Mycotoxins Biocontrol Methods

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Contamination of crops with phytopathogenic genera such as Fusarium, Aspergillus, Alternaria, and Penicillium usually results in mycotoxins in the stored crops or the final products (bread, beer, etc.).

biocontrol mycotoxins Fusarium Aspergillus Penicillium Alternaria

1. Introduction

Today’s agriculture relies on different agents to improve the health, yield, and nutritive value of crops. Small grain cereals (such as wheat, barley, oat, rye, and triticale) and maize are the main commodities grown all over the world in different climatic conditions. Areas affected by drought, humid areas, and high altitude areas can deliver favorable conditions to the population of pathogen fungi. Because of the wide spectrum of climatic conditions, cereals and maize can be contaminated with different pathogens resulting in mycotoxins. The application of chemicals may result in a reduction of fungal infection or mycotoxin contamination, but the sustainability of such application regarding ecological and environmental issues is not promising. Current trends question the safety of chemical agents used for the preservation of crops [1], as they are considered responsible for many carcinogenic and teratogenic toxic effects in humans and animals [2][3]. Natural and biological weapons applicable in the reduction of mycotoxigenic fungi and mycotoxins have been intensely investigated for many years. Not only the producers, but the consumers of cereal-based foods as well, are seeking natural ways to protect crops and to reduce the amount of fungicides in final products [4].

Global warming and climatic changes reshape the microbiome of cereals and maize in all corners of the world. Shifts in fungal species have already been reported by several authors across the globe [5][6][7][8][9]. Several *Fusarium* species are affected by rising temperatures, and not only in European countries. This became a serious marker for climatic changes follow-up and can be considered as an indicator of global warming. Shifts in fungal species and their adaptation to stressful conditions, such as drought and warmer temperatures, subsequently result in changes in secondary metabolites, mycotoxins, and plant defense metabolites that can be detected and quantified in small cereals and maize [10]. This challenges the possibilities of fungicide reduction. Namely, harsher environmental conditions intensify the production of different fungal and plant metabolites which calls for increased use of fungicide agents. However, the committed efforts of scholars are currently aimed toward the development of biological and natural agents that can be employed not only for the protection of crops from fungal infections, but to reduce the environmental damage to ecological systems where these crops are grown.

2. Mycotoxinogenic Fungi and Affected Grains

The most familiar fungal species that are related to mycotoxin contamination of maize and cereals belong to genera *Fusarium*, *Aspergillus*, *Alternaria*, and *Penicillium* [11]. Table 1 shows most common commodities, fungi, and mycotoxins worldwide. A more detailed overview of fungal species and their mycotoxins is given in the following sections.

Table 1. Most common commodities, fungi, and mycotoxins worldwide.

Cereal	World Region	Mycotoxin	Fungi	Source
Wheat	Europe	Ochratoxin A	*A. ochraceus*, *A. carbonarius*, *A. niger*, *A. westerdijkiae*, *A. steynii*, *P. verrucosum*	[12]
	Central/South America, Europe, North Asia and South-Eastern Asia	Zearalenone	*F. graminearum*, *F. culmorum*, *F. crookwellense*	
	Europe and North Asia	T-2/HT-2 toxins	*F. sporotrichioides*, *F. langsethiae*, *F. poae*	
	Europe	Deoxynivalenol	*F. graminearum*, *F. culmorum*	
Rye	Europe and North Asia	T-2/HT-2 toxins	*F. sporotrichioides*, *F. langsethiae*, *F. poae*	
Barley	Europe	Ochratoxin A	*A. ochraceus*, *A. carbonarius*, *A. niger*, *A. westerdijkiae*, *A. steynii*, *P. verrucosum*	
	Europe and North Asia	T-2/HT-2 toxins	*F. sporotrichioides*, *F. langsethiae*, *F. poae*	
	Worldwide	Deoxynivalenol	*F. graminearum*, *F. culmorum*	
Oats	Europe and North Asia	T-2/HT-2 toxins	*F. sporotrichioides*, *F. langsethiae*, *F. poae*	
Maize	Common in Central/South America, Africa, South-East Asia; Occassional in North America, Europe and North Asia	Aflatoxins B1, B2, G1, G2	*A. flavus*, *A. parasiticus*	
	Europe	Ochratoxin A	*A. ochraceus*, *A. carbonarius*, *A. niger*, *A. westerdijkiae*, *A. steynii*, *P. verrucosum*	
	Central/South America, Europe, North Asia and South-Eastern Asia	Zearalenone	*F. graminearum*, *F. culmorum*, *F. crookwellense*	
	Europe and North Asia	T-2/HT-2 toxins	*F. sporotrichioides*, *F. langsethiae*, *F. poae*	

