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Review paper

AFLATOXINS AND CLIMATE CHANGE, A SCOPING REVIEW OF MYCOTOXIN CONTAMINATION IN THE SOUTHWESTERN BALKANS

AFLATOKSINI I KLIMATSKE PROMJENE, PREGLED OPSEGA KONTAMINACIJE MIKOTOKSINIMA NA JUGOZAPADNOM BALKANU

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ABSTRACT

This review investigated mycotoxin contamination, focusing on aflatoxins, with a emphasis on the relationship between climate change and contamination levels in cereals and dairy products. The primary objective was to analyze data on aflatoxins and other mycotoxins in the region's agricultural commodities and assess the potential public health risks. Data was collected from studies published between 2010 and 2023, focusing on cereals, maize, and dairy products. The analysis synthesized findings from peer-reviewed literature and reports. Studies showed significant aflatoxin B1 and aflatoxin M1 contamination in maize, feed, and dairy products.

In maize and feed, 26.2% of samples had AFB1 levels above 5 µg/kg, but most remained within legal limits for animal feed. AFM1 in milk varied seasonally, with higher contamination in winter; Tuzla Canton showed the highest average (27.00 ng/L). Although most dairy products were safe, some isolated incidents exceeded limits. Other mycotoxins like ochratoxin A, fumonisins, and deoxynivalenol were also detected, often co-occurring, complicating management. Climatic conditions, particularly warmer, variable weather, correlated with increased mycotoxin levels. The review highlights the significant public health risk posed by mycotoxins in western Balkans. The findings suggest that ongoing

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climate change will exacerbate contamination levels, necessitating improved monitoring, storage practices, and regulatory frameworks to mitigate the risks. Further research is needed to develop effective mitigation strategies tailored to the region's unique climatic and agricultural conditions.

Keywords: Food contaminants, Mycotoxins, Climate, Balkans, Public Health Risk

SAŽETAK

Ovaj pregled istraživao je kontaminaciju mikotoksinima, s fokusom na aflatoksine, s naglaskom na odnos između klimatskih promjena i nivoa kontaminacije žitarica i mliječnih proizvoda. Primarni cilj bio je analizirati podatke o aflatoksinima i drugim mikotoksinima u poljoprivrednim proizvodima regije i procijeniti potencijalne rizike za javno zdravlje. Podaci su prikupljeni iz studija objavljenih između 2010. i 2023. godine, s fokusom na žitarice, kukuruz i mliječne proizvode. Analiza je sintetizirala nalaze iz recenzirane literature i izvještaja. Studije su pokazale značajnu kontaminaciju aflatoksinom B1 i aflatoksinom M1 u kukuruzu, stočnoj hrani i mliječnim proizvodima. U kukuruzu i stočnoj hrani, 26,2% uzoraka imalo je nivoa AFB1 iznad 5 µg/kg, ali većina je ostala unutar zakonskih granica za stočnu hranu. AFM1 u mlijeku varirao je sezonski, s većom kontaminacijom zimi; Tuzlanski kanton pokazao je najviši prosjek (27,00 ng/L).

Iako je većina mliječnih proizvoda bila sigurna, neki izolirani incidenti su premašili granice. Otkriveni su i drugi mikotoksini poput ohratoksina A, fumonizina i deoksinivalenola, koji su se često javljali istovremeno, što otežava preventivne mjere. Klimatski uslovi, posebno toplije i promjenjivo vrijeme, bili su u korelaciji s povećanim nivoima mikotoksina. Pregled ističe značajan rizik za javno zdravlje koji predstavljaju mikotoksini na zapadnom Balkanu. Nalazi ukazuju na to da će tekuće klimatske promjene pogoršati nivo kontaminacije, što će zahtijevati poboljšano praćenje, prakse skladištenja i regulatorne okvire za ublažavanje rizika. Potrebna su daljnja istraživanja kako bi se razvile efikasne strategije ublažavanja prilagođene jedinstvenim klimatskim i poljoprivrednim uslovima regije.

