6% to 118.7%.

2.8. Evaluation of dietary exposure and health risk

Another issue that is as important as determining the concentrations of mycotoxins in foods is the degree of exposure to these mycotoxins. Therefore, in our study, we also performed an exposure and health risk assessment on bee products with mycotoxin concentrations above the LOD value. For the exposure risk assessment from oral consumption from contaminated foods, the mycotoxin concentrations detected in bee products (μg/kg) were multiplied by the average consumption of bee products in Turkey. Daily consumption data were taken from the study by Aytop et al. (2019) (Table 4). Then, the Estimated Daily Intake (EDI) was determined by dividing it by the 70 kg average body weight value for adults recommended by EFSA (EFSA, 2012b). Briefly, determination of EDI of mycotoxins in each bee product group was calculated as described below (EFSA, 2012b): EDI (μg/kg bw/day) = *[Concentration of*

Table 2
Validation of mycotoxins determination by HPLC analysis.

	Concentration range (μg/mL)	Linear equation	Correlation coefficient (R)	LOD (μg/kg)	LOQ (μg/kg)	Recovery (%)
AFB1	0.125–1	y = 6105x	0.947	0.160	0.528	118.7
AFB2	0.125–1	y = 32448x	0.991	0.790	2.607	118.5
AFG1	0.125–1	y = 44690x	0.997	1.080	3.564	118.3
AFG2	0.125–1	y = 18328x	0.992	0.440	1.452	118.2
FB1	0.125–1	y = 11360x	0.993	0.270	0.891	103.6
FB2	0.125–1	y = 271.95x	0.962	0.007	0.023	103.1
CIT	2.5–10	y = 1256.4x + 2873.9	0.995	0.003	0.008	96.6
ZEA	0.5–8	y = 2444.2x + 1071.9	0.999	0.007	0.022	99.9

AFB1: Aflatoxin B1, AFB2: Aflatoxin B2, AFG1: Aflatoxin G1, AFG2: Aflatoxin G2, FB1: Fumonisin B1, FB2: Fumonisin B2, CIT: Citrinin, ZEA: Zearalenone.

mycotoxins (μg/kg) x food consumption (g/day)]/body weight (kg).

After calculating the EDI, the health risk characterization (%TDI) was calculated as shown below: %TDI = *EDI/TDI x 100*. TDI, a value determined by relevant institutions such as EFSA and JECFA, is the amount of a toxin that can be consumed over a lifetime without risk to health. %TDI<100 indicates a tolerable exposure level, while %TDI>100 indicates an intolerable exposure level (Wang et al., 2018). However, no TDI value has been determined for aflatoxins. The EFSA have recommended margin of exposure (MoE) for the risk characterization of genotoxic and carcinogenic mycotoxins such as aflatoxins, and we have applied this approach for the risk characterization of aflatoxins in this study. The MoE formula = *Benchmark dose lower confidence limit 10% ($BMDL_{10}$)/EDI*. Since $BMDL_{10}$ was specified only for AFB1 (170 ng/kg bw/day), only MoE for AFB1 could be measured in this study. For low health risk, MoE should be ≥ 10,000 (EFSA, 2020).

2.9. Statistics

Statistical analysis of data was performed using SPSS Statistics software, version 22.0 (IBM Corp., NY, USA). The mean ± standard error (SE) and percentage (%) were used to express the values. The Kruskal-Wallis test was used to analyze the differences in mycotoxin concentrations among the four bee products groups. Mann-Whitney *U* test was used to compare paired groups. A *p*-value of <0.05 was accepted for statistically significant differences.

3. Results

The bee product samples were analyzed for AFs (B1, B2, G1, and G2) FUMs (FB1, FB2), CIT, and ZEA. The LC-MS/MS chromatogram of some mycotoxins detected in a royal jelly sample is shown in Fig. 2. The average concentration values of mycotoxins in each bee product and total bee products were presented in Table 3. If the average concentrations of AFs, FUMs, CIT, and ZEA were below the LOD, the average concentration values of bee products were not included in the total average calculation.