Cereal	World Region	Mycotoxin	Fungi	Source
	Worldwide	Fumonisins B1, B2, B3	*F. verticillioides*, *F. proliferatum*	
	Worldwide	Deoxynivalenol	*F. graminearum*, *F. culmorum*	

2.1. Fusarium Spp.

2.1.1. Species Description

Fusarium spp. are designated as the most devastating species for small grain cereals, especially for wheat and barley, causing Fusarium head blight (FHB) [13][14][15][16][17][18][19][20]. Oats are generally less affected by *Fusarium* spp. than other cereals [21][22][23], but some regions (Scandinavia and Canada) encounter a serious problem with oat panicle blight [21][24]. Favorable conditions for head infections caused by *Fusarium* spp. include high humidity and temperatures above 20 °C [14][25][26][27]. According to Miller [28], *F. graminearum* is associated with wheat and maize grown in warmer areas, and *F. culmorum* with colder areas such as northwestern Europe, and the influence of temperature correlates with a prolonged period of warm weather with daytime temperatures above 30 °C. Even though several fungal species are related to head blight, *F. graminearum*, *F. culmorum*, and *F. avenaceum* are found to be dominant species in most parts of the world [19][27][29][30][31][32][33][34][35]. A significant increase in FHB caused by *F. poae* has been recorded for the last few years. It does not cause classical fusariosis-like symptoms (significant damage to kernel germination capacity), but still produces mycotoxins [34][36][37][38]. Other species can also be related to the pathogenesis of small cereals: *Fusarium sporotrichioides*, *Fusarium crookwellense*, *Fusarium roseum*, *Fusarium equiseti*, *Fusarium tricinctum*, *Fusarium oxysporum*, and *Fusarium langsethiae*, *Fusarium acuminatum*, *Fusarium fujikuroi*, and *Fusarium incarnatum* [23][27][39][40][41][42][43][44].

According to several sources [45][46][47], *Fusarium verticillioides* is a common fungal species that infects maize. The infection can occur via several routes. Often, the kernel gets infected through airborne conidia that can be found on the silks [48][49][50]. Usually, a small percentage of the infected kernels display symptoms of infection [51]. Another proposed infection pathway is systemically through the seed [52]. Systemic infection can start from fungal conidia or mycelia, inside the seeds, or on the seed surface. In this case, the fungus thrives inside the young plant, moves up from the roots to the stalk, and ends up in the cob and kernels. *F. verticillioides* is known to produce toxins that are potentially toxic to humans and animals. The most significant of these toxins produced by *F. verticillioides* are the fumonisins [46][49][53]. Fumonisins can be detected in symptomatic and asymptomatic maize kernels, and therefore the control of fumonisin contamination in maize has become a priority area in food safety research with distinct limits for maximum fumonisin levels in human food and animal feeds [54][55].

As reported by Oldenburg et al. [56], *Fusarium* species infecting European maize mostly belong to the sections Discolour and Liseola. Discolour prevails in colder and more humid areas and Liseola prefers a warmer and dryer climate. As in most grains, several *Fusarium* spp. can be detected on maize which can result in multi-contamination with mycotoxins.

