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Review

Good Agricultural Practices-Based Strategies for Mitigation of Aflatoxins in Maize with Focus on Smallholders—A Short Review

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Abstract

Maize is a major global staple food and feed. Maize grains are highly susceptible to *Aspergillus* infection and aflatoxin contamination both in the field and in storage. Aflatoxins, primarily produced by *A. flavus* and *A. parasiticus*, are highly toxic, carcinogenic, mutagenic, teratogenic, and immunosuppressive, posing serious risks to food safety and human and animal health. Moreover, they cause significant economic losses by reducing product quality, restricting trade and export, and increasing control and storage costs. Although historically most prevalent in tropical and subtropical regions, *Aspergillus* spp. and associated aflatoxins contamination are becoming increasingly important in temperate and Mediterranean regions under changing climatic conditions. To protect public health from severe food-safety hazards, most countries have set maximum allowable aflatoxin limits in maize and other crops. Continuous monitoring and the implementation of pre- and post-harvest mitigation strategies, including Good Agricultural Practices (GAP), are essential for reducing plant stress, limiting fungal development, and minimizing the risk of toxin accumulation. The use of climate-resilient and insect-tolerant maize hybrids represents an important component of pre-harvest aflatoxin management. Accordingly, sustainable maize production requires integrated, multidisciplinary, and cost-effective approaches to minimize fungal infection and aflatoxin contamination. This review examines traditional GAP alongside innovative mitigation strategies, such as biocontrol, to reduce fungal and aflatoxin contamination in maize, with a particular emphasis on their applicability to smallholder farmers in high-risk and climate-vulnerable regions, such as Serbia. It also highlights the importance of farmer training and incentives for improving maize production, food safety, and compliance with export regulatory standards.

Keywords: maize; *Aspergillus* spp.; aflatoxins; risk management; good agriculture practices; smallholder farmers; climate change; biocontrol

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1. Introduction

1.1. Aspergillus flavus and Aspergillus Ear Rot in Maize

Maize (*Zea mays* L.) is a major staple food and a primary source of animal feed worldwide. Fungal species from the genus *Aspergillus* cause Aspergillus ear rot (AER), which reduces both grain yield and quality [1]. This plant disease is particularly devastating

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because *Aspergillus* species produce various toxic secondary metabolites (mycotoxins), with aflatoxins representing the most prominent and hazardous group. The primary aflatoxin producers are *A. flavus*, *A. parasiticus*, *A. nomius*, and *A. pseudotamari*, which belong to the *Aspergillus* section *Flavi* [2,3]. However, *A. flavus* and *A. parasiticus* are the most extensively studied aflatoxin-producing fungi (molds) in agricultural commodities. Moreover, *A. flavus* is considered the predominant species in soil and agricultural areas and is more invasive than *A. parasiticus* [4], making it the primary target for pre- and post-harvest mitigation strategies.

1.2. Aflatoxins: Occurrence, Toxicity and Health Risks

Aflatoxins were discovered in the 1960s, when more than 100,000 turkeys died from an unknown disease called "turkey X disease" on a poultry farm in England. The cause was Brazilian peanut meal contaminated with a fungal toxin produced by *A. flavus*, called aflatoxin, named after the fungus [5]. Dietary exposure to aflatoxins can cause severe toxic, carcinogenic, mutagenic, teratogenic, and immunosuppressive effects in both humans and animals. Depending on the dose and duration of exposure, these toxins lead to distinct clinical manifestations; short-term, high-dose ingestion results in acute aflatoxicosis, whereas long-term exposure to lower concentrations induces chronic disease [4]. Among the major aflatoxins (B_1, B_2, G_1, and G_2), aflatoxin B_1 (AFB_1) is the most common and toxic. Aflatoxins M_1 (AFM_1) and M_2 (AFM_2) are hydroxylated metabolites of AFB_1 and aflatoxin B_2 (AFB_2), respectively, formed in the liver and excreted in the milk of lactating mammals. AFM_1 is the most commonly detected aflatoxin in milk and dairy products [6]. According to the International Agency for Research on Cancer (IARC), AFB_1 is classified as Group 1 (human carcinogen), whereas AFM_1 is classified as Group 2B (possible human carcinogen) [7].

1.3. Epidemiology of A. flavus

Given that the widespread distribution of *A. flavus* results from its ability to survive in diverse ecosystems and rapidly colonize crops, a comprehensive understanding of its epidemiology is essential for developing effective strategies to mitigate its occurrence. *A. flavus* is a cosmopolitan fungus that can infect crops such as cereal grains, legumes, and nuts both pre- and post-harvest, producing various toxic metabolites, including aflatoxins. Figure 1 illustrates potential routes of pre-harvest infection in maize. Primary infection by *A. flavus* occurs when wind and insects disseminate conidia produced from sclerotia to the maize cob via the silk. The maize silking stage is the most sensitive to *A. flavus* infection. Infection can also spread during the maturation stage under favourable environmental conditions. Insects play a prominent role in the epidemiology of this fungus. Common insect pests such as maize borer (*Ostrinia nubilalis)*, maize earworm (*Helicoverpa zea,* formerly *Heliothis zea),* and fall armyworm *(Spodoptera frugiperda*) can transmit fungal pathogens and damage grain and other tissues during feeding, thereby facilitating fungal infection and subsequent aflatoxin contamination of grains. Conidia (spores) produced on infected plants are a source of secondary inoculum. Wind and insects disperse spores, infecting susceptible plants. As a saprophyte, *A. flavus* survives in soil and crop debris as mycelium, asexual conidia, and sclerotia [8–11]; [Figure 1]. A diverse assemblage of *A. flavus* strains includes different vegetative compatibility groups (VCGs), sclerotial-type variants, and toxigenic and atoxigenic strains. The same VCG can rarely contain both aflatoxigenic and non-aflatoxigenic strains. *A. flavus* is a non-monophyletic species, comprising L strains (>400 µm in diameter) and S strains (<400 µm in diameter) classified by sclerotia size, which is a critical factor in determining their biological and ecological roles. S-morphotype strains

are more toxigenic than L-morphotype strains [4]. When aflatoxins are produced, *A. flavus* mostly produces aflatoxin B or, very rarely, both B and G [12].

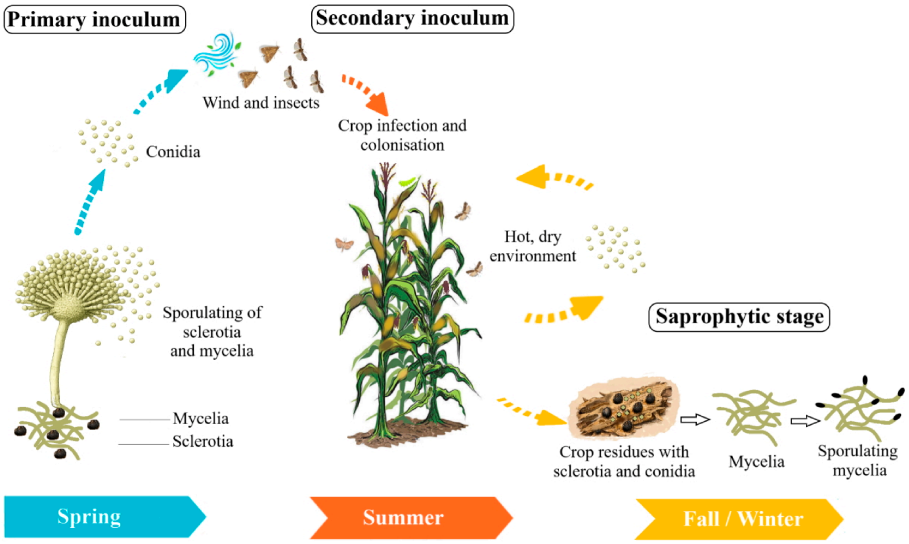

Figure 1. Pre-harvest infection of maize by *A. flavus*.