The mycotoxins most frequently detected in total bee products were ZEA (37.5%) and AFB2 (36.6%). The average concentrations of AFB1, FB2, and ZEA in all bee products were 0.595 μg/kg, 2.091 μg/kg, and 0.009 μg/kg, respectively. These values were found to be above the LOD levels for these mycotoxins. The mean concentrations of AFB2, AFG1, AFG2, FB1, and CIT in all bee products were below the LOD value (Table 3).

All mycotoxins analyzed in the honey samples were detected, but only AFB1 (0.671 μg/kg) and FB2 (4.671 μg/kg) concentrations were found above the LOD value.

CIT was not detected in the bee pollen samples. However, it was found that these samples were mostly contaminated with the mycotoxin FB1 (39.2%). Only AFB1 (0.441 μg/kg), AFB2 (0.087 μg/kg) and FB2 (0.561 μg/kg) concentrations were determined above the detection limit. FB2 contamination in honey samples was found to be significantly higher than in bee pollen samples ($p < 0.05$) (Table 3).

All mycotoxins, except for AFG1, were detected in propolis samples. AFB1 (0.449 μg/kg), AFB2 (0.327 μg/kg), and FB2 (1.757 μg/kg) concentrations were determined above the detection limit, similar to bee pollen samples. In royal jelly samples, ZEA was the most frequently detected mycotoxin (64.3%). Concentrations of AFB1 (0.659 μg/kg), FB2 (1.930 μg/kg), FB1 (0.241 μg/kg), and ZEA (0.009 μg/kg) were determined above the detection limit. Only one sample of royal jelly showed CIT (<LOD μg/kg) (Table 3).

The regional distribution of mycotoxins detected in honey (A), bee pollen (B), propolis (C), and royal jelly (D) is presented in Fig. 3. The distributions were determined by considering all bee products purchased from four provinces in each region, including samples that were detected below the LOD values.

When honey samples were examined on a regional basis, AFB2 was most frequently detected in the Aegean region and ZEA in the Eastern Anatolia region. AFG2 was detected only in the Black Sea and Eastern Anatolia regions. CIT was detected only in honey samples from some provinces of Central Anatolia and Southeastern Anatolia. Mycotoxin contamination (AFB2, ZEA, CIT) was found to be lower in the Central Anatolia region compared to other regions (Fig. 3A).

In bee pollen, AFB1 was detected in only one province each in the Aegean and Mediterranean regions. AFB2 was not detected in the Aegean and Central Anatolia regions, and AFG1 was not detected in the Marmara and Eastern Anatolia regions. While FB1 was found in all

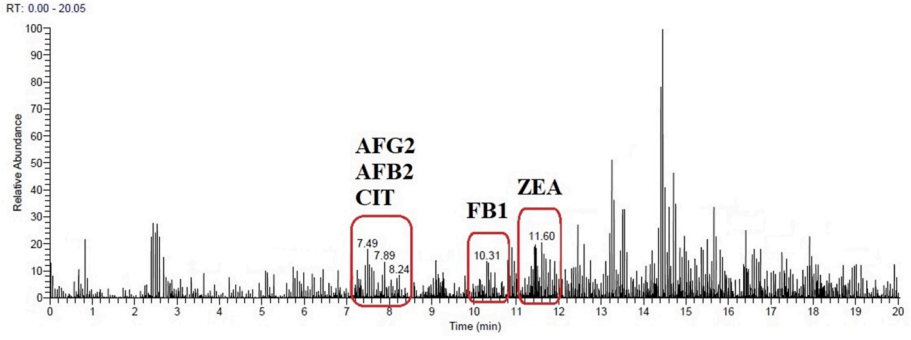

Fig. 2. The LC/MS-MS chromatogram of mycotoxins detected in a royal jelly sample.

Table 3
Content of mycotoxins detected in bee products.