2.1.2. Disease and Mycotoxin Producton

Fusarium spp. can also affect maize with two diseases described as “red ear rot” or Gibberella ear rot (*F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. cerealis*, *F. poae*, *F. equiseti*, and *F. sporotrichioides*), and “pink ear rot” or Fusarium ear rot, (*F. fujikuroi*) which takes place after pollination and is common in hot and dry climatic conditions [56][57][58][59].

2.1.3. Gibberella Ear Rot

Gibberella ear rot starts at the ear tip after entry of the fungi through the silks at female flowering [60][61]. The infection results in a grey-brownish to pink-reddish coloration of the infected parts of the rachis. The coloration usually indicates places where mycotoxins accumulate. Earlier ear tissue infection results in higher mycotoxin concentrations. Higher mycotoxin concentrations can be found at the ear tip if the infection occurred via the silks [62]. According to Oldenburg and Ellner [62], harvested kernels placed at the tip segment of maize ears, if the inoculation with *F. culmorum* or *F. graminearum* occurred during the flowering period, can contain DON, 3-a-DON, and ZEN. In comparison, rachis parts showed several times higher levels of the same mycotoxins (DAS, T2, and HT2 can be detected less often and in much lower concentrations) [34][63][64].

2.1.4. Fusarium Ear Rot

According to several sources [65][66][67], *F. temperatum*, can also be designated as a causative agent of ear rot in maize. Infection occurs more often through damaged tissue than through silks [68][69]. *F. verticillioides* causes tan to brown coloration, white or light pink mycelium on kernels, limited ear areas, or groups of kernels scattered over the ear [70]. Kernels can be infected with *F. verticillioides*, but show no visible symptoms of infection [62]. Common mycotoxins produced by *F. verticillioides* and *F. proliferatum* in maize ears are fumonisins (FB1 to FB4) [71][72][73]. FB1 synthesis in maize kernels correlates to the content of water, amylase, and starch [74]. FB1 accumulation in immature *F. verticillioides*-infected kernels was not observed due to the lack of starch [75]. Bluhm and Woloshuk [75] described amylopectin as a triggering substance to induce FB1 production. Higher FB1 concentrations were observed in kernels that suffered dual infection with *F. verticillioides* and *F. proliferatum* [76]. However, *F. verticillioides* produces significantly higher levels of FB1 than *F. proliferatum* [77]. Infections involving several other *Fusarium* species, *F. subglutinans*, *F. avenaceum*, or *F. equiseti*, commonly result in different concentrations of MON (moniliformin), BEA (beauvericin), ENNs (enniantins), and/or other mycotoxins [34][64][78][79][80][81][82].

Fusarium spp. also cause seedling diseases such as seed rot, root rot, or seedling blight of maize [83]. Common causes of seedling diseases are *F. verticillioides*, *F. proliferatum*, *F. subglutinans*, *F. graminearum*, *F. oxysporum*, and *F. temperatum* [84][85]. Low-quality seeds and seeds that withstood significant damage by insects or physical damage are especially susceptible to soil- and seed-borne pathogens. Seedling blight can be recognized by the brown coloration of the dead seedlings or by light-yellowish coloring and seeds that have lost the capacity to thrive [56].

As reported before, *F. graminearum* prefers warm and hot climatic conditions (T > 15 °C). However, it can proliferate in a milder climate with higher temperatures and high humidity. *F. graminearum* is currently reported as the most common causal agent of head blight in cereals and maize ear rot [13][14][15][16][17][18][19][20]. *Fusarium fujikuroi* also prefers a warmer climate with hot and dry vegetation seasons [86]. *F. avenaceum*, *F. culmorum*, and *F. poae* are seen in colder parts of the world [85][87][88][89][90] with an average annual air temperature between 5 °C and 15 °C and moderate precipitation. *F. culmorum*, however, is much more harmful to cereals at higher temperatures [15][24][87]. *Fusarium* spp. are the main reason for seedlings' death, foot rot, and head blight. Fusarium head blight (FHB) is a dangerous infection due to the subsequent mycotoxins contamination.

