# An overview of toxigenic fungi associated with corn seeds and milled corn products

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**ABSTRACT** The effects of climate change highlight concerns about food safety, including fungal contaminants, some of which could produce toxic compounds. Toxigenic fungi are expected to become more prevalent with climate change. This paper summarized the common fungal microflora of corn seeds and milled-corn products, focusing on the postharvest stage. The commonly produced mycotoxin groups and toxin-mitigating measures were also discussed. Commonly reported fungal species associated with corn seeds and milled products belong to the genera *Aspergillus* (*A. flavus, A. niger, A. fumigatus, A. terreus, A. parasiticus, A. ochraceous, A. candidus*), Fusarium (*F. verticillioides, F. proliferatum*), *Penicillium* (*P. citrinum*), Alternaria (*A. alternata*) and Eurotium (*E. chevalieri*). Six mycotoxin groups are associated with these fungal contaminants and have been detected in corn seeds. These mycotoxins were aflatoxin, fumonisin, ochratoxin, nivalenol, zearalenone, and cyclopiazonic acid. The presence of fungi does not necessarily indicate the presence of mycotoxins. Several factors affect these toxic metabolites' production, such as the fungal strains, substrate composition, moisture content, aeration, temperatures, and other storage conditions. This review provides a consolidated account of fungi detected and isolated from corn seeds and milled corn products.

**KEYWORDS:** mycotoxins, *Aspergillus*, *Fusarium*, *Penicillium*, seed pathology
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## INTRODUCTION

Corn (*Zea mays L.*) is the world's third most important crop, after rice and wheat. It is grown in many countries; some consider corn a staple (Roige *et al.*, 2009). Corn is used as a grain, starch products, oil, baby foods, popcorn, corn-based food items, corn flour, forage for animals, corn silage for winter animal feed in the cold temperate region, and corn stalks providing dry season feed for farm animals (Warr *et al.*, 2008). In the Philippines, corn grits are popularly consumed as a substitute for rice or mixed with rice due to their high protein level and low glycemic index (Jamias, 2014).

Corn plants are exposed to insects and microorganisms in the field. The wind blows fungal and bacterial spores into the air and soil. Fungi are abundant in corn fields, and corn ears are prone to fungal infection. Fungi may be dispersed by wind, insects, and floods (Egan *et al.*, 2014). They can grow on simple and complex food products producing various metabolites. Many fungal species have been classified as natural contaminants of agricultural and food products (Nleya *et al.*, 2018). Contamination of plant pathogenic fungi in seeds significantly contributes to crop losses and is a major hindrance to food security (Pangga *et al.*, 2015). Corn seeds are susceptible to mold invasion during pre-harvesting, processing, transportation, and storage (Ellis *et al.*, 1991). Fungal contamination is one of the leading causes of cereal spoilage. It contributes to potential economic losses and can cause acute or chronic intoxication in humans and animals, mainly through mycotoxin production (Muriuki, 1996). During postharvest operations, fungal contamination may result from long-term corn storage in poor environmental conditions, including high moisture and warm temperature. Corn stored for longer is more vulnerable to fungal contamination than freshly harvested corn. Moreover, storage insects and rodents may also contribute to the rapid deterioration of the grains and the spread of fungal microflora in storage (Hussein & Brasel, 2001).

The effects of climate change highlight concerns about food safety, including fungal contaminants, some of which could produce toxic compounds. Toxigenic fungi are expected to become more prevalent with climate change (Pangga *et al.*, 2015). Knowledge about the fungi associated with corn and milled corn products is important in assessing the risk of potential mycotoxin contamination (Balendres *et al.*, 2019). Generally, fungal species belonging mainly to the genera *Fusarium, Aspergillus,* and *Penicillium* are the most common mycotoxin producers. The three major fungal genera cause significant grain yield losses (Varga *et al.*, 2015). This paper provides a consolidated account of fungi detected and isolated from corn seeds and milled products. The commonly produced mycotoxin groups and some fungal contamination and toxin-mitigating measures are also discussed.