		Bee products				
		Honey (n = 28)	Bee pollen (n = 28)	Propolis (n = 28)	Royal jelly (n = 28)	Total (n = 112)[b]
AFB1	Positive samples, n (%)	7 (25.0)	2 (7.1)	2 (7.1)	1 (3.5)	12 (10.7)
	Mean ± SE (μg/kg)	0.671 ± 0.262	0.441 ± 0.136	0.449 ± 0.067	0.659	0.595 ± 0.153
AFB2	Positive samples, n (%)	8 (28.5)	25.0 (7)	16 (57.1)	10 (35.7)	41 (36.6)
	Mean ± SE (μg/kg)	0.033 ± 0.008	0.087 ± 0.349	0.327 ± 0.121	0.048 ± 0.022	0.161 ± 0.052
AFG1	Positive samples, n (%)	7 (25.0)	6 (21.4)	ND	10 (35.7)	23 (20.5)
	Mean ± SE (μg/kg)	0.042 ± 0.005	0.035 ± 0.006	ND	0.029 ± 0.007	0.034 ± 0.004
AFG2	Positive samples, n (%)	2 (7.1)	5 (17.8)	5 (17.8)	6 (21.4)	18 (16.1)
	Mean ± SE (μg/kg)	0.166 ± 0.049	0.116 ± 0.028	0.421 ± 0.140	0.188 ± 0.094	0.230 ± 0.056
FB1	Positive samples, n (%)	8 (28.5)	11 (39.2)	5 (17.8)	7 (25.0)	31 (27.6)
	Mean ± SE (μg/kg)	0.101 ± 0.034	0.224 ± 0.083	0.226 ± 0.104	0.241 ± 0.071	0.197 ± 0.038
FB2	Positive samples, n (%)	7 (25.0)	9 (32.1)	8 (28.5)	10 (35.7)	34 (30.3)
	Mean ± SE (μg/kg)	4.671 ± 2.017[a]	0.561 ± 0.334[a]	1.757 ± 0.984	1.930 ± 0.862	2.091 ± 0.573
CIT	Positive samples, n (%)	3 (10.7)	ND	3 (10.7)	1 (3.6)	7 (6.2)
	Mean ± SE (μg/kg)	0.0006 ± 0.0001	ND	0.0017 ± 0.0004	0.0015	0.002 ± 0.0001
ZEA	Positive samples, n (%)	7 (25.0)	9 (32.1)	8 (28.6)	18 (64.3)	42 (37.5)
	Mean ± SE (μg/kg)	0.0005 ± 0.00025	0.0004 ± 0.00010	0.0056 ± 0.00368	0.0094	0.0094

AFB1: Aflatoxin B1, AFB2: Aflatoxin B2, AFG1: Aflatoxin G1, AFG2: Aflatoxin G2, CIT: Citrinin, FB1: Fumonisin B1, FB2: Fumonisin B2, ZEA: Zearalenone, SE: standart error, ND: Not detected.

[a] Values with a same superscript in the same row are significantly different ($p < 0.05$).

[b] When the mean concentrations of AFs, FUMs, CITs and ZEA in the samples were below the LOD, the mean concentration values of bee products were not included in the total mean calculation.

regions, FB2 was not detected only in the Eastern Anatolia region. There is no presence of ZEA in the bee pollen from the Central Anatolia and the Southeastern Anatolia regions (Fig. 3B).

AFB1, AFG1 were not detected in propolis samples from all regions. AFB2 was not found only in the Central Anatolia region. While FB1 was not detected in the Mediterranean and Black Sea regions, FB2 was not found only in the Mediterranean region. In the Aegean and Mediterranean propolis samples, ZEA was not found. CIT is present in the regions of Central Anatolia, Eastern Anatolia and Southeastern Anatolia (Fig. 3C).

Royal jelly samples were exposed to more contamination than other

Table 4
Dietary exposure and health risk assessment of mycotoxins in bee product groups.