Infection of cereal heads with *Fusarium* spp. can occur at different times, but they are most susceptible to infection during the flowering phase and immediately after flowering. Warm and humid weather, dew, and higher precipitation during this period [26][29][91][92] enable the infection. Symptoms of infection show off on the infected spikes; they become white. The infected spikelets die out and block the development of kernels, resulting in a smaller, gray, shriveled, and loose consistency, and sometimes grains are covered with sporodochia and *Fusarium* spp. mycelium grains [26][29][36]. Infected grains are usually reddish in color.

Deoxynivalenol (DON)-producing chemotypes of *F. graminearum* are widespread around the world, while nivalenol (NIV)-producing chemotypes can be found in Asia and Europe. However, the occurrence of individual chemotypes is often affected by weather conditions [30][93][94].

2.2. Aspergillus Spp.

2.2.1. Species Description

The most infamous fungi belonging to genera *Aspergillus* are *Aspergillus flavus* and *Aspergillus parasiticus*. Even though *Aspergillus* spp. can be found in small grain cereals, they prevail in maize and cause damage especially during droughty and hot seasons [95][96]. The reported climatic changes predict an increase of this pathogen, more severe infections, and significantly higher mycotoxin levels in cereals and corn [96][97].

Aspergillus spp. are commonly referred to as the black fungi, and they are pathogenic for several crops. Their habitat varies from temperate climatic conditions to tropical and sub-temperate zones. They can be found in soil, where they decompose dead plant tissue [95]. *Aspergillus* spp. can infect and cause serious economic damage to grapes, onions, maize, and peanuts. On maize, they cause maize seedling blight and maize kernel rot.

When combined with different hosts, some symptomless endophytes can act as pathogens or as saprophytes but, in either state, they can become producers of mycotoxins. Symptomless *Aspergillus* spp. infections have been reported in the literature but information about their ability to produce mycotoxins and any associated pathology is scarce. Early publications designated *A. niger* as the main species that causes damage. According to current findings, identification of *Aspergillus* spp. was somewhat off and certain corrections have been made. For example, today we know that the nomenclature of *A. niger sensu stricto*, or, *A. niger* var. *niger*, was so far designated as *sensu lato* and usually refers to *A. niger*. There are more than 190 *Aspergillus* species that can be separated into several distinct morphospecies. Some of the separations were done according to their colors [96], but more accurate and precise separation via data sequencing resulted in eight subgenera [97], of which only *Circumdati*, the sections *Circumdati* (=*Aspergillus ochraceus* group), and *Nigri* (*A. niger* group) represent economically harmful subgeneras. *Aspergillus* in section *Nigri* have been taxonomically revised, which resulted with several new taxa, such as *A. niger* var. *niger*, *A. melleus*, *A. sulphureus*, *A. brasilensis*, *A. ostianus*, *A. petrakii*, *A. scletotium*, *A. carbonarius*, *A. aculeatus*, *A. japonicus*, *A. tubingensis*, *A. ibericus*, and *Eurotium herbariorum* [98][99][100], but none of them have been identified as responsible for any crop disease. The *Aspergillus* genus prefers the tropical belt and is even more frequent in subtropical to warm temperate zones [95]. They thrive in the forest and cultivated soils, and dislike desert soils. Nevertheless, *A. niger* var. *niger* can be found in forests, grasslands, wetlands, deserts, and cultivated soils [95]. As mentioned before, the rising global temperatures will greatly influence the population and shift the species within the *A. niger* group to the more northern geographical latitudes.

