Parasitic fungi of sweet grain sorghum in Burkina Faso: risks of its consumption in the absence of hygienic rules

Elise Sanon[1], *Kounbo Dabiré[1,2], Bénovana Bakiono[1], Daniel Boua[1], Hermann Tondé[1], Nerbéwendé Sawadogo[1], Philippe Sankara[3]

[1]Biosciences Laboratory, Department of Plant Biology and Plant Physiology, University Joseph Ki-Zerbo, 03 BP 7021 Ouagadougou 03, Burkina Faso
[2]University Centre of Tenkodogo, University Thomas Sankara, 12 BP 417 Ouagadougou 12, Burkina Faso
[3]Agronomy faculty, New Dawn University, 06 BP 9283 Ouagadougou, Burkina Faso
Corresponding author's email: dabirekounbo@gmail.com

Abstract

Sweet grain sorghum [*Sorghum bicolor* (L.) Moench] is a staple food in rural Africa and is mainly grown for its grains, which are consumed in a paste-like state. It is nowadays attacked by numerous parasitic fungi, hence this study was carried out with the objective to characterize the fungal pathogens associated with this crop from the seed, vegetative stage, to the doughy grain stage at harvest. For this purpose, 37 genotypes of seeds of this crop, obtained from the Genebank of the Plant Genetics and Breeding team, Biosciences Laboratory of the Joseph KI-ZERBO University of Burkina Faso, were subjected to sanitary analysis. All the genotypes were tested during the 2021 rainy season using an alpha lattice design with three replications. From the 30th day of the rainy season, symptomatic leaves were randomly collected from 11 genotypes of each replicate for the isolation and characterization of different fungal pathogens. Similarly, from day 90 onwards, the health status of the doughy grains was analyzed on the same genotypes. The results obtained revealed a diversity of fungal pathogens both on seeds in pre-sowing, on symptomatic leaves and on doughy grains in pre-harvest. Indeed, 10 fungal species were found on the selected genotypes, among which seven species of fungi were identified as pathogens of sweet grain sorghum, namely *Bipolaris* sp., *Curvularia lunata*, *Fusarium moniliforme*, *Phoma sorghina*, *Colletotrichum graminicola*, *Nigrospora oryzae* and *Exserohilum* sp. In addition, three saprotrophic fungi namely *Aspergillus flavus*, *Aspergillus niger* and *Rhizopus* sp., which account for 30% of the pre-sown seed and pre-harvest doughy grain, were also identified. *Curvularia lunata* (61.61%), *Fusarium moniliforme* (53.53%) and *Phoma sorghina* (42.42%) were the most abundant and common fungal pathogens at the development stages studied. To sum up, the existence of a number of parasites and saprotrophs fungi on grains of this crop, generally consumed in their fresh state, is a significant threat to humans because they could secrete mycotoxins, mainly fusariotoxins, which could lead to the occurrence of certain mycoses in humans in the long term. Hence there is a need to apply basic hygiene rules for a healthy consumption of these grains.
Keywords: Burkina Faso, Mouth seeds, Parasitic fungi, Sanitary analysis, Sweet grain sorghum.

Introduction

Fungal diseases are an important yield-limiting factor in cereals with estimated yield losses of 15–20%; when they occur to a large extent, losses may reach as high as 50% (Korbasn, 2009). Cereal fungal diseases have occurred in many parts of the world and are considered to be one of the main factors affecting yield and yield quality. Fungal diseases contribute both to a decrease in yields and to a deterioration of crop quality (Tekiela, 2008). Association of certain fungi with stored agricultural grains could lead to their contamination with fungi-derived toxins (mycotoxins) and these toxins due to their ability to suppress hormonal immunity and cause tissue breakdown have been implicated in many forms of allergies, immune dysfunctions, cancers, birth defects, and even death (Enyiukwu *et al*., 2014). Some plant pathogenic fungi implicated in human diseases are *Mucor mucedo*, *Aspergillus flavus*, *Curvularia lunata* and these and related organisms cause superficial, subcutaneous, systemic and opportunistic infections in humans (Manisha and Pawar, 2012).