		Bee products			
		Honey (n = 28)	Bee pollen (n = 28)	Propolis (n = 28)	Royal jelly (n = 28)
AFB1	Mean toxin concentrations (μg/kg)	0.671	0.441	0.449	0.659
	Consumption (g/day)	2.5	0.023	0.009	0.008
	EDI (μg/kg bw/day)	0.024	1.45×10^{-4}	0.58×10^{-4}	0.75×10^{-4}
	MoE[a,b]	>10.000	>10.000	>10.000	>10.000
AFB2	Mean toxin concentrations (μg/kg)	<LOD	0.087	0.327	<LOD
	Consumption (g/day)	2.5	0.023	0.009	0.008
	EDI (μg/kg bw/day)	NC[d]	0.29×10^{-4}	0.42×10^{-4}	NC[d]
	MoE[b]	–	–	–	–
AFG1	Mean toxin concentrations (μg/kg)	<LOD	<LOD	ND	<LOD
	Consumption (g/day)	2.5	0.023	0.009	0.008
	EDI (μg/kg bw/day)			NC[d]	
	MoE[b]	–	–	–	–
AFG2	Mean toxin concentrations (μg/kg)	<LOD	<LOD	<LOD	<LOD
	Consumption (g/day)	2.5	0.023	0.009	0.008
	EDI (μg/kg bw/day)			NC[d]	
	MoE[b]	–	–	–	–
FB1	Mean toxin concentrations (μg/kg)	<LOD	<LOD	<LOD	0.241
	Consumption (g/gün)	2.5	0.023	0.009	0.008
	EDI (μg/kg bw/day)	NC[d]	NC[d]	NC[d]	0.28×10^{-4}
	%TDI[c]	–	–	–	< %100
FB2	Mean toxin concentrations (μg/kg)	4.671	0.561	1.757	1.930
	Consumption (g/day)	2.5	0.023	0.009	0.008
	EDI (μg/kg bw/day)	0.166	1.84×10^{-4}	2.25×10^{-4}	2.20×10^{-4}
	%TDI[c]	<100%	<100%	<100%	<100%
CIT	Mean toxin concentrations (μg/kg)	<LOD	ND	<LOD	<LOD
	Consumption (g/day)	2.5	0.023	0.009	0.008
	EDI (μg/kg bw/day)			NC[d]	
	%TDI[c]	–	–	–	–
ZEA	Mean toxin concentrations (μg/kg)	<LOD	<LOD	<LOD	0,009
	Consumption (g/day)	2.5	0.023	0.009	0.008
	EDI (μg/kg bw/day)	NC[d]	NC[d]	NC[d]	0.1×10^{-5}
	%TDI[c]	–	–	–	<100%

AFB1: Aflatoxin B1, AFB2: Aflatoxin B2, AFG1: Aflatoxin G1, AFG2: Aflatoxin G2, bw: body weight, CIT: Citrinin, EDI: Estimated daily intake, FB1: Fumonisin B1, FB2: Fumonisin B2, MoE: Margin of exposure, NC: Not calculated, ND: Not detected, TDI: Tolerable daily intake, ZEA: Zearalenone.

[a] The MoE value was calculated using $BMDL_{10}$ (Benchmark dose) for AFB1 (170 ng/kg bw/day).

[b] Since BMDL is specified only for AFB1, the MoE was calculated only for AFB1 in this study.

[c] The TDI values used to calculate %TDI are 1 μg/kg/day, 1 μg/kg/day, 0.2 μg/kg/day, and 0.25 μg/kg/day for FB1, FB2, CIT, and ZEA, respectively.

[d] As the average mycotoxin concentration in the bee product was below the LOD value or could not be detected, the EDI was not calculated.

bee products. ZEA was found in all royal jelly collected in the Marmara Region. The Mediterranean region had the lowest contamination compared to other regions. CIT was detected only in one province of the Central Anatolia region and AFB1 in one province of the Black Sea region. Both fumanosins were not detected in the Aegean region (Fig. 3D).

When evaluated in terms of exposure and health risk, since the BMDL value was determined only for AFB1 among aflatoxins, the risk assessment (the MoE) of only this mycotoxin. As shown in Table 4, the EDI of AFB1 obtained by honey intake was the highest among bee product groups. The MoE scores of AFB1 for all these groups were >10.000. This means that the intake of AFB1 from bee products does not pose any health risk. Risk assessments for mycotoxins other than aflatoxins were evaluated using the %TDI approach. None of the EDI values for FB1, FB2, CIT, and ZEA exceeded the %TDI (<100%). This result indicates that there is no risk to health due to exposure to FBs, CIT and ZEA from dietary intake of bee products.