## FUNGAL MYCOFLORA IN CORN SEEDS

Corn cobs and kernels are relatively large (Pitt & Hocking 2009), and moist conditions at harvest often result in slow drying. Fungal contamination could be established during preharvest and postharvest. Among the major fungal contaminants, *Aspergillus* spp., section *Flavi* has been a significant concern to the industry. This genus constitutes a species that produces aflatoxins, a group of highly toxigenic compounds (Rodrigues *et al.*, 2011). *Fusarium* is another fungal genus with the most occurring species, including *Fusarium verticillioides,* in corn grains and milled products. Most *Fusarium* species invade through insect damage sites and may even enter the corn ear via silks (Marasas *et al.*, 1984).

About 40% of corn grains in Egypt were contaminated with several fungi, including *Aspergillus* species (Ismail *et al.* 2016). Egypt's corn grains' most dominant *Fusarium* species were *F. verticillioides, F. oxysporum, F. solani, F. proliferatum, F. udum,* and *F. nisikadoi* (Abdel-Hafez *et al.* 2014). In Kenya, *A. flavus, A. fumigatus, A. niger, A. ochraceus, A. terreus, A. versicolor,* and *A. clavatus* were reported in corn grains (Muthomi *et al.*, 2012). In Nigeria, stored maize grains is often used in processing Ogi products, in which *A. niger, A. tamarii, A. flavus, A. fumigatus, Penicillium chrysogenum, Penicillium* sp., *Fusarium* sp., *Rhizopus nigricans, Saccharomyces cerevisiae* have been isolated. *Aspergillus niger* had the highest percentage incidence, followed by *Penicillium* sp. and *A. flavus,* respectively (Jonathan *et al.*, 2019). *Aspergillus* species have been frequently isolated in Tunisia, followed by *Eurotium* sp. and *Penicillium* sp. The most relevant fungal species belonged to the *Aspergillus* section *Flavi*, followed by section *Nigri,* which showed high percentages of contaminated samples, 66.67% and 47.62 in corn, respectively (Jedidi *et al.*, 2018).

In the Kansas-Nebraska-Oklahoma area, *Fusarium* and *Penicillium* species occurred in approximately 50% of examined kernels (Bothast *et al.*, 1974). Some other species reported were *Chaetomium globosum, Trichoderma harzianum, Mucor* sp., *Rhizopus* sp., and *Absidia hesseltinii Helminthosporium maydis* (Bothast *et al.*, 1974). These species, however, are not known to produce mycotoxins. The fungal microflora associated with corn kernels and their industrial fractions obtained by dry milling in Argentina were *F. verticillioides* (=*F. moniliforme*), *A. flavus,* and *P. funiculosum.* Some isolates of dematiaceous fungi, such as *Curvularia lunata, Cladosporium cladosporioides,* and *Acremonium strictum,* were also detected. *Cladosporium cladosporioides, Mucor racemosus, Rhizopus stolonifera*, and *Ph. glomerata* have also been isolated from the whole corn kernel and fractions (Broggi *et al.*, 2002). Wicklow *et al.* (1998) also noted the predominance of *E. chevalieri* and *A. flavus* and the persistence of *F. verticillioides* in corn stored for up to 2 years under a range of temperatures (10 - 40 °C) and moisture (40 - 88 %RH) conditions. A similar range of fungi was reported in corn in Venezuela (Mazzani *et al.*, 2004).

In Pakistan, 56 fungal species have been isolated, where 70% of the samples were contaminated with species from the genera *Aspergillus* and *Penicillium* spp. The same fungal species have been reported on corn seeds globally, such as in the USA, Thailand, India, Canada, Australia, France, Nepal, the United Kingdom, Western Romania, and Hungary (Niaz & Dawar, 2009). In Saudi Arabia, yellow and white corn seeds were infested mainly with *Aspergillus* spp. and *Fusarium* spp. (*F. oxysporum* and *F. solani*), *Alternaria* spp., and *P. notatum* (Mahmoud *et al.*, 2013). In India, a high incidence of *Alternaria alternata*, *A. flavus,* and *A. niger* was observed (Kulkarni & Chavan, 2010).