Considering the epidemiology of *A. flavus*, an integrated approach must include both monitoring the pathogen population structure and controlling insects.

1.4. Symptoms of Aspergillus Ear Rot and Detection of Aflatoxin Contamination in Maize

A. flavus infection on maize cob manifests as characteristic morphological changes in the form of rot, giving rise to the name Aspergillus Ear Rot (AER). These visible symptoms typically develop between the late milk stage and the dough stage of maize grain maturity. *A. flavus* colonization appears as a yellow-green or olive-green powdery mycelium on the maize grains. *Aspergillus* sporulation may occur anywhere on the cob, especially at the tip or localized along insect-feeding areas, and is commonly associated with *Fusarium* infection (white to pale pink mycelium) (Figure 2). *Aspergillus* spores are easily dispersed over long distances from the parent mycelium [1].



Figure 2. Aspergillus ear rot (yellow-green mycelium) on maize cobs associated with *Fusarium* infection (original photo by authors).

Apparent mycelial growth and spore formation are not necessary predictors for aflatoxin occurrence in maize grains. Similarly, maize grains without visible mycelium or injury may also contain aflatoxins [13,14]. Thus, the phenotypic expression of AER disease is not a reliable indicator of aflatoxin presence in maize grain. This separation between macroscopic colonization and toxin accumulation indicates a complex interaction among pathogens, maize, and environmental factors (Figure 3).

Therefore, maize producers should not rely on visual inspection alone to assess crop safety. Instead, the implementation of reliable scientific methods for monitoring and control is essential.

1.5. Environmental and Epidemiological Factors

Environmental and biological factors regulate *A. flavus* development and aflatoxin synthesis. The occurrence of AER disease and aflatoxin production are influenced by interactions among the host plant (maize hybrid properties, maize growth stages), the toxigenic potential of *A. flavus*, and environmental factors such as temperature, humidity, water activity, pests, locality, agricultural practices, weeds, microorganisms, etc. (Figure 3). However, high air temperatures and drought stress are the greatest contributors to *A. flavus* growth and subsequent aflatoxin production in maize [15]. Temperatures between 25 °C and 35 °C and relative humidity above 80% are ideal conditions for aflatoxin production. These conditions are typical in tropical and subtropical regions of Africa, Asia, and South America, where aflatoxins are most abundant [16,17].

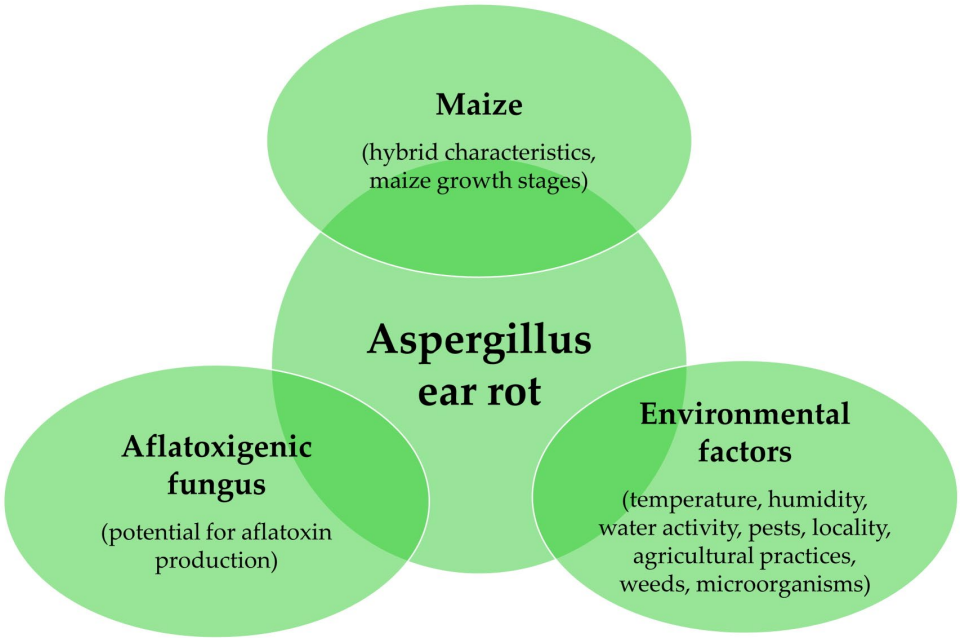

Figure 3. Factor interaction relationships for Aspergillus ear rot occurrence in maize.

1.6. Aflatoxin Risk in Serbia and Other Temperate Regions

Global climate change has raised temperatures and prolonged droughts, favouring the spread of potentially toxigenic fungi and expanding the geographic range of aflatoxin production. This trend is increasingly affecting regions where aflatoxin in maize was previously rare or low, such as temperate areas and Mediterranean regions of Europe, including Serbia. Physiologically, these climatic extremes are primary drivers of pre-harvest contamination. Elevated temperatures (optimally between 30 °C and 35 °C) and prolonged drought drastically accelerate the proliferation of *A. flavus* and trigger the upregulation of regulatory genes responsible for aflatoxin biosynthesis [18]. In addition,

severe drought stress disrupts the maize plant's water balance and weakens its natural defense mechanisms, rendering grains significantly more susceptible to fungal infection and insect damage, which subsequently creates entry points for the mold [19].

In Serbia, an epidemic outbreak of *A. flavus* and aflatoxins in maize crops occurred in 2012 (Table 1). High temperatures during the anthesis and maturity stages of maize, followed by a prolonged drought period during the vegetative season and low seasonal rainfall, caused this outbreak [20–25]. A similar epidemiological pattern of maize infection by *A. flavus* was observed in Northern Italy during 2012–2013, due to favorable weather conditions [4]. Since 2012, Serbia has also recorded increased aflatoxin levels in maize. Kos et al. [22,26] reported elevated total aflatoxin levels (above 10 µg/kg) in more than 3% of maize grain samples collected in Northern and Central Serbia in 2013 (8.7%), 2015 (8.8%), and 2022 (3.8%). Furthermore, 14.29% and 23.5% of investigated maize grain samples harvested in 2021 in the City of Belgrade district [27] and in 2024 in the Autonomous Province of Vojvodina [28], respectively, contained more than 10 µg/kg of total aflatoxins. Accordingly, the emergence and spread of *A. flavus* indicate that Serbia and the wider region are no longer low-risk zones for aflatoxins. These areas now exhibit significant and variable contamination, driven by specific climatic extremes. This knowledge supports the development of prognostic models and early warning systems to prevent mass infection and toxicogenesis. It also integrates agronomy, public health, and regulatory policy into a unified approach to risk management.

Table 1. Surveys of incidence rates and total aflatoxin (AFs) and AFB_1 levels in maize samples harvested in 2012 in Serbia.

Number of Samples *	Incidence Rate (%)	Aflatoxin	Method	Mean Level (µg/kg)	Reference
200	15	AFs	ELISA	4.67	[20]
200	24	AFs	ELISA	26.2	[20]
200	29.5	AFs	ELISA	64.9	[20]
29	44.83	AFB_1	ELISA	13.95	[24]
29	55.17	AFB_1	ELISA	>40	[24]
40	17	AFs	ELISA	3.66	[21]
40	54	AFs	ELISA	26.5	[21]
40	29	AFs	ELISA	64	[21]
40	25	AFs	HPLC	5.34	[21]
40	58	AFs	HPLC	28.2	[21]
40	17	AFs	HPLC	63.6	[21]
600	27.7	AFs	HPLC	37.4	[22]
37	97.3	AFB_1	ELISA	60.3	[25]
48	94	AFB_1	LC–MS/MS	44	[23]

ELISA—Enzyme-Linked Immunosorbent Assay; HPLC—High-Performance Liquid Chromatography; LC–MS/MS—Liquid Chromatography–tandem mass spectrometry; * Sampling was performed according to the European Commission Regulation (EC/401/2006).