4. Discussion

Mycotoxins are compounds that can cause toxic effects in vertebrate organisms when taken into the body through a natural route such as the mouth, respiratory system or skin (Marin et al., 2013). The formation of mycotoxins in food and feed is a major concern in both developed and developing countries due to its potential health and economic impacts (Alshannaq & Yu, 2017; Nawaz et al., 2017). The health effects of mycotoxins are toxic and vary depending on their chemical structures and concentrations in foods. These effects include acute or chronic damage to the liver, kidneys, gastrointestinal system, central nervous system and immune system (Karaica et al., 2020; Kharayat & Singh, 2018; Polak-Śliwińska & Paszczyk, 2021).

In the literature, mycotoxin analysis has been mostly carried out in various foods such as grains, grain-based foods, oilseeds, spices, dried fruits, dairy products and coffee (Ali & Degen, 2019; Alshannaq & Yu, 2017; Kharayat & Singh, 2018). However, it has been observed that the presence of mycotoxins in bee products, which are accepted as bio-indicators today, has not been investigated in detail, and the existing analysis studies have only been carried out in a very small number of bee pollens. Based on this information, in our study, unlike other studies, toxin analysis was performed on three bee product groups as well as bee pollen. In Turkey, there is only one study to determine mycotoxin levels in bee products. In our new study, different mycotoxins (deoxynivalenol, T2 toxin, HT2 toxin, ochratoxin A) were analyzed from our current study. For this reason, our study aimed to create a database on the possible presence of AFs, FUMs, CIT and ZEA mycotoxins in these four bee product groups obtained from throughout Turkey (seven different regions, twenty-eight different provinces).

It has been determined that there are a limited number of international research on mycotoxin contamination in bee products. We have not found any studies that can compare our findings on propolis, honey, and royal jelly samples. The materials of the limited number of studies in the current literature were only bee pollen. Eight *Fusarium* toxins were investigated in fifteen bee pollen samples using the gas chromatography tandem mass spectrometry (GC-MS/MS) in Spain. Two of the fifteen samples analyzed were contaminated with high concentrations of neosolaniol and nivalenol. It was reported that the concentrations of other mycotoxins analyzed were measured below the detection limits (0.3–1.2 μg/kg) determined for each toxin (Rodríguez-Carrasco et al., 2013). However, in another study conducted in Spain, a total of twenty pollen samples purchased from beekeepers and herbalists were analyzed by HPLC-FLD for the detection of aflatoxin (B1, B2, G1, G2) and ochratoxin A, unlike the previous study, and no mycotoxin contamination was found in the pollen samples (Garcia-Villanova et al., 2004). Similar results were revealed in the study conducted in China by Xue et al. (2014). Aflatoxins (B1, B2, G1, G2) and ochratoxin A were examined in twenty bee pollen samples collected from the Northern China using a different analysis method (LC-MS/MS). LOD and LOQ values for all five mycotoxins have been reported to be in the range of 0.01–0.05 μg/kg and 0.25–0.5 μg/kg, respectively. However, measurable concentrations were not determined for AFB1, AFB2, AFG1, AFG2, and ochratoxin A in all pollen (Xue et al., 2014). Total aflatoxin, total fumonisin, DON, T-2 toxin, ochratoxin A and ZEA mycotoxins were analyzed by ELISA in forty-five bee pollen samples (three groups: poppy, rapeseed and sunflower pollen; n = 15 for each group) collected from three beekeepers in different regions of Western Slovakia. Other mycotoxins except fumonisin were detected in all samples. While the most common mycotoxins in poppy pollen were (356.6 μg/kg), DON (228.5 μg/kg), and T-2 toxin (214.3 μg/kg), respectively, in rapeseed and sunflower pollen, this order changed to T-2 toksin (231.3 μg/kg; 276 μg/kg), DON (229.9 μg/kg; 187.9 μg/kg), and ZEA (159.4 μg/kg; 132.4 μg/kg). In addition, the most common fungal species causing mycotoxin formation in all samples were investigated and fungi belonging to the species *Aspergillus* (predominantly *A. flavus*), *Fusarium* (*F. verticillioides*, *F. sporotrochoides*), *Alternaria* (*A. alternata*) and *Penicillium* (*P. verrucosum*) were detected in all samples (Kačániová et al., 2011). Similar findings were seen in the study conducted by Gonzalez et al. (2005). The study analyzed a total of 90 bee pollen samples, 87 from Spain and 3 from Argentina, and drew attention to the formation of fungi in pollen. The samples were found to contain *P. verrucosum*, *A. niger aggregate* (*A. niger* + A. *tubingensis*), *A. carbonarius*, *A. ochraceus*, *A. flavus*, *A. parasiticus,* and *Alternaria* strains. It was determined that