Corn from Southeast Asia is also heavily invaded by *A. flavus*, which was present in more than 85% of 150 samples of Thai corn and up to 100% of grains in some infected samples (Pitt *et al.*, 1993). Similar figures were obtained from Indonesia and the Philippines (Pitt *et al.*, 1998). In Malaysia, *A. flavus, A. niger, Fusarium verticillioides, F. graminearum, F. proliferatum, F. equiseti,* and *Penicillium* sp. were the prevalent fungi in all corn samples (Reddy & Salleh, 2011). The commonly isolated storage fungi in corn samples from Thailand were the *Eurotium* species (*E. chevalieri*, *E. rubrum*, and *E. amstelodami*). Nevertheless, *Wallemia sebi*, *A. wentii*, *A. tamarii,* and *A. niger* were also present in a significant number (Pitt *et al.*, 1993). Similar results were obtained from 148 corn samples from the Philippines and 82 from Indonesia. *Fusarium* species, particularly *F. verticillioides, F. semitectum, and F. proliferatum*, persisted in high numbers in stored corn from all three countries (Pitt *et al.*, 1993; 1998). A recent publication from Widiastuti *et al.* (2020) reported that *F. verticillioides, F. proliferatum, F. graminearum,* and *F. asiaticum* were isolated from corn kernels in Java and Lombok, Indonesia. Also, *Penicillium* species in corn from Thailand, the Philippines, and Indonesia were *P. citrinum, P. funiculosum, P. pinophilum, P. oxalicum*, and P. *raistrickii* (Pitt *et al.*, 1993; 1998).

## FUNGAL MYCOFLORA IN CORN-MILLED PRODUCTS

Fungi not only contaminate corn seeds, but they also contaminate various corn products (Jonathan *et al.*, 2019). Contamination was high in broken and damaged kernels (Karlovsky *et al.*, 2006). Fungi acquired in the field, particularly *F. verticillioides, F. proliferatum, F. oxysporum*, and *Aspergillus flavus*, can persist in corn and molds. Their toxins may be carried through corn products such as flour, grits, corn chips, tortillas, and breakfast cereals (Pitt & Hocking, 1997). *Aspergillus* has also been reported as a contaminant of feedstuffs (Abarca *et al.*, 1994).

Fungal contaminants from eighty samples taken from corn-based feeds (poultry, pig, and rabbit feeds) in Córdoba province, Argentina, have been identified. The predominant species isolated were *A candidus*, followed by *A. flavus* and *A. terreus* from poultry and pig feeds. *A. flavus* and *A. parasiticus* were isolated frequently in rabbit feeds. Other *Aspergillus* species isolated were *A. fumigatus, A. awamori, A. ustus, A. foetidus, A. ochraceous, A. aculeatus, A. japonicus,* and *A. clavatus* (Magnoli *et al.*, 2005). In 2009, *Penicillium* (70%), *Fusarium* (47%), and *Aspergillus* (34%) were the most frequent and abundant genera in corn grain samples used for cattle and pig feed in Argentina (Roige *et al.*, 2009). This report agrees with Tapia *et al.* (2005). They reported that *Penicillium* sp. was the most prevalent species in corn-based feed in Minnesota.

Rosa *et al.* (2006) analyzed 96 Brazilian poultry feed samples. *Aspergillus flavus* was the most prevalent, followed by *A. niger, A. ochraceus, A., P. verrucosum*, and *A. carbonarius*. In Ecuador, corn-based pellet feed destined for poultry, pig, and cow feeds showed that *A. flavus* was the most prevalent fungus present, followed by *F. graminearum, F. verticillioides,* and *A. parasiticus* (Magnoli *et* *al.*, 2007). Moreover, Purwoko *et al.* (1991) and Dutta and Das (2001) also observed that *A. flavus* and *A. parasiticus* were the most dominant fungi in corn feed from Indonesia and India, respectively. In Iran, animal and poultry feed samples were infected with *A. flavus, A. parasiticus, A. ochraceous, A. niger, Penicillium glabrum, Fusarium verticillioides, F. proliferatum, F. fujikuroi, F. concentricum, Rhizopus oryzae, Absidia corymbifera, Rhizomucor* sp., *Trichothecium* sp., and *Alternaria alternata* (Darvishnia *et al.*, 2019).

A survey was conducted in Rio de Janeiro, Brazil, to evaluate the fungal microflora in 144 corn samples, initial poultry feeds, pelleting poultry feed, and trough feed samples. Fungal counts decreased significantly as the pelleting process was completed. In corn and initial poultry feed samples, *A. flavus* and *E. chevalieri* were the most prevalent species isolated, followed by *E. amstelodami, A. candidus*, and *A. niger*. In trough feed samples, *A. flavus, E. amstelodami,* and *E. chevalieri* were the most frequent species, isolated from 22% of the samples, followed by *A. niger, A. sydowii,* and *A. versicolor* (Fraga *et al.*, 2007).