1.7. Aflatoxin Management and Regulations

A. flavus and aflatoxins can occur at any stage of pre- or post-harvest handling, including cultivation, harvesting, storage, transportation, processing, and packaging [29]. Among cereal crops, maize is particularly susceptible to aflatoxin contamination. Figure 4 shows the pathways of aflatoxin contamination through animal feed and human food. Aflatoxins enter the food chain through contaminated animal feed, especially maize grains and other cereal-based feeds, which are primary sources of exposure for farm animals such as cows, turkeys, chickens, and pigs. After ingestion, the most carcinogenic and prevalent aflatoxin, AFB_1, is metabolized in the animal liver into several hydroxylation products, principally AFM_1, and transferred indirectly to humans through the consumption of animal-derived foods (milk, meat, eggs) [30,31]; [Figure 4]. Humans can also be affected by consuming

plant-based foods such as cereals, maize, wheat, nuts, and dried fruits that contain residual mycotoxins or their metabolites [32]; [Figure 4]. Aflatoxins are relatively heat-stable and can withstand processing conditions; for example, common heat treatments used in the food industry, including pasteurization and sterilization, are insufficient to degrade aflatoxins. Long-term exposure in humans is associated with serious health consequences, including acute and chronic aflatoxicosis, immune suppression, liver cancer, liver cirrhosis, and stunted growth in children [33]; [Figure 4].

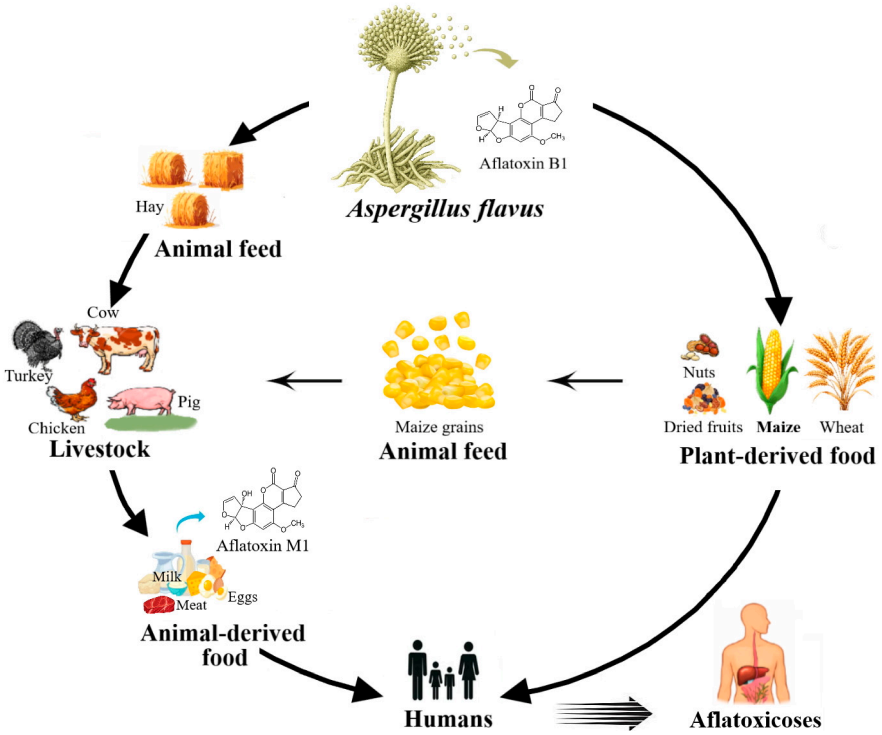

Figure 4. Pathways of aflatoxin contamination through animal feed and human food.

In many African countries, where maize and milk are staple foods, there is a risk to consumer health [34]. Furthermore, a lack of awareness of *A. flavus* and aflatoxin contamination increases risks to human and animal health, as well as economic losses in crops and livestock [35]. Regulations, pre- and post-harvest strategies, decontamination, and detoxification are therefore necessary to prevent human exposure to aflatoxins through the diet. Additionally, certain foodstuffs are subject to strict import controls. The European Union (EU) has established maximum limits for aflatoxins in agricultural food and feed products and their commodities (Commission Regulation EU/574/2011, EU/915/2023, and amendments). Thus, under Commission Regulation EU/915/2023, the maximum limits of AFB_1 and total aflatoxins (AFB_1, AFB_2, AFG_1, and AFG_2) for unprocessed maize intended to be subjected to sorting or other physical treatment before human consumption or use as an ingredient in foodstuffs are 5 µg/kg and 10 µg/kg, respectively, while the maximum limit of AFM_1 in raw milk is 0.05 µg/kg in EU countries. In contrast, the US Food and Drug Administration has imposed action levels for total aflatoxins of 20 µg/kg in human food and AFM_1 of 0.5 µg/kg in raw milk, respectively, in the USA [US FDA Compliance Policy Guide (CPG Sec. 555.400; CPG Sec. 527.400)]. In Serbia, as of 2013, the maximum limit for AFM_1 in milk was revised every 6 months, ranging from 0.05 to 0.5 µg/kg [36]. However, according to the latest Regulation on Maximum Concentrations of Certain Contaminants in Food (Sl. glasnik RS, no 73/2024, 90/2024, 47/2025, 61/2025, and 103/2025), the maximum limit of AFM_1 will be aligned with the EU Regulation from

1 December 2026 at 0.05 µg/kg. The Serbian regulatory framework sets maximum limits for AFB_1 and total aflatoxins in maize that are fully aligned with EU legislation.

These data indicate that aflatoxins represent not only an agronomic and health problem but also a regulatory and market challenge. In addition, climate change, agronomic practices, awareness among producers and consumers, and national and international policies directly affect both the extent of aflatoxin risk and the scale of economic consequences. Based on a comprehensive review of peer-reviewed articles in English from databases such as Web of Science, Scopus, PubMed, CABI, SCImago, Google Scholar, ScienceDirect, and DOAJ, using keywords such as maize, *A. flavus*, aflatoxin risk management, and Good Agricultural Practices, the main aim of this review is to present risk management strategies for controlling *A. flavus* and aflatoxin in maize. It focuses on Good Agricultural Practices (GAP), including innovative, eco-friendly methods that are especially relevant to regions with a high risk of aflatoxin contamination in the food chain, as well as to Serbia and smallholder farmers. In addition, GAP strategies are considered in relation to Good Manufacturing Practices (GMP) and the Hazard Analysis and Critical Control Point (HACCP) system as integrated frameworks for food safety. Ultimately, multidisciplinary approaches, traditional GAP, and novel methods improve global food and feed safety, ensuring high maize productivity and profitability.

2. Good Agricultural Practices (GAP) for Control of *A. flavus* and Aflatoxins in Maize for Smallholder Farmers

Good Agricultural Practices (GAP) are effective means for controlling *A. flavus* and aflatoxins in rural, commercial, and sustainable agroecosystems, ensuring the safety of both food and non-food products. GAP aims to reduce *A. flavus* and aflatoxin contamination in maize production and covers the pre-harvest, harvest, and post-harvest stages (Table 2).

Table 2. Good Agricultural Practices (GAP) for control of *A. flavus* and aflatoxins in maize.

GAP for Control of *A. flavus* and Aflatoxins in Maize		
Pre-Harvest Strategies	**Harvest Strategies**	**Post-Harvest Strategies**
Crop rotation Tillage and residue management Intercropping Resistant/tolerant maize hybrids: drought-resistant; insect-tolerant; resistant to AER and aflatoxins. Optimal sowing time Optimal plant density Balanced fertilization Irrigation/drought stress management Pest management: insect and chemical disease control; biological control (atoxigenic *A. flavus* strains); weed control.	Timely harvest Sorting damaged/infected cobs De-husking	Drying Shelling Storage: proper storage conditions; hermetic storage; regular monitoring of grain moisture and storage conditions; cleaning and sanitation of storage facilities.

Note: The listed GAP strategies are compiled and synthesized based on the studies described in the text, primarily including Xu et al. [11], Daou et al. [13], Bruns [15], and Joutsjoki and Korhonen [34], among others.