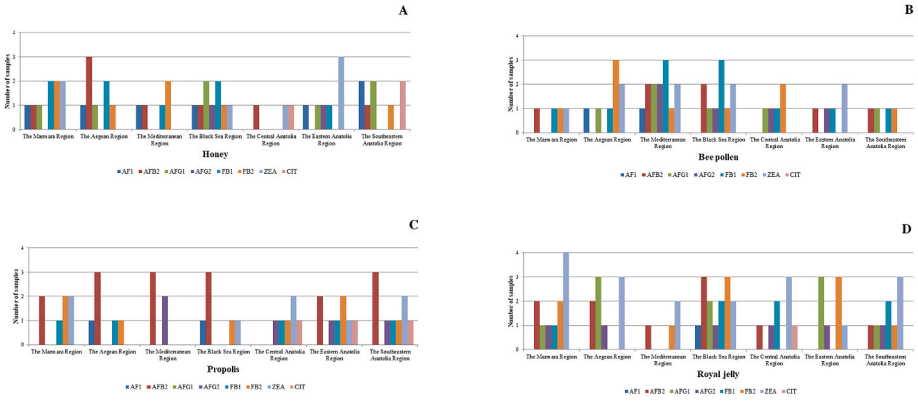

Fig. 3. The distribution of mycotoxins detected in honey (A), bee pollen (B), propolis (C) and royal jelly (D) by region.

these isolated fungal species caused the production of ochratoxin A, AFB1, and AFB2. The factor that makes this study different from the previous study is the analysis method used, and the analyzes were carried out with HPLC- Fluorescence. In Serbia, high concentrations (ELISA; 3.15–17.32 μg/kg) of AFB1 were found in all 26 bee pollen collected from the Central and Northwestern regions of the country. Microbiologically, *Penicillium* sp., *Alternaria* sp., *A. flavus* and *Fusarium* species were detected in ten samples, similar to previous studies, and differently, *Mucor* sp., *Rhizopus* sp. and *Trichoderma* sp. has been determined. These fungal species produce mycotoxins (Kostić et al., 2017). A similar finding was seen in a recent study by Carrera et al. (2023). The most common mycotoxin in a total of 80 bee pollen samples obtained from 28 countries was AFB1 (98.7%). The samples also contained deoxynivalenol (66.2%), ZEA (55%) and ochratoxin A (28.7%), while T-2 toxin was not detected. Finally, in a recent study conducted on 29 bee pollen samples in Italy, the presence of DON and total aflatoxin was investigated by ELISA. While aflatoxin was found in all bee pollen, DON was detected in 86.2% of all samples (Nuvoloni et al., 2021). The only study on mycotoxin analysis in bee products in Turkey was conducted by Keskin and Eyupoglu (2023) and deoxynivalenol, ochratoxin A, T2 toxin, and HT2 toxin were detected in bee pollen, propolis, honey, and royal jelly. However, apart from this study, there are no studies at the national level with which we can compare our findings.