Three mills in the Kansas-Nebraska-Oklahoma area were surveyed for corn grits and flour. Total mold counts were significantly lower in grits (440 per g.) than in corn (9,700 per g.) and flour (3,200 per g.) (Bothast *et al.*, 1974). In the Philippines, *A. flavus* was reported in corn grits, *A. oryzae* var. *viride,* and *A. candidus* were reported in cracked kernels, and *A. pseudoglaucus (E. chevalieri)* was reported in corn for popping (Lozada, 1995). Yamashita *et al.* (1995) reported that *F. moniliforme* and *F. proliferatum* contaminated corn grits. Fungal contaminants in corn grits, corn flour, cornmeal, corn germ, and bran from a corn mill in Argentina were contaminated with *F. verticillioides (=F. moniliforme*). *Fusarium* counts were highest in the germ and bran samples, followed by corn meals and the flaking grits (Broggi *et al.*, 2002). Sule *et al.* (2015), who analyzed corn grains, cornflour, and corn bran from a market in Kaduna state in Nigeria, showed that corn bran samples had the highest level of aflatoxin with a mean aflatoxin level of 213 ppb. In a similar report by Mutungi *et al.* (2008), dehulling corn grain samples reduced the aflatoxin levels. However, they also reported that the aflatoxin contents in the by-products, comprising hulls and fines, were 2-7 times higher than the whole-grain corn levels (Jonathan *et al.*, 2019).

Other fungi reported in corn products include *P. funiculosum, P. duclauxii,* and *P. brevicompactum* in corn meal and corn starch in Brazil (Ribeiro *et al.*, 2003*), Aspergillus parasiticus, P. citrinum, P. funiculosum,* and *Alternaria alternata* in milled corn in Argentina (Broggi *et al.*, 2002), and *A. flavus, A. sulphureus, Penicillium stoloniferum,* and *P. aurantiogriseum* in cornflour in Kenya (Muriuki & Siboe, 1995).

## COMPOUNDS FROM TOXIGENIC FUNGI

Mycotoxins, toxic secondary metabolites some fungi produce, can contaminate food and feed worldwide. Their prevalence depends on various factors, such as the commodity, climatic conditions, agricultural practices, and storage conditions (Nyangi *et al.*, 2016). The UN's Food and Agriculture Organization estimated that 25% of harvested crops are contaminated with mycotoxins (Aoun *et al.*, 2020). About 60-80% of foods carry detectable levels of mycotoxin (Eskola *et al.*, 2019). Over 5 billion people, mainly in the developing world, are chronically exposed to aflatoxins (Strosnider *et al.*, 2006, Wild & Gong, 2010). Mycotoxin may represent a public health hazard, especially in rural subsistence farming communities where mycotoxin problems are rarely regulated (Zain, 2011).

Aflatoxins (AFs), fumonisins (FBs), ochratoxins (OTs), deoxynivalenol (DON), and zearalenone (ZEN) are the most detected mycotoxins in cereal grains and cereal product-derived processed food. Mycotoxins can be transferred from raw grain materials to processed food products. Food processing is an essential value-adding activity for cereal grains. It is also the last defense step to prevent mycotoxin contamination in human consumption foods (Wan *et al.*, 2020). Aflatoxins occur in corn and products in many countries, including Brazil, China, India, Italy, Nigeria, Turkey, the USA, and Greece (Pitt & Hocking, 2009). Various corn products have also detected aflatoxins (Arim *et al.*, 1999). Amra *et al.* (2017) detected *Aspergillus flavus* and *A. parasiticus* that produce aflatoxin from field samples from the Philippines. Out of over 180 species within the *Aspergillus* genus, *A. flavus, A. parasiticus*, and *A. nomius* have been reported as the primary producers of aflatoxins in agricultural commodities.

Currently, more fungal strains have been implicated in aflatoxin production. These include sections *Flavi A. oryzae, A. bombycis, A. pseudotamarii, A. tamarii, A. parvisclerotigenus, A. nidulans, A. niger, A. arachidicola, A. togoensis,* and *A. minisclerotigenes* sp., and some newly emerging strains in Section *Nidulantes*; *Emericella venezualensis* and *E. astellata, E. olivicola* from the genera Emericella; and Section *Ochraceorosei* (*A. ochraceoroseus* and *A. rambellii)* (Varga *et al.*, 2009; Rank *et al.*, 2011; Akinola *et al.*, 2019).

