Annex D – Pre- and post-harvest strategies to mitigate mycotoxin contamination in sorghum

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Sorghum

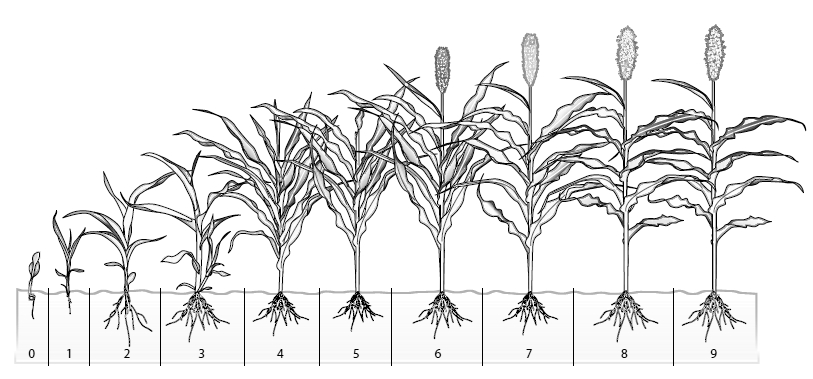
Sorghum (*Sorghum bicolor* L. Moench) is considered the fifth most important crop among the world’s cereals after maize, wheat, rice, and barley. More than half of the world’s sorghum is grown in semiarid or subtropical regions due to its resistance to harsh weather conditions. Total world production of sorghum reached 57 million tons in 2017, of which more than 80% was produced in Africa and Americas, while only a small part in Europe (1.6%). In 2017, sorghum covered 295,853 ha in the EU-28 and EU ranked sixth among global sorghum importers. Just the 40% of the sorghum global production goes toward human consumption, primarily African and Asian countries where it represents an important staple food. Whereas, about 45% of global sorghum production is used as raw material for bovine, poultry, and swine feeds (FAOSTAT, 2017). Besides being an important food, feed and forage crop, sorghum also provides raw material for the production of starch, fiber, biofuels and other products. Within the EU, sorghum is not widely used as livestock feed, although there are areas where it is grown and may be more extensively used in livestock rations (EFSA, 2012).

Sorghum as other cereals is not free of mycotoxin contamination. It is attacked by several mycotoxigenic fungi that affect crop productivity and security for animal and human health. Several genera have been frequently recovered from sorghum grains including *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp., *Alternaria* spp. and *Claviceps* spp. (Hussaini et al., 2009; Alves dos Reis et al., 2010; Yassin et al., 2010; Mahmoud et al., 2013; Lahouar et al., 2015). Consequently, sorghum is usually contaminated by aflatoxins (AFs) and fumonisins (FBs). Whereas zearalenone (ZEN) and ochratoxin A (OTA) as well as a variety of other toxins (i.e. ergot alcaloids (EAs) and moniformin) are reported less (Leslie, 2014). AFs and FBs contamination reported in sorghum is usually much lower than the contamination encountered in other cereals, like maize. The reasoning behind this information is not yet refined. Thus, the mechanisms of plant-pathogen interaction and infection cycle of fungal species in this crop are not yet defined. The main findings on occurring mycotoxigenic fungi in sorghum and the associated mycotoxins retrieved from the literature are reported in the following paragraph. Furthermore, more details are collected in a summary table (**Table D.1**).

At present, relatively little is done to manage mycotoxin contamination in sorghum except for good agricultural practices and good storage practices. However, the development of control methodologies requires additional knowledge on sorghum-pathogen systems. The few information retrieved are reported in the dedicated paragraphs.

* 1. Sorghum phenology

A poster describing the phenological development process of sorghum has been published by K-State Research and Extension and it is available online (Ciampitti, 2015). We reported the nine stages of growth of the sorghum plant and related figure (**Fig D.1**): (0) emergence (coleoptile visible at soil surface); (1) three-leaf stage (collar of third leaf visible); (2) Five-leaf stage (collar of fifth leaf visible); (3) growing point differentiation (about eight-leaf stage with a visible collar); (4) flag leaf visible (final leaf visible in whorl); (5) boot stage (head extended into flag leaf sheath); (6) half-bloom (half of the plants at some stage of bloom); (7) soft-dough (grains are soft with little or no liquid present when squeezed); (8) hard-dough (grains are hard when squeezed); (9) physiological maturity (black layer on the bottom of kernel).