Ključne riječi: Kontaminanti hrane, Mikotoksini, Klima, Balkan, Rizik za javno zdravlje

INTRODUCTION

Mycotoxins are toxic secondary metabolites produced by various species of fungi, such as *Aspergillus*, *Penicillium*, and *Fusarium*. These fungi contaminate crops like maize, wheat, and dairy products at multiple stages of production, including pre-harvest, post-harvest, storage, and processing. In the Balkans, the combination of climatic variability, poor storage conditions, and high dependence on cereals and maize as staple foods exacerbates

the contamination risk. Mycotoxins, particularly aflatoxins, ochratoxin A (OTA), fumonisins, and deoxynivalenol (DON), are major public health concerns due to their toxic effects on humans and animals (Peraica *et al.*, 2002). The contamination of agricultural commodities with mycotoxins is not unique to the Balkans; it represents a global challenge. According to the Food and Agriculture Organization (FAO), approximately 25 % of the world's food crops are contaminated with mycotoxins, resulting in substantial economic and health impacts worldwide (Eskola *et al.*, 2020; Ostry *et al.*, 2017). Mycotoxins are classified as unavoidable contaminants in foods and feed due to the challenges of completely preventing fungal growth during the various stages of food production (Eskola *et al.*, 2020). This problem is compounded in regions like Southeastern Europe, where environmental conditions such as high humidity and temperature variability create ideal conditions for fungal proliferation (Schatzmayr and Streit, 2013).

The most studied and concerning mycotoxin is aflatoxin, particularly aflatoxin B1 (AFB1), which is produced by *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxins are known for their hepatotoxicity, mutagenicity, and carcinogenicity, with AFB1 classified as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC) (Ostry *et al.*, 2017). In addition to causing liver cancer, aflatoxins can suppress the immune system, making populations more susceptible to infections (Saha Turna *et al.*, 2023). Aflatoxin M1 (AFM1), a metabolite of AFB1, frequently contaminates milk and dairy products when animals are fed contaminated feed (Peraica *et al.*, 2002). In the Balkans, this has become a critical issue due to the high consumption of maize-based animal feeds (Omeragic *et al.*, 2020).

Ochratoxin A (OTA), produced by *Aspergillus ochraceus* and *Penicillium verrucosum*, also pose significant health risks. It primarily affects cereal grains such as wheat and barley but can also contaminate coffee, grapes, and wine (Bui-Klimke and Wu, 2015). OTA is nephrotoxic, immunosuppressive, and a potential carcinogen, with chronic exposure linked to kidney disease (Bui-Klimke and Wu, 2015). Fumonisins and deoxynivalenol (DON), primarily produced by *Fusarium* species, are common in maize and wheat. Fumonisin B1 (FB1), the most toxic of the fumonisins, has been associated with esophageal cancer and neural tube defects (Arumugam and Chuturgoon, 2021). DON, also known as "vomitoxin," can cause gastrointestinal distress and immune suppression in humans and animals (Roter, 1996). Mycotoxins are heat stable and resistant to traditional food processing methods, making their removal from food products particularly challenging (Wood, 1992). They often co-occur, increasing the complexity of their detection and management in the food supply chain (Streit *et al.*, 2012).

Preventive measures and regulations have been implemented to control mycotoxin contamination. In the European Union (EU), strict regulatory limits have been set for mycotoxins in food and feed, with continuous monitoring enforced by food safety authorities. For example, the maximum permissible limit for AFB1 in cereals is 2 µg/kg, and for AFM1

in milk, it is 0.05 µg/kg (European Commission, 2006). In Southeastern Europe, efforts to mitigate mycotoxin contamination have focused on improving storage practices, promoting the use of resistant crop varieties, and enhancing monitoring systems (Milicevic and Pleadin, 2023) Yet, as climate change continues to alter environmental conditions, the frequency and severity of mycotoxin contamination are expected to increase (Battilani *et al.*, 2016, Moretti *et al.*, 2019). The adoption of integrated pest management (IPM) strategies and the development of novel mycotoxin decontamination techniques are crucial for safeguarding food security and public health in the region (Krska *et al.*, 2022, A Abbas *et al.*, 2009).

1. MATERIALS AND METHODS

This review compiles data from various studies conducted between 2010. and 2023. Search was conducted using the Google scholar search engine using keywords: “mycotoxin contamination”, “climate change”, “Balkans”. Data were collected from peer-reviewed journals, reports from food safety authorities, and other research articles focusing on mycotoxins in cereals, maize, and dairy products. Preliminary search identified 2780 papers, of which there were 204 review articles. We identified 10 papers dealing with data from the western Balkans and this data is presented in the results. The data were synthesized using the guidelines of The Conduct of Systematic Reviews in Toxicology and Environmental Health Research (COSTER) (Whaley *et al.*, 2020).