2.1. Pre-Harvest Strategies

Pre-harvest strategies for controlling aflatoxin production in maize crops are crucial because *A. flavus* infection begins at this stage. Crop rotation, tillage and residue management, intercropping, resistant/tolerant hybrids, optimal sowing time, optimal plant density, balanced fertilization, irrigation/drought stress management, and pest management (insect

and chemical disease control, biological control, and weed control) are the most common pre-harvest agricultural practices in sustainable maize production.

Crop rotation. Crop rotation (growing different crops in succession) can improve soil health and increase soil nutrient content by reducing plant stress, breaking pest and disease cycles, and enhancing pest resistance and soil fertility [11]. However, crop rotation is an effective strategy only when combined with other agrotechnical practices. Implementing appropriate crop rotation, in synergy with drought-alleviating practices such as hybrid selection, tillage, earlier sowing, plant density, irrigation, and weed, insect, and disease control, drastically reduces infection rates and lowers aflatoxin levels to safe minimums [37]. According to Abraham et al. [38], the successive cultivation of crops highly susceptible to *A. flavus* and aflatoxin, such as maize, groundnuts, cotton, sorghum, and pigeon pea, is not recommended.

Tillage and residue management. Tilling and deep plowing are important agrotechnical practices for field preparation before planting. These practices reduce *A. flavus* inoculum by removing plant residues from previous crops, increasing aeration and water availability for plants, and reducing weed populations. Deep tillage improves soil structure and crop vitality, resulting in lower aflatoxin concentrations. Removing and ploughing maize residues after harvest can also eliminate fungal spores, promoting a healthier agricultural ecosystem and reducing the long-term pressure of toxigenic *A. flavus* strains in production systems [11,13,39]. However, no-till farming practices can reduce soil erosion and water runoff, increase soil organic carbon levels, and improve ecosystem stability [39]. No-tilling preserves the topsoil but increases soil contamination with *A. flavus* spores, requiring a balance between soil protection and aflatoxin control [40]. Therefore, the choice of tillage system should be based on local conditions and integrated with other management measures to ensure both soil conservation and effective control of *A. flavus* and aflatoxins.

Intercropping. Intercropping is a traditional practice used to improve maize primary productivity. In recent decades, farmers have applied excessive rates of N fertilizer to increase yields, thereby altering agroecosystem stability. As a result, sustainable agriculture now emphasizes environmentally friendly practices such as intercropping. Intercropping legumes, which fix N, with maize is an effective approach to sustainable maize production. Maize-legume intercropping can help control diseases, pests, and weeds. Additionally, intercropping can improve N-use efficiency and utilization of nutrients, water, and light, as well as soil biodiversity and crop yield [41]. However, this recommendation requires qualification regarding aflatoxin management, since *A. flavus* is not host-specific. For instance, cultivating highly susceptible legumes such as groundnut or pigeonpea can inadvertently increase *A. flavus* soil populations and exacerbate aflatoxin levels [38]. Intercropping can also increase beneficial insect populations and reduce damage from pests, such as the maize earworm and maize borer, lowering *A. flavus* populations and aflatoxin production in maize crops [42]. Accordingly, intercropping simultaneously improves resource utilization and agroecosystem stability while indirectly reducing conditions that favor the development of aflatoxigenic fungi.

Resistant/tolerant hybrids. The use of drought-resistant hybrids is a primary measure in *A. flavus* and aflatoxin management, focusing on maize hybrids well adapted to local agro-ecological conditions [43]. Improved water management is therefore an important component of aflatoxin risk mitigation. Consequently, developing drought-resistant hybrids with higher water and nitrogen use efficiency will enhance their adaptation to global warming [44]. Maize resistance to AER and aflatoxins is a desirable strategy in sustainable agriculture. However, resistant germplasm is limited, and no commercially available maize hybrids have complete resistance to *A. flavus* and aflatoxins yet [45,46]. Early screening by Williams et al. [47] identified only a few sources of aflatoxin resistance. Therefore,

modern breeding programs rely on indirect selection through maize hybrids resistant to environmental stressors and biological agents that facilitate fungal entry. Drought tolerance mitigates environmental stress, while insect-tolerant maize hybrids prevent mechanical damage. For instance, transgenic Bt maize hybrids provide targeted insect resistance, significantly reducing aflatoxin accumulation in regions where such cultivation is permitted [48]. Likewise, maize hybrids with long cobs and thick husk cover are more resistant to insect attack and suffer less aflatoxin contamination [40]. Furthermore, genetic modification of maize hybrids to increase nutritional value (biofortification) is now an integral part of modern breeding programs. Suwarno et al. [49] reported that maize hybrids with elevated beta-carotene, beta-cryptoxanthin, and provitamin A contents possess natural antioxidant properties that inhibit aflatoxin synthesis, simultaneously enhancing vitamin A in maize. Selecting genetically superior, locally adapted hybrids is a long-term strategy to increase the resilience of the production system and reduce the risk of *A. flavus* and aflatoxins in maize grains. However, genetic resistance alone is not sufficient. The greatest effect is achieved when it is combined with other GAP practices.

Optimal sowing time. Sowing maize at the optimal time and under favourable soil conditions is essential for successful production. Soil texture, crop residues, and temporal variations influence strain composition and *A. flavus* population size. Rising soil temperatures increase the proportion of aflatoxigenic strains. Likewise, the *A. flavus* population is higher in soil where maize residues are left after harvest [39]. Proper sowing time helps avoid high temperatures and drought stress during seed development and maize maturation. Early sowing is especially beneficial in water-stressed regions. In addition, selecting maize hybrids suitable for water stress and lower temperatures, together with an adjustable sowing time, can help to avoid wet periods during early flowering and the detrimental effects of mycotoxins [50]. Early sowing is important in arid and water-limited regions because it reduces drought stress during critical stages of growth and development, thereby lowering the risk of *A. flavus* infection and aflatoxin accumulation in maize.

Optimal plant density. Plant density influences microclimatic conditions within the crop. High plant densities increase wet and warm conditions, as well as the number of insects, pathogens, and diseases. They can also reduce yield due to interplant competition for soil nutrients and sunlight. Alvarado-Carrillo et al. [51] reported higher aflatoxin levels and lower grain yield at high plant densities in maize crops. In contrast, Abbas et al. [52] found no reduction in aflatoxin levels in maize grain at lower plant density. These inconsistencies could result from differences in the genotypic and phenotypic properties of the maize hybrids and their ability to adapt to the stress imposed by high plant density. Likewise, unpredictable environmental factors and high fungal adaptability can promote *A. flavus* growth and aflatoxin production under lower crop density. Hence, the optimal crop density should follow the hybrid manufacturer's recommendations, available resources, and agroecological conditions. Although the effect of plant density on aflatoxin levels is not always consistent, an optimal density adapted to the maize hybrid and growing conditions is essential for maintaining a balance between productivity and crop phytosanitary status.

Balanced fertilization. Fertilization is a crucial practice for increasing maize grain yield and quality. Balanced fertilization with nitrogen, phosphorus, potassium, and micronutrients supports plant vigor and reduces susceptibility to abiotic and biotic stresses associated with *A. flavus* and aflatoxin occurrence. Conversely, insufficient or excessive nutrient levels can disturb plant physiological balance, increasing vulnerability to insect pests and diseases and disrupting essential biochemical pathways and physiological processes. Fertilization must be applied in optimal amounts and at the right time to prevent plant stress and reduce susceptibility to pests and pathogenic fungi [13]. Research results on the influence of nitrogen (N) fertilization on pathogen presence and mycotoxin levels are inconsistent. While

some studies report that N increases these parameters [53], others report decreases [54] or no effect [55]. Differences in N amounts and forms, along with local agroecological conditions, cause these conflicting results. Regarding the effect of N fertilization on aflatoxin levels in maize, Blandino et al. [56] and Mwalugha et al. [57] reported that N fertilization suppressed aflatoxin in maize. According to Nyandi and Pepó [58], selecting less susceptible maize hybrids was a more effective agrotechnical practice for reducing mycotoxin levels in maize grains than N fertilization, which remains a significant agronomic measure for maximizing yield. As with plant density, these inconsistencies could result from differences in agronomic and climatic interactions.