Our findings in bee pollen samples have both similarities and differences to the studies mentioned above. When compared to studies conducted in other countries, it was determined that our bee pollen samples were exposed to AFs and ZEA contamination, which is consistent with the results found in Slovakia. However, while the presence of FUMs was not observed in the same study, FB1 and FB2 toxins were detected in our study. In our study, unlike the two studies conducted by Xue et al. (2014) and Rodríguez-Carrasco et al. (2013), AFs (B1, B2, G1, G2) were found in our bee pollen samples. While AFB1 and AFB2 were above the detection limit, AFG1 and AFG2 were below the detection limit. The most frequently detected mycotoxin in bee pollen was FB1 (32.1%). Unlike other studies, In our study, we conducted CIT analysis on bee products for the first time. There was no data for CIT contamination in bee products.

The detection of mycotoxins in both the literature and the bee pollen samples in our study can be attributed to the presence of fungi that were isolated from the bee pollen samples, as demonstrated in previous studies. At the same time, the differences between our study and the studies whose results are given above may be due to the following reasons: The differences in the countries where bee products are supplied, each country's unique geographical and climatic conditions, the physical structure of the bee pollen (fresh, dried, frozen, etc.), differences in the agricultural policies of the countries (harvest and storage conditions, harvest methods, etc.) and cross-contamination of bee products through environmental factors such as insects, bees and/or wind. The analysis methods we used can be shown as another factor that makes our study findings different from other studies. While analysis methods such as ELISA, GC-MS/MS and HPLC-Fluorescence are generally used in studies in the literature, in our study, bee products were analyzed with HPLC-UV, and then the samples with mycotoxin detected were confirmed with the LC-MS/MS. Compared to other studies, it is thought that the ability to detect mycotoxin contamination in bee products in our study, even at proportionally and quantitatively lower concentrations, may be due to the combined use of two analysis methods, which have been described as the most sensitive, selective and sensitive in recent years.

It is known that bee products are rich in bioactive compounds and that is why they are called superfoods. It has been widely consumed in all age groups in recent years due to its beneficial health effects on improving and protecting health. Today, studies on the presence of mycotoxins in bee products against their positive effects on health are very limited. Based on this information, mycotoxin exposure and health risk assessment was also carried out in our study. In a review study that only discussed risk assessment in bee pollen, it was reported that there was insufficient data to draw a comprehensive conclusion. Only one study has shown that concentrations of T-2 toxin, deoxynivalenol, ochratoxin A, and zearalenone detected in pollen may pose a health risk as a result of consuming 25 g/day of bee pollen (Végh et al., 2021). Unlike this study, Keskin and Eyupoglu (2023) published that the MoE values for deoxynivalenol, ochratoxin A, T2 toxin and HT-2 toxin for bee products consumption in Turkey was >10.000 (not pose a risk). Similarly, in our study, it was determined that AFB1, FBs, CIT and ZEA in all bee products did not have a negative effect on health. However, the daily bee products consumption data used in the study were taken from a cross-sectional study. For an accurate and clear exposure assessment, a bee products consumption study conducted on a large population across Turkey is required.

5. Conclusion

Contamination of the food chain constitutes a significant food safety problem worldwide. Therefore, studies that determine mycotoxin levels are crucial for both public health and environmental pollution. The quality and safety of bee products are important due to their direct consumption and use in formulated nutritional supplements worldwide. The study's findings indicate that bee products in Turkey have been exposed to AFs, FUMs, ZEA, and CIT contamination for the first time. The most commonly detected mycotoxins in all bee product groups are ZEA and AFB2. AFB2 and FB1 in honey, FB1 in pollen, AFB2 in propolis and ZEA in royal jelly were determined as the most common mycotoxins. The exposure and health risk results showed that there was no health risk for Turkish consumers of bee products. These results are an indication that toxicological analysis of bee products is necessary for food safety. As with grains and oilseeds, it is important to conduct studies and establish standards to regulate acceptable mycotoxin concentrations in bee products by national and international authorities. It is unclear from our study whether the detected contaminations occurred during the production of bee products or in storage conditions. Therefore, all measures declared by local authorities, especially temperature, humidity, and hygiene, must be meticulously implemented and followed at every stage of the food process. Based on these issues, there is a need for comprehensive research on the relationship between bee products and mycotoxins.

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CRediT authorship contribution statement

Eda Keskin: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Ozan Emre Eyupoglu:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, 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.

Data availability

The authors do not have permission to share data.

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