2. RESULTS

Of the ten papers reviewed, six papers (60%) identified levels of mycotoxin contamination in food or feed above the legal limits. In a study analyzing 434 samples of food and feed imported to the Republic of Srpska from 2013. to 2015. for the presence of AFB1 using enzyme-linked immunosorbent assay (ELISA). Results showed that 60.5 % of the samples were contaminated, with 34.3 % containing AFB1 levels between 2-5 µg/kg and 26.2 % exceeding 5 µg/kg. Importantly, none of the analyzed samples surpassed the legal limits for AFB1 in animal feed (20 µg/kg), although 5.8 % of samples exceeded limits for human consumption in 2014 (Trkulja *et al.*, 2016). A second study analyzed 418 feed samples from 2014 to 2016 using the ELISA method. The study found that 2.57 % of the samples had AFB1 concentrations higher than 20 µg/kg, the legal limit for animal feed. The highest contamination levels were found in concentrated feed, reaching up to 30 µg/kg, while other food products like nuts and dried fruits showed much lower concentrations of AFB1 (Dojčinović *et al.*, 2017).

A study in the Bihać region investigated 54 animal feed samples for the presence of AFB1. The analysis found that all feed samples were below the maximum allowable concentration of AFB1 (0.02 mg/kg), with most samples showing non-detectable levels of

aflatoxin. These findings indicated that feed safety measures are being followed adequately, reducing the risk of aflatoxin contamination in the food chain via animal products such as meat, eggs, and milk (Huska *et al.*, 2022). A study conducted on 83 corn grain samples in Republic of Srpska found that *Aspergillus* species, particularly *Aspergillus flavus* and *Aspergillus parasiticus*, were the most frequent contaminants, present in over 80% of samples. Only two samples had AFB1 concentrations above the legal limit, with levels of 3.24 and 7.29 $\mu\text{g}/\text{kg}$ (2,40%). The majority of the samples had AFB1 concentrations below the defined maximum allowable levels, suggesting that while *Aspergillus* contamination was common, it did not always lead to significant aflatoxin production under the observed conditions (Trkulja *et al.*, 2014). A study of milk collected from Bosnia and Herzegovina assessed aflatoxin M1 (AFM1) contamination and analyzing samples from different regions, the study found that the estimated daily intake (EDI) of AFM1 ranged from 0.045 to 0.256 ng/kg body weight per day, depending on the region and season. The highest contamination levels were detected in the Tuzla Canton, with a mean concentration of 27.00 ± 18.00 ng/L in raw milk, while the lowest levels were found in the Romanija region (6.00 ± 2.00 ng/L). Risk assessments for hepatocellular carcinoma (HCC) were relatively low across all regions, indicating that AFM1 exposure in milk did not pose a significant health risk to the general population (Omeragic *et al.*, 2020).

A study investigating raw cow's milk from 13 collection sites, including farms in Vitez, Kalesija, and Butmir, found AFM1 levels exceeding regulatory limits in 2 % of the samples, especially during the winter months. The highest recorded concentration was 0.161 $\mu\text{g}/\text{L}$ in Kalesija in February 2014 (Busuladzic and Saric, 2019). This contamination was linked to poor feed storage, where maize silage provided ideal conditions for *Aspergillus* fungi, producing aflatoxins. Pearson's correlation coefficient analysis showed no significant relationship between milk quality parameters and AFM1 levels, though seasonal variations were evident, with higher contamination in winter (Trkulja *et al.*, 2014). A study done in 2018. analyzed 27 raw cow milk samples from the Una-Sana Canton in Bosnia and Herzegovina using the ELISA method to detect AFM1. The results showed that AFM1 concentrations in the samples ranged between 0.010 to 0.032 $\mu\text{g}/\text{kg}$, which is below the maximum permissible limit of 0.05 $\mu\text{g}/\text{kg}$ set by both EU regulations and local Bosnian standards. No samples exceeded the allowed limit, ensuring the milk met safety regulations for consumption (Jukic *et al.*, 2020).

A study conducted on 85 meat product samples from Zenica-Doboj Canton, Bosnia and Herzegovina, analyzing them for AFB1, nitrites, chlorides, lead, and cadmium found the average concentration of AFB1 in these samples was 0.048 $\mu\text{g}/\text{kg}$, well below the EU recommended maximum limit of 1 $\mu\text{g}/\text{kg}$ (Hasanbasic and Ibrahimagic, 2024). In a study conducted on 145 samples of Bosnian "sudžuk" (a type of dry fermented sausage) were analyzed for contamination by *Aspergillus flavus* and *Aspergillus parasiticus*. The study

detected aflatoxigenic genes in 8 of the 11 isolates. All aflatoxigenic strains were found in artisanal “sudžuk”, while none were present in industrially produced samples.