Sorghum [*Sorghum bicolor* (L.) Moench] is a semi-arid sub-Saharan African staple crop, cultivated in Africa and well adapted to the semi-arid tropics due to its hardiness and moderate water requirements. Within it, two major groups can be recognized, such as unsweetened grain sorghum and sweet grain sorghum. Sweet grain sorghum, which is the subject of this study, is mainly grown for its grains, which are consumed in a pasty state. It is generally harvested before the main food crops and constitutes a supplementary food during the lean season in some rural localities of sub-Saharan Africa (Nebié *et al*., 2012). In addition, its leaves and stems

are used as fodder or fuel. Compared to the main cereal crops (unsweetened sorghum, millet, maize), sweet grain sorghum is a minor crop in the agricultural system of Burkina Faso. However, it is not exempted from parasitic attacks, especially of fungi, which can lead to yield losses and also serve as a reservoir for fungal agents that could induce the secretion of mycotoxins harmful to human and animal health through their metabolism. For this reason, this groundbreaking research was undertaken to survey for fungi associated with sweet grain sorghum at three stages of its development *viz.* seed (pre-sowing stage), vegetative stage and grain maturation stage (pre-harvest). This work is intended to be a warning to producers and consumers of sweet grain sorghum of the need to observe hygienic measures before consuming grains in their fresh state.

Materials and Methods

Study site

A field experiment was conducted at the experimental station of the Institute du Rural Development Rural (IDR) of Gampèla during the 2021-2022 agricultural season. The experimental station is located about 18 km east of Ouagadougou and lies between 12°24'29" North latitude and 1°21'9.6" West longitude. It covers an area of 490 ha and is crossed from west to east by a tributary of the Massili River (Thiombiano and Kampmann, 2010). The anatomical and morphological analyses and identification of the fungi of the different samples took place in the laboratory of the Phytopathology and Tropical Mycology team, Department of Plant Biology and Plant Physiology, UFR/SVT, Joseph KI-ZERBO University.

Plant material

The plant material used in this study was seed of 37 genotypes (SKA3; YOU4; KBA1; PBO5; BKO3; YOU1; YOH4; BZI1; GBI4; YOH3; SPI2; PLA1; PBO4; BKO1; YOU5; PGO3; BIP4; BKB1; BKB2; BKB4; KBZ1; KBZ4; MBO7; MDE5; MTC2; SBR1; SBR5; SBR7; STO5; STO2; YOH8; YOH2; GCO5; LOU10; MBO8; SKA2; STO6) of symptomatic leaves and doughy grains of 11 sweet grain sorghum genotypes (BKB1, PGO3, PBO4, KBZ4, LOU10, SKA2, SKA3, MBO8, PLA1, SBR5, STO6) randomly selected from the 37 genotypes. These genotypes were obtained from the Genebank of the Plant Genetics and Improvement Team of the Biosciences Laboratory of the Joseph Ki-Zerbo University. They were selected according to their agro-morphological performance and the very sweet flavor of the grains revealed during previous studies (Tiendrébéogo, 2015; Tondé, 2020).

Experimental design

The experimental design was a completely randomized alpha-lattice with three replicates, 916.8 m^2. The distance between replicates was 2 m. Each replication was subdivided into two sub-blocks 1 m apart with 5.2 m long rows, 80 cm between rows and 40 cm between bunches, with 14 bunches per line.

Survey and sample collection

The survey and collection of samples was carried out at 40 days after sowing (DAS). Symptomatic leaf fragments of 11 genotypes were collected, labelled and packed in plastic bags and stored in a cooler and sent to the laboratory for analysis (Fig. 1). Also, from 80th DAS onwards, samples of pasty grains of the different genotypes were also collected for health analysis.