**Fig D.1:** Sorghum Growth and Development (Ciampitti, 2015)

* 1. Infection cycle of *Aspergillus* spp. in sorghum and plant pathogen interaction

The two main sections isolated in sorghum are *Aspergillus* *Flavi* and *Nigri*, being respectively *A. flavus* and *A. niger* the prevalent species (Lahouar et al., 2015; Lahouar et al., 2016; Taye et al., 2016). In particular, *A. flavus* and *A. parasiticus* show a lowest frequency of isolation from sorghum grains compared to other cereals (Alves dos Reis et al., 2010; Yassin et al., 2010). On the contrary, strains from the *Aspergillus section Nigri*  have shown to be dominant in sorghum from Saudi Arabia, India, Egypt and Tunisia (Hussaini et al., 2009). *A. niger* is considered the main source of OTA in sorghum. However, recently another *A. niger* aggregate strain, namely *A. tubingensis,* was identified as main OTA producer in this crop (Lahouar et al., 2017). Furthermore, *A. carbonarius* showed a higher distribution in Indian sorghum samples (Priyanka et al., 2014).

Current scientific literature on the ecology of the fungi as well as its infection cycle and plant-pathogen interaction in this crop is very poor. A recent study describes the effect of water activity (aw) and temperature on *in vitro* growth and OTA production of *A. tubingensis* strains isolated from Tunisian sorghum samples. The aw useful range was 0.88-0.99 aw and the maximum growth rates were observed at 37 °C and 0.99 aw. Optimum OTA production was observed at 0.97 aw × 37 °C (Lahouar et al., 2017). Hussaini et al. (2009) reported that *A. niger* was the most frequent fungal contaminant of sorghum in the hot, dry season, whereas *A. flavus* was the predominant species during the rainy season in Nigeria (Hussaini et al., 2009). However, no specific ecological data are reported.

Resistance of sorghum to fungal infection is related to grain characteristics such as pericarp color or hardness of heads. According to (Ratnavathi and Sashidhar, 2003) the physical characteristics and biochemical composition of the sorghum genotypes with red pericarps make the grains less susceptible to *A. flavus* infection and to AFs contamination. In particular, the authors showed that in red lines there was a significant negative correlation between polyphenol content and AFs produced after infection, suggesting that polyphenols and pigments may offer some resistance to fungal infection.

Sorghum lines with compact heads are more prone to mycotoxin contamination due to higher moisture accumulated in the heads compared to the one with more open heads (Alves dos Reis et al., 2010).

* 1. Infection cycle of *Fusarium* spp. in sorghum and plant pathogen interaction

Several species of *Fusarium* are associated with the contamination of sorghum grains with an incidence ranging from 10 to 100%, according to the literature retrieved (da Silva et al., 2004; Alves dos Reis et al., 2010; Sharma et al., 2011; Lahouar et al., 2015). A number of *Fusarium* species associated with sorghum belong to the *Gibberella fujikuroi* species complex (e.g. *F. verticillioides*, *F. thapsinum*, *F. proliferatum* and *F. pseudonygamai*). The species of this section have the capacity to produce FBs, moniliformin, fusaric acid and beuvericin (BEA) (Leslie et al., 2005).

*F. thapsinum* is the predominant species in India (Sharma et al., 2011), and has been reported to be common in sorghum grain (Leslie et al., 2005). (Lahouar et al., 2015) found in Tunisian and Egyptian sorghum 50 isolates of *Fusarium* mainly belonging to the *F. equiseti* complex with capacity to produce detectable levels of ZEN at concentrations ranging from 0.017 to 4.61 mg/kg.

A recent study describes the effect of aw and temperature on *in vitro* growth and ZEN production of *F. incarnatum* strains isolated from Tunisian sorghum samples. The useful aw range was 0.91-0.99 and the maximum growth rates were observed at 25 °C and 0.99 aw. On the contrary, it was not possible to define the optimal conditions for ZEN production since they varied from one isolate to another in terms of temperature and activity water; the maximum ZEN concentration reported was of 99.97 ng/g. The authors also states that there was no correlation between the growth of *F. incarnatum* and ZEN production (Lahouar et al., 2017).