This suggests a higher risk of aflatoxin contamination in artisanal food production compared to industrial methods (Smajlovic *et al.*, 2020). In a study in Macedonia Out of a total of 3,635 tested raw milk samples, 105 samples (2.9%) contained aflatoxin M1 at concentrations exceeding the maximum permitted limit. The highest recorded concentration was 408.1 ng/kg (Dimitrieska-Stojkovic *et al.*, 2016). A study conducted in five regions of Kosovo during winter and summer of 2016, 74 out of 192 tested raw milk samples (38%) were contaminated with aflatoxin. Among them, 11 samples (5.7%) exceeded the European Union's maximum permitted level. (Camaj *et al.*, 2018).

Table 1. Summary of Aflatoxin Data from Reviewed Papers

Study	Sample Type	N (Samples)	Contaminant	% Positive Samples	Notes
Trkulja <i>et al.</i> , 2016	Food and Feed (import)	434	AFB1	60.5%	34.3% (2–5 µg/kg), 26.2% > 5 µg/kg; none > legal limit for animal feed
Dojčinović <i>et al.</i> , 2017	Animal feed	418	AFB1	2.57% > 20 µg/kg	Highest level: 30 µg/kg
Huska <i>et al.</i> , 2022	Animal feed	54	AFB1	0%	All < 0.02 mg/kg
Trkulja <i>et al.</i> , 2014	Corn grain	83	AFB1	2.4%	2 samples > limit: 3.24 and 7.29 µg/kg
Omeragić <i>et al.</i> , 2020	Raw milk	Not stated	AFM1	Not stated	Mean Tuzla: 27.00 ± 18.00 ng/L; Romanija: 6.00 ± 2.00 ng/L
Busuladžić & Sarić, 2019	Raw milk	13 sites	AFM1	2% > 0.05 µg/L	Highest: 0.161 µg/L; peak in winter
Jukić <i>et al.</i> , 2020	Raw milk	27	AFM1	0%	Range: 0.010–0.032 µg/kg; all below 0.05 µg/kg limit

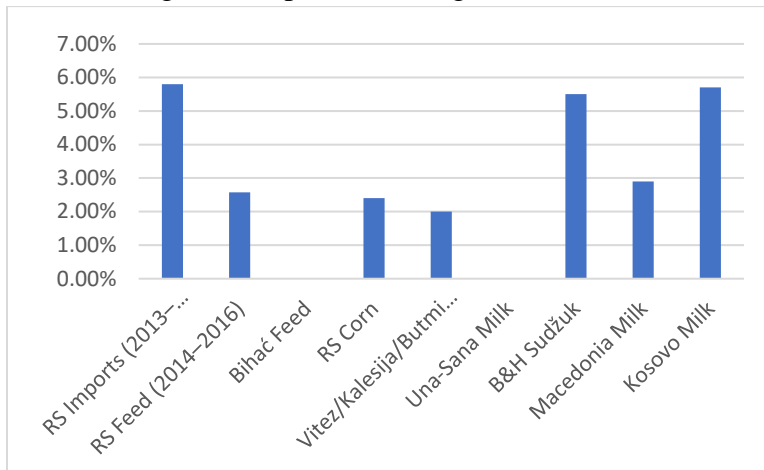
Hasanbašić & Ibrahimagić, 2024	Meat products	85	AFB1	Not stated	Mean: 0.048 µg/kg; below 1 µg/kg EU limit
Smajlović et al., 2020	“Sudžuk” sausage	145	Aspergillus (genes)	5.5% (8/145)	Aflatoxigenic genes in 8 isolates; all in artisanal, none in industrial samples
Dimitrieska-Stojković et al., 2016	Raw milk (Macedonia)	3,635	AFM1	2.9% > 0.05 µg/kg	Highest: 408.1 ng/kg
Camaj et al., 2018	Raw milk (Kosovo)	192	AFM1	38% positive; 5.7% > 0.05 µg/kg	Higher contamination in winter

Source: own editing

Geographical and Climatic Variations

The prevalence of mycotoxins varied significantly based on geographical location and climatic conditions. In a 2019. study it was found that countries in the southern Balkans, such as Albania and North Macedonia, showed higher contamination levels during warmer months, while wetter periods were associated with increased contamination in Serbia and Bosnia (Milicevic *et al.*, 2019). Climate change, marked by increased temperatures and extreme weather conditions, is expected to exacerbate mycotoxin contamination across the region (Milicevic and Pleadin, 2023). The presence of mycotoxins in cereals and dairy products is particularly concerning as these are dietary staples in these countries (Ambrus *et al.*, 2013).