Irrigation/drought stress management. Maize is highly sensitive to moisture stress during critical growth stages such as silking and grain filling, which severely predisposes the crop to fungal infection. Properly timed irrigation management can successfully mitigate *A. flavus* outbreaks and aflatoxin occurrence by preventing severe drought stress and maintaining optimal crop water status [59]. In a recent field study conducted in Serbia, Savić et al. [60] reported no significant differences in aflatoxin levels between irrigated and non-irrigated maize treatments across the 2016 and 2017 harvest seasons. In contrast, in the USA, Payne [61] reported that irrigation reduced aflatoxin contamination in maize crops. However, in Mexico, Rodriguez-del-Bosque [62] reported that a combination of pre-harvest practices, early sowing, reduced plant density, irrigation, and insect control reduced aflatoxin levels compared with late-sowing, non-irrigated maize at higher plant density without insect control. Therefore, when integrated with crop rotation, optimized nitrogen fertilization, and other pre-harvest strategies, irrigation reduced both plant stress and fungal contamination in maize. Precision irrigation technologies, such as soil moisture monitoring and sensor-based scheduling, enhance water-use efficiency while substantially mitigating drought-driven risks of aflatoxin contamination.

Pest management/insect and chemical disease control. Insects such as maize earworm, fall armyworm, and maize borer damage the crop and transmit fungi that produce aflatoxin. Timely control of insects with registered insecticides reduces damage and the risk of fungal development on maize grains. Because anthesis is the most susceptible stage for fungal infection, chemical controls should be applied to protect maize grains from fungal pathogens. Fungicides have not been widely used to reduce *A. flavus* and aflatoxin in maize. However, azole-based fungicides may have potential as part of an integrated approach to *Aspergillus* control, with sustainable and responsible pesticide application [11]. Nevertheless, their widespread use in agriculture is not recommended due to the risk of resistance developing in non-target fungi; specifically, *Aspergillus fumigatus* is a species resistant to all azoles and is a human pathogen [63].

Pest management/biological control strategies. Microorganisms (fungi, bacteria, and yeasts) are widely used as biocontrol agents to protect crops from plant diseases. They have been tested and shown to be effective in suppressing *A. flavus* and reducing aflatoxin contamination in maize crops [64]. These biological agents mitigate aflatoxin risks through several distinct mechanisms: competitive exclusion, antibiosis, and enzymatic degradation. Among fungal agents, non-aflatoxigenic *A. flavus* strains are highly effective, primarily through competitive exclusion, outcompeting toxigenic strains for carbon sources, essential nutrients, and physical space within the maize canopy, thereby preventing the proliferation of toxin-producing strains. Additionally, *Trichoderma* species, such as *Trichoderma harzianum*, are widely distributed microbial antagonists that inhibit *Aspergillus* growth via resource competition and metabolic interference. Beyond inhibition, *Trichoderma* spp. can actively degrade existing aflatoxins through the secretion of specialized extracellular enzymes, breaking down the toxin molecule into less toxic or non-toxic metabolites [65]. Bacterial and yeast antagonists offer further protective pathways through antibiosis. Bacteria from

the genera *Bacillus*, *Lactobacillus*, *Pseudomonas*, and *Streptomyces*, alongside yeasts such as *Saccharomyces* spp., *Aureobasidium* spp., *Pichia* spp., and *Rhodotorula* spp., produce secondary metabolites and volatile organic compounds that suppress *Aspergillus* growth and downregulate aflatoxin biosynthesis genes [65]. Furthermore, specific *Lactobacillus* strains and yeasts can be used for post-harvest decontamination, efficiently removing aflatoxins from food and feed commodities (such as milk) through physical binding to their cell wall peptidoglycans or polysaccharides [66].

The successful translation of laboratory-scale biocontrol into field applications has led to the commercialization of several atoxigenic *A. flavus* formulations. These products are strategically applied before maize harvest to maximize competitive exclusion in the field. Atoxigenic *A. flavus* strains marketed under trade names such as AflaSafe in Africa [1], AF-X1 in Europe, primarily applied in Italy [67,68], and Afla-Guard in the USA are most effective at reducing aflatoxin levels before maize harvest [10]. Although laboratory studies have demonstrated the antagonistic and inhibitory potential of various microorganisms or their metabolites against *A. flavus* [69], their commercial application has remained limited, resulting in few products available on the market (Table 3).

Table 3. Commercial microorganism-based biopesticides for controlling *A. flavus* and aflatoxins in maize.

Microorganisms	Trade Name	Dosage	Company	Effectiveness	Method of Application	Reference
Fungi						
Aureobasidium pullulans	Botector®	1 g/L H_2O	BIO-FERM® (Herzogenburg, Austria)	On the seed in vitro and pre-harvest	Spraying	[64]
Trichoderma harzianum	Trianum®	3 g/L H_2O	Koppert® (Berkel en Rodenrijs, The Netherlands)	On the seed in vitro	Spraying	[64]
Non-aflatoxigenic strains of *A. flavus*	AF-X1®	25 kg/ha	CORTEVA Agriscience (Wilmington, DE, USA)	Pre-harvest	Broadcast in the field	[70,71]
Non-aflatoxigenic strains of *A. flavus*	AflaGuard®	10–20 lb/A (4.5–9 kg/0.4 ha)	SYNGENTA® (Basel, Switzerland)	Pre-harvest	Broadcast in the field	[70,72]
Non-aflatoxigenic strains of *A. flavus*	AflaSafe	4 kg/A (10 kg/ha)	IITA (Ibadan, Nigeria) and USDA-ARS (Beltsville, MD, USA)	Pre-harvest	Broadcast in the field	[73,74]
Bacteria						
Bacillus subtilis	Serenade Max®	4 g/L H_2O	Bayer® (Leverkusen, Germany)	On the seed in vitro	Spraying	[64]
Streptomyces griseoviridis	Mycostop®	0.5 g/L H_2O	Verdera® (Vancouver, BC, Canada)	On the seed in vitro and pre-harvest	Spraying	[64]

IITA—International Institute for Tropical Agriculture; USDA-ARS—United States Department of Agriculture—Agricultural Research Service.

Despite being environmentally friendly, the widespread adoption of biopesticides faces several major barriers. First, these biological agents are often more expensive to produce and apply compared to conventional products. Second, there is still a critical lack

of safety data regarding the toxicity of the metabolites formed after enzymatic mycotoxin degradation [75]. Addressing these challenges requires extensive laboratory research to develop more stable and high-efficiency strains. Furthermore, in developing countries, including Serbia, the biopesticide market remains highly underdeveloped. To overcome these economic and regulatory bottlenecks, close cooperation between the scientific community, government bodies, and the private sector is crucial for establishing sustainable maize production [76].

Pest management/weed control. Weed control supports optimal maize growth and prevents competition. This can be accomplished through mechanical removal or applying herbicides. Pre- and post-emergence herbicides are effective for weed management. To prevent herbicide resistance in weeds, herbicides from the same chemical group should not be used repeatedly. Sustainable measures, such as reducing crop rows, increasing plant density, reducing tillage, retaining crop residues, and diversifying crops, also involve avoiding herbicides, which are expensive and can disrupt the agroecosystem. However, weed management strategies must be integrated into sustainable maize production [77]. Accordingly, pest management should integrate insect control, biological control, resistant hybrids, and sustainable agronomic practices.

All control measures must be considered as components of an integrated aflatoxin risk management system rather than as isolated interventions. The future of control depends on a balanced approach that integrates biological control, agrotechnical measures, and the rational use of pesticides, in line with the principles of integrated and sustainable maize production.