According to Frederiksen et al. 1982 fungal contamination of sorghum grains probably starts in the apical portion of flower tissues such as glume, lemma and palea, with pigmentation of these structures being the first visible symptom. Mycelial growth occurs in the flower tissue in the direction of the base or in the spaces between tissues and may interfere with grain filling. The detection of *F. verticillioides* in soil and atmospheric air by few studies indicates that these routes of contamination were responsible for the presence of the fungus in sorghum grains in the field (Alves dos Reis et al., 2010).

* 1. Infection cycle of *Claviceps* spp. in sorghum and plant pathogen interaction

Sorghum ergot caused by *Claviceps africana* is of particular concern in sorghum growing areas worldwide (EFSA, 2012). Diseased sorghum with this fungus can be contaminated with ergot alkaloids (EAs), of which the most prevalent was identified as dihydroergosine (DHES) (Molloy et al., 2003; Blaney et al., 2006). Recently also dihydroergotamine was found as significant component within the *C. africana* sclerotia in Israeli sorghum (Shimshoni et al., 2017). Other species that infect sorghum include *C. sorghi* and *C. sorghicola* (Tooley et al., 2010). *C. africana* infects plants during flowering, particularly in cold weather, when it begins to replace the ovaries with dark mycelial masses known as sclerotia. The disease cycle is thought to be completed with asexually produced conidia and asexual secondary airborne conidia. Insect transmission seems to play no significant role in spreading sorghum ergot (Miedaner and Geiger, 2015).

**Table D.1.** Studies on mycotoxigenic fungi isolated from sorghum grains and the associated mycotoxins.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Samples origin** | **Fungi genera isolated** | **Incidence (%) on total fungi** | **Fungi species isolated** | | | **Incidence (%) of species in genus** | **Mycotoxin (s)** | **Reference (s)** |
| Saudi Arabia | *Aspergillus* | 32.49 | *A. flavus* | | | 54.73 | ND | (Mahmoud et al., 2013) |
| *A. niger* | | | 36.49 |
| *Fusarium* | 31.24 | *F. verticillioides* | | | 29.33 |
| *F. oxysporum* | | | 24 |
| *Penicillium* | 6.66 | *F. solani* | | | 22 |
| *F. semitectum* | | | 14 |
| *Alternaria* | 2.49 | *P. citrinum* | | | ND |
| *A. alternata* | | | ND |
| Saudi Arabia | *Aspergillus* | 10.3 | ND | | | ND | AFs | (Mahmoud et al., 2014) |
| *Penicillium* | 9.8 |
| *Fusarium* | 9.5 |
| *Alternaria* | 7.7 |
| Saudi Arabia | *Aspergillus* | ND | *A. niger* | | | 28.47 | AFBs  AFGs  FBs  ZEN  Altenuene | (Yassin et al., 2010) |
| *Fusarium* | ND | *F. verticillioides* | | | 0.78 |
| *Penicillium* | ND | *F. nygamai* | | | 0.27 |
| *Alternaria* | ND | *F. semitectum* | | | 0.78 |
| *P. funiculosum* | | | 9.55 |
| Egypt  Tunisia | *Fusarium* | 95.3 | *F. incarnatum* | | | 62.7 | AFs  OTA  ZEN | (Lahouar et al., 2015) |
| *Aspergillus* | 87.5 | *F. verticillioides* | | | 6.8 |
| *Alternaria* | 81.2 | *F. thapsinum* | | | 3.4 |
| *Penicillium* | 64.0 | *F. proliferatum* | | | 3.4 |
| *F. pseudonygamai* | | | 3.4 |
| *A. flavus* | | | 90.1 |
| *A. parasiticus* | | | 9.9 |
| *A. niger* | | | 34.3 |
| *A. tubingensis* | | | 23.05 |
| *P. citrinum* | | | 66.7 |
| Egypt | *Fusarium* | ND | *F. nygamai* | | | 4.66 | ND | (Alves dos Reis et al., 2010; Abdel-Hafez et al., 2014) |
| *F. verticillioides* | | | 5.72 |
| *F. solani* | | | 3.18 |
| India | *Fusarium* | ND | *F. equiseti* | | | 9.5 | ND | (Sharma et al., 2011) |
| *F. proliferatum* | | | 19.04 |
| India | *Aspergillus* | ND | *A. niger* | | | 100 | AFB1  OTA | (Priyanka et al., 2014) |
| *A. flavus* | | | 50 |
| *A. carbonarius* | | | 100 |
| *A. oryzae* | | | 17 |
| *A. fumigatus* | | | 83 |
| Ethiopia | *Aspergillus* | 100 | ND | | | ND | AFB1  FBs | (Taye et al., 2016) |
| *Fusarium* | 100 |
| Brazil | *Fusarium* | 25.1 | *F. verticillioides* | | 15.1 | | FB1 | (Alves dos Reis et al., 2010) |
| *Aspergillus* | 7 | *F. proliferatum* | | 0.2 | |
| *Alternaria* | 4.2 | *F. subglutinans* | | 3.7 | |
| *Penicillium* | 1.4 | *A. parasiticus* | | 4.0 | |
| *A. flavus* | | 3.0 | |
| Brazil | *Fusarium* | ND | ND | | ND | | AFB1  FB1 | (da Silva et al., 2004) |
| *Aspergillus* | ND |
| Nigeria | *Fusarium* |  | *A. flavus* | | 50 | | AFB1  OTA  ZEN | (Hussaini et al., 2009) |
| *Aspergillus* |  | *A. niger* | | 60 | |
| *F. equiseti* | | 21.4 | |
| Israel | *Fusarium* | 75 | ND | | | | ZEN  FB1-2  FUSARIC ACID  BEA  MON  EQUISETIN  ENN A1  ENN B | (Shimshoni et al., 2017) |
| Uruguay | *Aspergillus* | 65 | *A. flavus* | 60 | | | AFB1  FBs | (Del Palacio et al., 2016) |
| *Fusarium* | 35 | *F. nygamai* | 68 | | |
| *F. graminearum* | 26 | | |
| *Penicillium* | 70 | *P. citrinum* | 34 | | |
| *P. purpurogenum* | 11 | | |