Pascual *et al.* (2016) studied *Fusarium* species associated with corn ear rot. They found that most of the *F. verticillioides* species are mycotoxigenic, regardless of their origin. Dela Campa *et al.* (2005) state that the environmental conditions during silking are crucial for fumonisin accumulation. Other fungi known to produce fumonisins are *F. proliferatum*, *F. nygamai, F. subglutinans, F. oxysporum, F. globosum, Aspergillus niger*, *A. welwitschiae*, and *Bipolaris maydis* (Leslie *et al.*, 2005; Waalwijk *et al.*, 2008; Kocube *et al.*, 2013; Varga *et al.*, 2015). Ochratoxins are a family of mycotoxins produced mainly by *A. ochraceus*, *A. carbonarius*, *A. westerdijkiae, A. niger, A. welwitschiae, A. sclerotioniger, Penicillium verrucosum,* and *P. nordicum* (Ostry *et al.*, 2013; Varga *et al.*, 2015). Ochratoxin A has also been reported in corn products (Muriuki & Siboe, 1995).

Nivalenol and zearalenone were also found in corn, where fumonisin and aflatoxin were detected (Yamashita *et al.*, 1995). Of 208 corn samples from 18 provinces collected from the Philippines, 8.2% were positive for zearalenone (Cumagun *et al.*, 2019). Several species of *Penicillium* and *Aspergillus* also produce cyclopiazonic acid. In the Philippines, cyclopiazonic acid has been monitored in a single published report. Hayashi & Yoshizawa (2005) reported cyclopiazonic acid in one of six corn samples from the Philippines.

## MITIGATING FUNGAL AND MYCOTOXIN CONTAMINATION

Implementing good agricultural practices is necessary for reducing fungal contamination and the mycotoxin levels in corn and corn-milled products. Contact of the corn with the soil should be avoided during harvest and drying to avoid contamination with the fungal inoculum present in the soil. Drying corn to safe moisture levels of less than 13% and cleaning storage facilities at the end of each season would reduce the chances of infection and mold growth.

Other methods of reducing mold and mycotoxin contamination include drying corn on mats and polythene sheets to avoid contact with the soil surface (Muthomi *et al.*, 2009). In addition, the exclusion of highly contaminated or infected kernels before processing is encouraged. Contaminated kernels can be excluded by manual sorting or, when available, using sorting machines based on particle weight or optical techniques (Neuhof *et al.*, 2008; Karlovsky *et al.*, 2016). A recent technology utilized to help manage fungal and mycotoxin contamination is non-aflatoxigenic *A. flavus*. However, it relies on competition between non-toxigenic and toxigenic strains for infection sites and essential nutrients during crop development (Savic *et al*., 2020).

On the other hand, ozonation is considered the most promising chemical method for detoxifying AFB1. ClO2 gas could inhibit mycelium growth and spore germination (Yu *et al*., 2020). Generally, sanitation and exclusion of the samples from all possible sources of fungal contamination are needed.

## CONCLUSION

*Aspergillus flavus* is the most prevalent species reported worldwide in corn seeds and milled products. The other commonly isolated and detected fungi are *Aspergillus niger*, *Fusarium verticillioides, A. parasiticus, Aspergillus fumigatus, A. ochraceous, Alternaria alternata, Eurotium chevalieri, Fusarium proliferatum, Aspergillus terreus*, and *Penicillium citrinum*, and other *Penicillium* species. Proper corn seed storage and good management practices are necessary for contamination in raw and processed materials.

Strict implementation of sanitation and good management practices can help eliminate or at least reduce mycotoxin contamination to a relatively acceptable level. Further, more fungal contaminants in corn seeds and milled corn products are yet to be identified and characterized. Nevertheless, molecular biology assays that help discriminate species with similar morphology and cultural characteristics can be helpful in species identification. In addition, molecular-based assays could detect fungi with the potential to produce a toxin before toxins are even produced. These methods offer a more robust and early detection technique for possible fungal and mycotoxin contamination.

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