2.2. Harvest Strategies

Harvest strategies for smallholder maize farmers include timely harvest, sorting damaged/infected cobs, and de-husking.

Timely harvest. High maize yield and quality are ensured by timely harvesting. Harvesting, either by hand or machine depending on weather conditions and maturity coincide, usually occurs 14–28 days after physiological maturity. In many countries worldwide, maize is harvested with a high moisture content of 22% to 37%. Producers should also avoid over-drying the grain in the field, as this can cause it to shrivel and break during harvesting [15,78]. Likewise, if maize remains in the field after maturity, it causes yield losses and increases the risk of *A. flavus* infestation and aflatoxin production. Research shows that aflatoxin content can increase if maize harvest is delayed by one month after full maturity [79]. Variability in weather conditions, such as sporadic and late-growing-season rainfall, also increases fungal development, aflatoxin production, and insect infestation and damage in maize [80]. Therefore, timely harvest, and even earlier harvest in areas with high aflatoxin risk, is recommended because it reduces the exposure time to fungi in the field and the risk of aflatoxin accumulation [15,40]. Timely harvesting is one of the simplest and most effective preventive measures for reducing the risk of *A. flavus* and aflatoxin contamination in maize grains.

Sorting damaged/infected cobs. Sorting and removing visibly moldy, damaged, or deformed cobs before storage can significantly reduce aflatoxin contamination. Sorting cobs that are free of insects or visible fungal growth may be an even more effective approach. However, such sorting can be labour-intensive. It may result in discarding quality grain, a large number of cobs, and significant direct financial losses [81]. Therefore, sorting intensity needs to ensure both food safety and the economic sustainability of production. Laboratory analyses during sorting are necessary, since mycotoxins are not evenly distributed across maize bins. Mycotoxin-contaminated grains are commonly localized within a maize lot, making it difficult to quantify mycotoxin levels. Thus, sorting helps to manage potential

foci of fungal infection and mycotoxin levels. Physical characteristics of maize grains, such as size, color, and weight, are indicators of mycotoxin contamination and can be used for grain sorting. Currently, in low- and middle-income countries, the most common maize grain sorting method is based on manually removing discolored, broken, and insect-damaged cobs and grains. Low-cost sorters, which separate grains based on their physical and spectral properties, have proved highly successful in reducing aflatoxins in maize [82]. In Tanzania, research by Wuiya et al. [83] found that sorting by color significantly reduced aflatoxin intensity, emphasizing the importance of proper maize sorting and investment in project design for color sorters. On the other hand, in extensive farming systems in developed countries, optical and high-speed sorters can separate damaged and discolored grains using pneumatic machines and real-time image processing, reducing aflatoxin contamination with high effectiveness (>80%) [84]. Accordingly, combining visual inspection, laboratory monitoring, and modern sorting technologies improves maize safety while maintaining economic sustainability.

De-husking. Maize hand-picking is common among smallholder farmers in developing countries due to fragmented landholdings, limited access to mechanized equipment, and financial constraints [85]. In rural farming communities, de-husking is mostly performed manually, often by women. This traditional method is time-consuming, associated with poor hygiene, laborious, and results in significant waste. In contrast, sophisticated de-husking machines are very expensive and difficult to use and maintain, and most rural farmers cannot afford them [86]. Advisers often recommend that smallholders harvest maize cobs directly from the plants, without removing the cob leaves, because the leaves protect the cobs during transport and reduce damage [11]. However, this practice alone is not sufficient and must be accompanied by adequate drying. It is a component of an integrated risk management system, alongside proper drying and storage.

2.3. Post-Harvest Strategies

A. flavus development and aflatoxin production in stored maize are strongly influenced by temperature, humidity, and insect abundance. Storage temperature and moisture availability are the most critical factors in controlling potential mycotoxin issues in dried grain. The ideal temperature for storing dry grain is 1 to 4 °C, while during the summer months, temperatures between 10 and 15 °C are safe for storing maize. To prevent mold and aflatoxin contamination, the water availability of dried grains must be below 0.70 (=14.0%), and relative humidity below 65–70% [37,87]. Additionally, inadequate storage facilities can lead to mycotoxin problems in the grain. Consequently, aeration is critical for temperature regulation and preserving the overall quality of stored maize. Likewise, frequent observations and real-time monitoring are imperative to prevent moisture migration and condensation, insect proliferation, and subsequent mycotoxin accumulation [37]. Drying, shelling, and storage are effective post-harvest strategies in maize management.

Drying. After harvesting, grain is dried to an optimal moisture content for storage (13–14%). Spreading cobs or grains on the ground for sun exposure or hanging the cobs on wooden racks for air drying are common practices in low- and middle-income countries, whereas mechanical drying prevails in more intensive systems [88]. Drying the harvested cobs as soon as possible, with or without husk, is very important. Simple sun-drying or open-air drying of cobs is a traditional, low-cost practice for smallholder farmers. It is advisable to dry the cobs on polyethylene sheets on the ground rather than directly on the soil. Mechanical and solar dryers are also common in many countries. When drying cobs or de-hulled grains, maize layers should not be too thick, because the bottom layers dry more slowly and retain higher moisture content than the top layers [11]. Delayed drying, leaving maize cobs in containers for more than six hours after harvest, significantly contributes to

the occurrence of *A. flavus* and aflatoxins. Hence, maize should be dried on the cob before shelling [89].

Maize grains should ideally be dried using dryers. There are dryers for service drying and storage; however, their rental costs can reduce the earnings of agricultural holdings by up to 25%, depending on the quantity of goods. Small dryers made by smallholder farmers would be the optimal solution for small and medium-sized farms. They are more affordable than used imported dryers, made of quality materials, and can also serve as storage for grains after drying. It is recommended to cool the grain after drying and before storage to avoid condensation when storing warm grains in cold storage [90].

This measure is a key control for preserving the quality, safety, and market value of maize grains. Drying should not be prolonged and should be completed within 48 h after shelling. Accordingly, mechanical dryers and grain-drying facilities can dry grains more quickly without depending on weather conditions, unlike traditional sun-drying. In addition, the controlled temperature of heated air provides uniform moisture content in maize grains [91]. While traditional open-air drying remains an affordable option for small producers, mechanical and smart solar dryers provide faster, more uniform, and more reliable drying regardless of weather conditions.

Shelling. Improper machine settings, high shelling speeds, or high grain moisture can break and damage maize grains during shelling. Broken maize grains are more susceptible to fungal infection because they provide more maize germ surface for fungal development. Therefore, smallholder farmers should exercise caution when implementing this practice. Proper harvesting machine settings can also minimize grain breakage during shelling or threshing [11,15]. Shelling equipment should be thoroughly cleaned before use to prevent cross-contamination from previously contaminated grain.

Storage. Smallholder farmers commonly store maize in hermetic bags and metal silos. Reduced oxygen and increased carbon dioxide in airtight (hermetic) storage technologies decrease grain respiration and weight loss and are unsuitable environments for fungal and insect infestations. Hermetic technologies can also block external relative humidity [92,93]. Hermetic systems allow longer storage periods (up to six months) than conventional non-hermetic systems. In developing countries, Purdue Improved Crop Storage (PICS), SuperGrain IV-R, Agro-Z, Agro-Z Plus, and Elite bags are the most commonly used for storing cereal grains. ZeroFly insecticide-incorporated hermetic bags also protect cereal grains from post-harvest insects [94]. Globally, continued modernization and improvement of hermetic bags are necessary to enhance aflatoxin management during maize storage [95]. Williams et al. [96] reported that PICS bags were more effective than non-airtight containers in preventing fungal growth and aflatoxin production across a wide range of moisture contents in maize grains, emphasizing that the end use of the grain determines the target moisture content of dried grains before storage. In Serbia, farmers store maize in several ways, depending on whether it is kept as cobs or grains, as well as on production volume and available farm equipment. The traditional method, especially on smaller family farms, involves storing maize cobs in open-air wooden or wire barns (locally known as kotobanja, koš or čardak) [97]. The cobs are kept in facilities with good natural ventilation, where they gradually dry. Larger, commercial producers and farms with appropriate machinery shell, clean, and dry the maize after harvest, and then store the grain in tall vertical concrete or modern steel silos. Smaller farms use floor warehouses or other adapted facilities for grain storage [98]. It is crucial that the grain is sufficiently dry and that there is adequate ventilation. Livestock farms also ensile wet grain, either by grinding and ensiling it or by ensiling whole grain. In this way, conventional drying is avoided. However, a common problem for small maize producers is uneven moisture and temperature control

during storage, which makes maize more susceptible to *A. flavus* growth and aflatoxin production [11,98].