-\*ND= not determined

- abbreviations: aflatoxin B1 (AFB1), fumonisin B1 (FB1), zearalenone (ZEN), ochratoxin A (OTA), BEA (beauvericin), moniliformin (MON), enniatin A1 (ENN A1), enniatin B (ENN B)

1. Mycotoxin occurrence

Several mycotoxins are known to occur in sorghum, and AFs are the most studied. It is known that AFs are produced in sorghum grains, but usually at lower level than found in maize (Leslie, 2014). Other reported mycotoxins occurring in sorghum are FBs, ZEN, OTA, EAs, Alternaria toxins and moniliformin (Shimshoni et al., 2017)(**Table G.1**). However, determining the level of contamination is not easy due to the lack of available data in the literature. In particular, not many data have been found in Europe on the occurrence of mycotoxins in sorghum. Most of the available papers analyse samples coming from no-EU countries, mainly Africa. Only one paper reports occurrence data of red sorghum cultivated in EU and destined to feed use. The authors reported the presence of T2, Diacetoxyscirpenol (DAS) and ZEN, of which DAS showed the highest occurrence rate (90%)(Ediage et al., 2015). On the contrary, quite an increasing interest has been noted among African countries where, as said, sorghum is one of the principal staple cereals. A study published in 2018 reported that of 1533 sorghum samples coming from sub-Saharan African countries, 33% were contaminated with at least one of the following mycotoxins: AFs, FBs, sterigmatocystin, Alternaria toxins, OTA and ZEN, being FBs (17%), sterigmatocystin (15%), and AFs (13%) the most prevalent (Ssepuuya et al., 2018).