Chart 1. Percentage of samples above legal limits in examined studies.



Source: own editing

3. DISCUSSION

3.1. Aflatoxin Contamination and Climate Change

Climate change exacerbates this problem by increasing temperatures and reducing rainfall, creating optimal conditions for *Aspergillus flavus* growth. Over the past several decades, Bosnia and Herzegovina (BiH) has experienced steady warming. Analyses from Sabrija Čadro et al. (2024) indicate that average summer air temperatures have been increasing at a rate of approximately 0.4 to 0.8 °C per decade. Simultaneously, precipitation has been decreasing by up to 8 mm per decade. The frequency of drought, spring and autumn frosts, hail, and floods has heightened, posing significant threats to agriculture and ecosystems in BiH. Complementing this, Popov et al. (2023) detail climate trends in the Republic of Srpska and broader BiH from 1961–2015, finding significant upward trends in mean, maximum, and minimum annual and seasonal air temperatures—especially pronounced during summer. Precipitation trends were found to be spatially and seasonally variable, generally non-significant, although interannual variability and extreme rainfall events have increased. Under the RCP8.5 scenario, projected changes by the end of the 21st century include annual temperature increases of up to 5 °C, annual precipitation reductions of up to 30 %, and summer precipitation deficits reaching 40 %—all pointing to intensified drought and water scarcity risk

For the entire Western Balkans region, Energy, Sustainability and Society (2021) reports that mean air temperature has increased by approximately 1.2 °C over the past 50 years, with the strongest warming (around 1.0–1.5 °C) occurring in the northern portion of the region and more modest warming (0.5–1.0 °C) further south. Under climate scenario RCP4.5, average warming by 2081–2100 is projected at 2.0 °C, whereas under RCP8.5 it is

estimated at 4.4 °C—with summer heat waves becoming more frequent and intense. Regarding precipitation and drought across the broader region, Islami et al. and Schewe et al. (cited in a regional adaptation report) conclude that in a 4 °C warmer world, annual rainfall is projected to decrease by 20–30 %, while the number of dry days is projected to rise by 20 %. River runoff in summer may drop by as much as 45 %, raising concerns over water availability for agriculture, hydropower, and ecosystems. Seasonal shifts—including reduced snow cover days—are also expected to worsen drought in summer and increase winter–spring flood risk along major river systems such as the Danube and Sava. These predictions are consistent with the findings of studies done in Bosnia and the region, which identified aflatoxin contamination in maize samples.

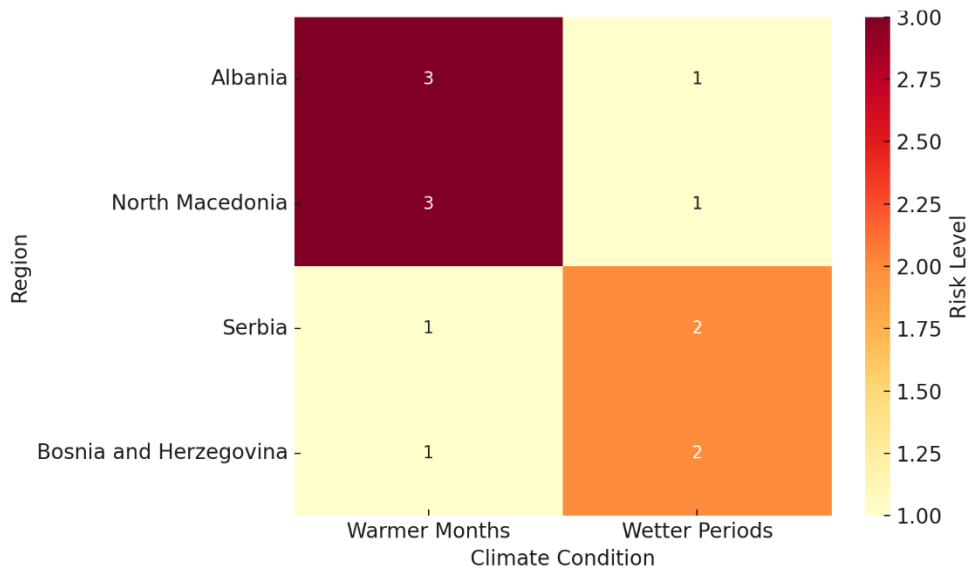
Aflatoxins, particularly AFB1, were identified as a significant risk in the Balkans, especially in maize and dairy products. This pattern mirrors global trends, where aflatoxins are the most toxic and prevalent mycotoxins in maize, peanuts, and tree nuts. The presence of AFM1 in dairy products in Serbia and Bosnia and Herzegovina is consistent with findings in other regions where livestock feed is contaminated by AFB1. A study found that aflatoxin contamination in animal feed is common across Europe, especially in maize (Streit *et al.*, 2012). In several studies, aflatoxin contamination peaks during drought periods, emphasizing climate as a key factor driving fungal growth and toxin production (Milicevic *et al.*, 2019).