Store maize in clean, dry, well-ventilated premises to prevent fungal growth and insect infestations. Before storing fresh maize grains, remove dust and old grains. Effective management of stored maize follows the first-in, first-out principle. Use older stored maize first [99]. Controlling temperature and relative humidity is crucial to preserving maize quality in storage [100]. Storage strategies to reduce fungal and mycotoxin contamination of maize grains can be beneficial. Developing and implementing cost-effective, practical methods to prevent fungal growth, such as improved storage conditions, are initial steps in managing maize contaminants.

Drying and storage are key post-harvest strategies for regulating moisture content. Drying maize with either natural or heated air can alter the grain's physical and nutritional quality. The rate of biochemical and metabolic reactions, as well as grain respiration, decreases in dried grains, which is unfavorable for the development of fungi and insects. Drying at higher temperatures accelerates the reduction of grain moisture content to the required storage level. Storing maize at temperatures above 20 °C for six months can reduce its physicochemical quality, while storage at 10 °C can maintain its physical and physicochemical properties. Storing maize in bags, either aerated or hermetically sealed, did not affect the grain's physical properties but maintained chemical quality during storage. Broken and damaged grains were of poor quality in both aerated and hermetic storage systems [100].

These post-harvest measures should also be viewed as part of an integrated system. Post-harvest control is just as important as agronomic measures in the field.

3. Emerging Strategies for *A. flavus* and Aflatoxins in Stored Maize

Beyond GAP-based strategies, the toxic effects of aflatoxins can be significantly mitigated by detoxification approaches such as the use of antioxidants (vitamins, minerals, phenolic compounds, carotenoids, plant extracts, etc.), followed by mineral (hydrated sodium and calcium aluminosilicate, bentonite, zeolite, activated carbon) and biological binders (bacteria, yeast). Antioxidants reduce oxidative damage in exposed animals by neutralizing free radicals. Microorganisms that detoxify aflatoxins can bind them or enzymatically degrade them into less toxic compounds [101]. Esterified glucomannans derived from the cell wall of the yeast *Saccharomyces cerevisiae* can adsorb, inactivate, and neutralize aflatoxins, especially AFB_1, in the gastrointestinal tract of broiler chickens [102]. Mineral adsorbents are added to moldy animal feed to bind aflatoxins in the gastrointestinal tract and prevent aflatoxicoses in livestock. This mitigation strategy is widely used in the feed industry as a low-cost and effective method for reducing bioavailable aflatoxin levels in animals [17]. Additionally, nanoparticle-based adsorbents (carbon-based materials and magnetic nanoparticles) are being developed for superior effectiveness, possessing a higher surface area than conventional adsorbents. In addition, nanozymes are synthetic nanoparticles with properties similar to natural enzymes. They can remove contaminants, including aflatoxins, through filtration, adsorption, and catalysis. Ultraviolet radiation is also an effective physical method for decontamination of fungal spoilage, damaging fungal DNA and degrading aflatoxins through photochemical reactions [65]. Furthermore, the antimicrobial oxidant ozone (O_3), in gas or liquid phases, and cold plasma, a neutral-ionised gas, are emerging technologies showing high potential for microbial deactivation and aflatoxin degradation in maize [65,103].

Although mineral adsorbents have high potential for aflatoxin sequestration, several physicochemical factors, such as changes in pH, temperature, and toxin levels, can contribute to their variable performance. Limited adsorption efficiency and lack of selectivity,

which lead to co-binding of essential nutrients (vitamins, minerals, and proteins) from feed or food materials together with toxins, are disadvantages. However, their laminar structures, the large interlayer surface area for AFB_1 and AFM_1, and low cost are particularly suitable for animal feed, making them highly effective and more applicable than organic-biological adsorbents. In contrast, the practical application of nanoparticle-based adsorbents is limited due to high production costs and scalability barriers. Tiny particles can enter the food chain and deteriorate health. Likewise, there are limited data on the efficacy of cold plasma and nanozymes in practice. Therefore, further studies are needed to confirm their effectiveness. UV treatment is considered an advanced decontamination method because it is low-cost, causes no secondary pollution, and is easy to apply in the food industry. However, limitations include oxidation of valuable nutrient components and low penetration into solid materials such as grains [65,104]. Recently, there has been a trend towards an increasing number of newly discovered natural and chemical antioxidants as inhibitors of aflatoxins. However, the mechanisms of action of most of them are still poorly understood. Therefore, extensive, long-term studies of their metabolites in aflatoxin control are necessary for such chemical compounds to be commercialized and widely used [105].

4. Integrated Management of Fungal Contamination and Aflatoxins in Maize Production Systems

4.1. Global Health and Economic Impacts of Aflatoxin Contamination

Aflatoxin-contaminated maize poses a risk to human health worldwide, particularly in tropical and subtropical regions of Africa, Asia, and South America. Human exposure is particularly high in regions where maize is a staple food and climatic conditions favor fungal growth. Accordingly, cases of aflatoxicosis have been frequently reported in East African countries, where maize is a major component of the local diet. In addition, extreme weather conditions, limited implementation of preventive agronomic practices, improper grain storage, inadequate knowledge, and insufficient training of smallholder farmers in aflatoxin prevention and management may contribute to increased aflatoxin contamination in the food chain [106,107]. Because aflatoxin contamination is difficult to predict and can be unevenly distributed in both field-grown and stored maize, its detection and management remain challenging. The associated economic costs can be substantial, including losses resulting from product rejection, trade restrictions, and product recalls when contaminated maize and maize-based products reach the market [40].

4.2. Integrated Food Safety Frameworks: GAP, GMP, and HACCP Interconnection

A range of control measures targeting both pre- and post-harvest stages, including physical, chemical, and biological approaches, can substantially reduce aflatoxin contamination when appropriate interventions are applied. Hazard Analysis and Critical Control Point (HACCP), as recommended by the Food and Agriculture Organization (FAO) of the United Nations [108], is a key component of integrated food safety management for the prevention and control of mycotoxins. HACCP principles are widely applied by food business operators involved in processing and distribution, while primary producers are encouraged to implement preventive practices such as Good Agricultural Practices (GAP) and Good Hygiene Practices (GHP). Certification programs promote the implementation of GAP, thereby reducing the risk of *Aspergillus* infection and aflatoxin contamination through the control of key factors at pre- and post-harvest stages. The implementation of programs based on GHP, GAP, Good Storage Practices (GSP), and Good Manufacturing Practices (GMP) provides an important foundation for the effective implementation of HACCP and for ensuring a high level of food safety [109,110]. These systems are based on the identi-

fication and assessment of food safety hazards, the determination of appropriate control measures, and the implementation of preventive controls throughout the production chain.

4.3. Requirements for Advanced Decontamination Techniques

Decontamination techniques for reducing aflatoxin contamination should be carefully selected to prevent deterioration of the nutritional quality and organoleptic properties of maize. Preventive measures alone may not be sufficient to completely prevent aflatoxin contamination in food and feed products. Therefore, the development and implementation of effective decontamination strategies remain important for protecting human and animal health [103]. Effective methods for decontaminating food and feed should remove or inactivate mycotoxins without producing toxic residues or adversely affecting the technological properties, nutritional quality, or sensory characteristics of the products. These methods should also be cost-effective, practical to apply, and suitable for implementation at an appropriate scale.