2.2.2. Disease and Mycotoxin Production

Mycotoxins associated with *Aspergillus* subgenera or specific species pose a toxic threat to livestock, poultry, fish, and human health. Severe cases of poisoning, such as the Turkey-X disease of peanuts, caused by *A. flavus* and *A. parasiticus*, have been described in the literature [101]. The identification of aflatoxins as the toxicological agent [102] was the first step towards the solution of ensuring food safety. Another group of mycotoxins, ochratoxins, was also related to this genera. Today, they are reported as carcinogenic mycotoxins and are included in the legislation. Recently, ochratoxins have been reported in several other species of *Aspergillus* sections *Circumdati* (*A. ochraceus* group), and by *Eurotium herbariorum*, a member of the *Aspergillus* section (*A. glaucus* group) [103].

2.3. Alternaria Spp.

2.3.1. Species Description

Genus *Alternaria*, in particular *Alternaria alternata*, is a frequent contaminant of different small cereals causing “black point” disease. Favorable conditions for *Alternaria* spp. include high humidity and frequent precipitation [76].

2.3.2. Disease and Mycotoxin Production

A common symptom of this disease is the coloration of ears and grains with dark pigment, and melanin [104]. The black point mostly causes a decrease in milling quality of wheat, barley, and oats, and is not as significant as a yield reducer. However, the changes in flour and bran color have significant economic importance. Besides the discoloration, decreased nutritive value, and the loss of taste also significantly reduce the technological quality of cereal products [105].

Alternaria triticina can cause damages to ears and grains, but the disease can occur on leaves in the form of leaf blight lesions. *Alternaria* spp. are often reported as storage fungi, where they cause spoilage of small grains and small-grains-based products. Even though they enjoy humid (high water activity) and warm storage conditions, *Alternaria* spp. can proliferate worldwide, in both humid and semi-arid climatic areas. *Alternaria* spp. Have been reported on wheat, barley, oat, and rye [106][107][108][109]. In the Mediterranean countries, as well as in Estonia, Slowakia, and Argentina, the prevalent species are *A. alternata* and *Alternaria tenuissima*, reported on wheat [110][111][112][113], while *Alternaria infectoria* was reported in Norway [105]. *Alternaria triticina*, originally from Indian wheat, was also reported in Argentina [114][115]. Toth et al. [116] reported *Alternaria hungarica* as a novel species on Hungarian wheat, considering it a minor foliar pathogen with small economic importance. Serbia reported *A. alternata* and *Alternaria logipes* as wheat pathogens, while *A. alternata* and *A. tenuissima* were noted on spelt wheat [117][118].

Alternaria spp. produce mycotoxins with different toxicological properties. Since there were reports that certain *Alternaria* toxins could exhibit carcinogenic effects [119], the European Commission (EC) requested that the European Food Safety Authority (EFSA) provide a scientific opinion on the risks to animal and human health related to the presence of *Alternaria* toxins in food and feed [120]. Besides, *Alternaria* spores are considered to be one of the most prolific fungal allergens, and have been associated with respiratory allergies and skin infections [121][122][123].

According to different sources [124][125][126], *Alternaria* toxins can be sectioned into three main structural classes:

- dibenzo-α-pyrone derivatives: alternariol (AOH), alternariol monomethyl ether (AME), altenuen (ALT), altenuisol (AS);
- tetramic acid derivates: tenuazoic acid (TEA);
- perylene derivatives: altertoxins I, II, III (ATX-I,-II,-III).

So far, *Alternaria* toxins have been detected in small cereal grains and small-grains-based products (bread and rolls, muesli, fine bakery wares, pasta, etc.) [120]. AOH, AME, and TEA in “black point wheat” on the German market [127][128]; AOH, AME, and ALT in Slovakian [113] and Czech grains [129]; AOH and AME in small cereal grains in Poland were reported [109]. Li and Yoshizawa [130] found wheat kernels significantly infected with mostly *A. alternate*; AOH was detected in 20 of 22 tested samples between 116–731 µg/kg. AME was at a mean level of 443 µg/kg (range = 51–1426 µg/kg) in 21 samples. TEA, the most abundant *Alternaria* toxin, was detected with an average level of 2419 µg/kg and with a maximum quantity of 6432 µg/kg. The presence of *Alternaria* strains in Argentinean wheat also designated TEA as the most abundant toxin [131].