Ochratoxin A (OTA) contamination, primarily in cereals and wine, is another significant health concern. Investigators identified OTA as a common contaminant in cereal grains across the world (Schatzmayr and Sreit, 2013). The co-occurrence of OTA with other mycotoxins, such as fumonisins and zearalenone (ZEA), is a notable concern, as co-contamination can exacerbate the overall toxicity of food products (Streit *et al.*, 2012). This issue is compounded by improper storage practices, which are prevalent in many parts of the Balkans and other European countries.

Fumonisin and Deoxynivalenol (DON) contamination in maize and wheat is a global issue, and the findings from the Balkans are consistent with trends reported elsewhere. Fumonisin, particularly fumonisin B1 (FB1), have been widely detected in maize, with contamination levels ranging between 200 and 400 µg/kg. Fumonisin contamination poses significant risks to human health, including esophageal cancer and neural tube defects (Moretti *et al.*, 2019). Deoxynivalenol contamination in wheat and barley is another major concern. Research has shown that DON levels are expected to rise due to climate-driven changes in *Fusarium* species distribution (Krska *et al.*, 2022). This finding is in line with the current study's detection of DON contamination in wheat samples from the Balkans, particularly during wetter seasons. The co-occurrence of DON with fumonisins and other *Fusarium* toxins complicates the management of mycotoxin contamination.

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Chart 2. Heatmap showing the relative risk of mycotoxin contamination under different climate conditions across selected Balkan countries according to examined research.



Source: own editing

3.2. Co-Contamination and Risk to Public Health

One of the most concerning aspects of mycotoxin contamination is the frequent co-occurrence of multiple mycotoxins in food products. Research has reported that more than 75% of feed samples contain more than one mycotoxin, with aflatoxins, fumonisins, and DON commonly co-occurring (Streit *et al.*, 2012). Co-contamination significantly increases the risk to human and animal health, as mycotoxins can interact and enhance each other's toxicity. The findings suggest that co-contamination is prevalent in the Balkans, particularly in cereals and dairy products.

3.3. Mitigation and Future Directions

The findings of this study underscore the need for robust monitoring systems and regulatory frameworks to mitigate the risks associated with mycotoxins. Research emphasizes the use of predictive models to anticipate mycotoxin contamination under various climate scenarios (Battilani *et al.*, 2016, Krska *et al.*, 2022). Such models can help policymakers prioritize interventions and adapt agricultural practices to minimize the impact of mycotoxins. Additionally, biological control methods, such as the use of non-toxigenic *Aspergillus flavus* strains to displace toxigenic strains, have shown promise in reducing aflatoxin contamination in maize (Khan *et al.*, 2021).

CONCLUSIONS

This review demonstrates that mycotoxins present a significant health risk in the Balkans, with aflatoxins, ochratoxins, fumonisins, and DON frequently detected in cereals, maize, and dairy products, and to some degree in meat and meat products. Contamination levels occasionally exceed regulatory limits, particularly during periods of extreme weather, which is likely to worsen with ongoing climate change. Robust monitoring systems, regulatory enforcement, and improved agricultural practices are critical for reducing mycotoxin exposure in the region. Future research should focus on developing better detection methods and mitigating strategies tailored to the Balkans' unique climatic and agricultural conditions.

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