1. Cropping system and harvest management

Starting from 2016, the European Union enhanced a series of important initiatives promoting the use of sorghum in Europe, especially for its technical and economic assets in terms of production and its potential uses and outlets for human food (gluten-free segment), animal feed and non-food outlets (energy biomass, bioethanol, biomaterials). In 2017, subsidies were received from the European Union for the promotion of sorghum in seven countries, including Spain, France, Italy, Romania, Bulgaria, Ukraine and Russia. Moreover, it was created an European sorghum association to lead all main initiatives, named “Sorghum ID”. On the dedicated website of the mentioned association, same guidelines for good agricultural practices in sorghum management were published, however none of these good practices were directly linked to the problem of mycotoxins in this crop. In particular, same recommended conducts are suggested for sowing density, fertilisation, irrigation and parasite pressure as well as harvesting and drying. Regarding the latter two, it is suggested to harvest while humidity levels are between 18 and 25% to facilitate drying processes which are considered essential because sorghum grains need to be stored below 15% humidity. However, as previously said, despite the increasing effort in optimizing sorghum cultivation, little research has been done on the management of mycotoxins in this crop either in field and after harvesting; available information is rather scarce and patchy, and mainly influenced by the different pathosystems. Wet grain at harvest as well as stressing factor to the plant (i.e. drought or poor nutrition) are the only in field conditions correlated to AFs contamination in sorghum so far (Leslie, 2014). Few information is available on the management of *Claviceps* spp. and it has been recently reviewed by (Miedaner and Geiger, 2015). The paper states that immediate pollination of receptive stigmas and closed flowering are the main plant traits reducing ergot infection, and that fungicide application in field have limited efficacy in addition to being constrained by weather conditions (Miedaner and Geiger, 2015).

Some biological control agents have been tested in sorghum, however no in field studies have been found. The biological control of *A.flavus* by *Rhodococcus erythropolis*, *Bacillus subtilis*, *Pseudomonas fluorescens* and *Trichoderma viride* has been tested *in vitro* in naturally contaminated sorghum seed by (Reddy et al., 2010). In particular the percentage of inhibition growth was respectively of 100%, 72%, 74% and 65%.

1. Post-harvest management

**Cleaning** In sorghum, seeds are often sticky from honeydew, which makes mechanical cleaning difficult, as well as expensive and time consuming (Miedaner and Geiger, 2015).

**Storage** The mycoflora and occurrence of AFB1 and FB1 during storage has been studied in Brazilian sorghum stocked in jute sacks and kept for 12 months in a well-ventilated warehouse. *Aspergillus* spp. were prevalent at moisture content and aw below 14% and 0.73, respectively. Concerning the genera *Fusarium*, the highest numbers of CFU/g were recorded at levels that ranged from 14 to 16% and from 0.65 to 0.74 aw. According to the study, mean temperature and time of storage were the variables that most influenced *Aspergillus* growth. In particular, the highest numbers of CFU/g for *Aspergillus* were recorded after 224 days of storage, with a mean temperature and relative humidity between 17 and 24 °C and 69 and 82%, respectively. Notwithstanding the large number of isolates, high levels of mycotoxins were absent. On the contrary, *Fusarium* species were the most frequent isolates on recently harvested grains and stored grains up to 196 days. The greatest levels of contamination were recorded for recently harvested and 28-day-stored samples (29 x 103 to 36 x 103 CFU/g); during this period, the mean temperature and relative humidity levels varied from 19 °C and 72- 78%, respectively (Silva et al., 2000). A marked increase in *Aspergillus* spp. and AFB1 concentrations between fresh and stored sorghum, was also observed in Nigerian samples of sorghum stored in underground pits. In samples that were collected at 5–6 months of storage, *Aspergillus* spp. invasion was higher (1.95–2.85 log cfu g−1) than in fresh sorghum grain samples (1–1.4 log cfu g−1). The moisture content (13–19.8%) of the grain during storage, the storage temperature (22–30°C) and relative humidity (65–84%) of the underground pits were optimal for the fungi species to grow. The maximum value of AFB1 recorded in freshly harvested sorghum and in stored grains was 17.0 μg kg−1 and 33.1 μg kg−1, respectively (Taye et al., 2016).

In conclusion, sorghum is produced in Europe on a very limited scale, but it is attracting ever-growing interest. Indeed, research has demonstrated the presence of mycotoxins in sorghum, but still limited efforts have been undertaken to improve the management of mycotoxins in this crop and no regulations for mycotoxins or groups of mycotoxins in sorghum have been settled in this respect in EU.

Abbreviations

|  |  |
| --- | --- |
| AFB1 | Aflatoxin B1 |
| aw | Activity water |
| BCAs | Biological control agents |
| BEA | beauvericin |
| DAS | Diacetoxyscirpenol |
| DHES | dihydroergosine |
| ENN A1 | enniatin A1 |
| ENN B | enniatin B |
| EAs | ergot alkaloids |
| FB1 | fumonisin B1 |
| MON | moniliformin |
| OTA | Ochratoxin A |
| PP | Propyl paraben |
| T2 | T-2 toxin |
| ZEN | zearalenone |

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