Given the rapid development of agricultural technologies, farmers may increasingly benefit from innovative practices that support sustainable agricultural production. Precision agriculture technologies, remote sensing, weather-based forecasting systems, decision-support platforms, and other digital tools can contribute to the timely assessment of aflatoxin risk in maize production [111–113]. In addition, knowledge transfer through well-organized training and agricultural extension services is important for improving farmers' knowledge and skills [114]. Agricultural programs related to maize production should therefore consider training on aflatoxin prevention and management throughout the production chain, from field to fork. GAP and climate-resilient agricultural practices, including the use of drought-tolerant hybrids and improved storage and drying methods, combined with integrated pest management (IPM), represent important strategies for reducing the risk of toxigenic fungi and mycotoxin contamination and limiting production losses in high-risk maize-growing regions [45].

4.4. Regulatory Standards and Support Schemes in Serbia

In Serbia, food safety is regulated by the Law on Food Safety ("Sl. glasnik RS", Nos. 41/2009 and 17/2019), which is aligned with the general principles of EU food law, including those established by Regulation (EC) No. 178/2002 of the European Parliament and of the Council. In addition, smallholder farmers seeking access to certain markets may benefit from aligning their production with GlobalG.A.P. standards. Compliance with such standards can strengthen links between producers and supply chains and facilitate access to competitive markets. GlobalG.A.P. certification provides evidence that production processes comply with defined standards related to food safety, environmental protection, and responsible agricultural practices [115].

Growing market demand for high-quality agricultural products produced according to integrated production principles and with reduced pesticide residues encourages the adoption of improved production technologies, appropriate genotypes and agrotechnical measures, quality certification, adaptation to market requirements, production diversification, brand development, improved infrastructure, and cooperation among stakeholders in the food production chain. Food standards have therefore become increasingly important in agricultural supply chains. Farmers' associations, together with improvements in processing, packaging, promotion, and protection of quality labels, can contribute to the supply of sufficient quantities of safe agricultural products and facilitate the implementation of relevant standards [115].

The Agricultural Advisory and Expert Services of the Republic of Serbia organize training programs on the implementation of GAP and food standards, providing farmers

with knowledge to improve production practices and food safety [116]. The Law on Incentives in Agriculture and Rural Development ("Sl. glasnik RS", Nos. 10/2013, 142/2014, 103/2015, 101/2016, 35/2023, 92/2023, and 94/2024) provides a legal framework for agricultural and rural development incentives financed through the agricultural budget of the Republic of Serbia. Government support schemes can facilitate investments in irrigation, agricultural machinery, and crop protection, while improved agronomic practices may indirectly contribute to reducing aflatoxin risk by improving crop management and post-harvest practices. Such incentives are intended to support agricultural productivity and technological development [117].

4.5. Mitigation Efficacy of Pre-Harvest Agrotechnical Practices

Numerous studies have demonstrated the potential of GAP strategies to reduce *A. flavus* infection and aflatoxin contamination in maize. Traditional pre-harvest GAP practices applicable to smallholder farmers, such as crop rotation, soil fertility management, appropriate tillage, the use of resistant or tolerant hybrids, optimal sowing time, irrigation, and pest control, can help reduce plant stress caused by abiotic and biotic factors. Although the economic feasibility of individual pre-harvest strategies varies across farming systems, breeding programs have contributed to the development of maize germplasm with improved resistance or tolerance to environmental stresses and aflatoxin contamination [118]. Farmers should select maize hybrids adapted to local growing conditions, particularly those characterized by drought, insect pressure, and fungal diseases. Breeding for drought tolerance has achieved substantial progress, while developing consistent resistance to *A. flavus* infection and aflatoxin accumulation remains challenging. Selecting hybrids well adapted to prevailing environmental conditions may reduce the risk of fungal infection and aflatoxin contamination by limiting crop stress.

4.6. Biological Control Strategies and Socio-Economic Barriers

Biological control products, primarily based on atoxigenic strains of *A. flavus*, can reduce aflatoxin contamination in maize [1,60,64,67,68] through competitive exclusion of toxigenic strains. However, for smallholder farmers, the use of such products represents an additional investment, and their adoption may depend on regulatory approval, economic accessibility, and adequate extension services and farmer training. The adoption of biological control and other advanced agricultural practices by smallholder farmers may also be constrained by implementation costs, limited access to financing and credit, insufficient extension services, and limited awareness of aflatoxin-related risks. Consequently, appropriate regulatory support, financial incentives, and education may facilitate their broader adoption. In addition, irrigation, appropriate pest management, and optimal sowing time can help reduce plant stress and susceptibility to *A. flavus* infection and subsequent aflatoxin contamination [15]. Furthermore, integrating GAP, appropriate food safety management systems, resistant or tolerant hybrids, biological control, and farmer education can contribute to more effective aflatoxin risk management. Continuous improvement of GAP and risk-based management approaches is important for enhancing food safety, reducing aflatoxin-related risks, and strengthening consumer confidence.

4.7. Analytical Advances and Future Research Directions

Because mold growth and aflatoxin contamination remain persistent risks in maize production, future control strategies should address the entire food chain, from primary production to end users. Rapid, inexpensive, and reliable screening methods are needed alongside regular food safety risk assessments. Significant progress has recently been made in techniques for the detection and monitoring of mycotoxins in agricultural products, foods, and feeds. LC-MS/MS, which stands as the gold standard for quantitative analy-

sis, can be complemented with other techniques, such as immunoassays and biosensor technologies that are suitable for rapid, rapid large scale-screening and on-site applications [119]. Further development and evaluation of effective and safe physical, chemical, and biological decontamination methods are needed to reduce aflatoxin contamination. Eco-friendly approaches based on microbial and enzymatic detoxification represent promising alternatives to conventional chemical and physical methods, while low-cost approaches, such as the use of mineral adsorbents in animal feed, may be more accessible in some production systems. In addition, further research on aflatoxin contamination in regions affected by changing climatic conditions is needed. Future research should focus on improving aflatoxin management in maize production through improved detection methods, precision agriculture, biological control, and effective risk prediction tools.

5. Conclusions

Drought stress during the reproductive growth stage can weaken the natural defense mechanisms of maize, thereby increasing its susceptibility to *A. flavus* infection and aflatoxin accumulation. Mitigating this risk requires a comprehensive and integrated approach that encompasses the entire maize production cycle, from field management to harvesting and post-harvest handling.

Pre-harvest practices aim to reduce conditions that favor *A. flavus* infection and subsequent aflatoxin accumulation. These practices include the selection of appropriate maize hybrids, adequate crop and soil management, insect control, irrigation where available and agronomically justified, and biological control. After harvest, timely harvesting, adequate drying, careful handling of maize grains, and appropriate storage conditions are important components of aflatoxin management because fungal development and aflatoxin contamination may continue or increase when post-harvest conditions are favorable.

In addition to traditional GAP practices, several emerging post-harvest approaches, including UV radiation, ozone treatment, cold plasma, nanoparticle-based adsorbents, and biological methods, have been investigated for their potential to reduce fungal growth and/or aflatoxin contamination. However, their wider implementation, particularly in smallholder production systems, depends on several factors, including effectiveness, cost, technological requirements, safety, and practical feasibility. Therefore, further evaluation is needed before some of these approaches can be considered suitable for routine application by smallholder farmers.

Improving aflatoxin management at the smallholder level requires not only effective mitigation practices but also appropriate farmer training, extension activities, and, where applicable, economic or institutional support. Raising awareness of aflatoxin risks and available control measures should involve different actors throughout the maize value chain, including producers, storage operators, processors, traders, and consumers. Clear and locally applicable guidelines may facilitate the adoption of appropriate practices and contribute to improved maize safety. Effective aflatoxin risk management therefore requires cooperation among scientific institutions, producers, extension services, regulatory authorities, and other relevant stakeholders, supported by appropriate risk assessment, monitoring, and risk communication.

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