2.4. Penicillium Spp.

2.4.1. Species Description

Several *Penicillium* spp. (*Penicillium citrinum*, *Penicillium expansum*) have been reported as foodborne contaminants, but *Penicillium verrucosum* is one of the most concerning species belonging to genera *Penicillium*. It is generally assumed that *P. verrucosum* is a common producer of OTA in temperate and cold climates [132]. Even though much research has been conducted on *Penicillium* spp. on cereals and maize [133][134][135][136][137][138], this fungal genera is not as popular as the other selected genera in this review.

2.4.2. Disease and Mycotoxin Production

Penicillium spp. are commonly saprophytic microorganisms that invade plant tissue and soil debris. Penicillium ear rot on maize usually occurs on ears already damaged by birds or insects [139][140]. In silage, the most frequently isolated *Penicillium* spp. is *Penicillium roqueforti* [141][142][143][144]. Based on rDNA genes analysis and chemotaxonomic profiles, a recent finding confirmed *P. roqueforti* as three species, *P. roqueforti*, *Penicillium paneum*, and *Penicillium carneum* [145]. Subsequently, only *P. roqueforti* and *P. paneum* have been reported in silage [143][145]. Both species, however, produce ROC (roquefortine C) and *P. roqueforti* also produces PR-toxin (Penicillin Roquefort toxin) and MPA (mycophenolic acid), while *P. paneum* produces PAT (patulin) as well [145][146][147]. Silage microbiota includes *P. expansum*, which produced ROC and PAT, *P. crustosum* and *P. commune*, both producers of CPA (cyclopiazonic acid), and ROC [148]. PAT and ROC can cause toxicoses in livestock (ROC has been reported as a suspected causal agent in several cases of paralysis, abortion, and placental retention in cattle) [141][142][149][150]. As with any other fungi, *P. roqueforti* can produce several mycotoxins at once, which makes it difficult to confirm that solely ROC is the toxin responsible for the reported symptoms. Several types of research suggested that ROC caused toxicosis in dogs after they had ingested food colonized by *P. roqueforti.* Reportedly, they suffered from paralysis, tremors, and convulsions [151][152][153], which indicates a neurotoxic effect. Here, too, it is impossible to claim that ROC is the main cause since another toxin, penitrem A, was detected as well. PAT was involved in cattle health disorders, causing tremors, paralysis, and death [154]. However, in this case, PAT was synthesized by *Aspergillus clavatus*, not *Penicillium* spp. Cattle suffered extensive damage to the nervous system. MPA is recognized as a potent immunosuppressant but does not possess the properties of acutely toxic compounds [155]. It is commonly utilized as an immunosuppressive agent for patients in need [156]. CPA, on another hand, is not well investigated but, in poultry, CPA exposure can result in tremors, liver, kidney, and gastrointestinal tract damage [157]. It can be excreted in milk, withstands pasteurization temperatures, and remains stable for extended periods of storage [158]. The potential dangers of *Penicillium* mycotoxins in the feed are yet to be fully discovered since not much information regarding their toxicity is available. CPA, MPA, PAT, and ROC are the most familiar toxins originating from *Penicillum* species (*P. roqueforti*, *P. paneum*, *P. commune*, *P. crustosum*, and *P. expansum*) [159].

Toxins are generally produced during storage time (low water activity, low pH, and oxygen concentration) [145][160][161]. For example, *P. roqueforti* and *P. paneum* can be found in silage. There they can thrive even if the silage is not visibly covered in mycelium, and they can also produce mycotoxins which is why they pose such a threat to animal and human health [141][145].

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