Isolation of fungi from collected samples

Once in the laboratory, the symptomatic leaves were disinfected with 3% sodium hypochlorite, rinsed with distilled water and left to dry on blotting paper for 15 min. They were then cut into small fragments of 1 to 2 cm and placed in Petri dishes containing potato dextrose agar (PDA) medium and sealed with parafilm and incubated at 25 °C for 7 days. Likewise, the sanitary analysis of the seeds and pasty grains was carried out according to the blotter test method recommended by ISTA (1999). For this, 25 grains of each batch (seeds and pasty grains) were selected at random and then placed in a Petri dish with a bed of blotting paper. The entire contents were moistened with sterile distilled water and incubated at 25 °C in an incubation room for 7 days.

Fungal strains identification

After several transplantations, the pure strains of fungi obtained were observed with a binocular magnifying glass and a Ci Nikon light microscope at X400 and X1000 magnifications and identified on the basis of their morphological characteristics (mycelium and fruiting bodies). The keys of Pitt and Hocking (1997) and Mathur and Kongsdal (2003) were used for the identification of fungi.

Results

Diversity of fungal flora identified on seeds (pre-sowing)

The sanitary analysis of the seeds of the 37 sweet grain sorghum genotypes in pre-planting showed that all the seeds were infected by fungi. Ten fungal species were identified as *Curvularia lunata* (20.75%); *Aspergillus niger* (11.72%); *Aspergillus flavus* (7.40%); *Fusarium moniliforme* (15.81%); *Phoma sorghina* (15.19%); *Rhizopus* sp. (10.49%); *Penicillium* sp (7.40%); *Bipolaris* sp. (7.17%); *Colletotrichum graminicola* (3.46%) and *Nigrospora oryzae* (0.61%) (Fig. 2). Among them, there are six (6) fungal species belonging to the group of

parasites: *Curvularia lunata* (Fig. 3), *Fusarium moniliforme* (Fig. 4), *Phoma sorghina* (Fig. 5), *Colletotrichum graminicola* (Fig. 6), *Nigrospora oryzae* (Fig. 7) and *Bipolaris* sp. (Fig. 8).

Diversity of fungal flora identified on symptomatic leaves

Fungal species detected on symptomatic leaves were 7 with the following infection rates: *Curvularia lunata* (100%); *Colletotrichum graminicola* (63.64%); *Fusarium moniliforme* (54.54%); *Aspergilus niger* (45.45%); *Phoma sorghina* (36.36%); *Nigrospora oryzae* (9.10%) and *Fusarium* sp.1 (9.10%) (Fig. 9). Symptomatic leaves of genotype SBR5 were found to harbour the highest number of fungi such as *Curvularia lunata*, *Phoma sorghina*, *Colletotrichum graminicola*, *Aspergilus niger* and *Fusarium* sp.2 in contrast to genotypes BKB1, SKA2, PLA1 and STO6 which were infested by 2 types of fungi (Table 1). Apart from *Aspergillus niger* which is saprotrophic, all 6 species are parasites.

Diversity of fungal flora identified on doughy grains

Table 2 gives the results of the sanitary analysis of doughy grains of sweet grain sorghum genotypes at pre-harvest stage. Eight fungal species were identified with the following contamination rates: *Aspergillus niger* (9.09%), *Fusarium moniliforme* (63.63%), *Rhizopus* sp. (36.36%), *Curvularia lunata* (45.45%), *Phoma sorghina* (36.36%), *Bipolaris* sp. (9.09%) and *Penicillium* sp. (36.36%) (Fig. 10). *Fusarium moniliforme* was identified in 7 genotypes (BKB1, KBZ4, LOU10, MBO8, PLA1, SBR5 and STO6) with the highest infestation rate (63.63%) in contrast to *Aspergillus niger* and *Bipolaris* sp. which each had an infestation rate of 9.09%. Among the fungal species identified, four species were parasitic: *Curvularia lunata*, *Fusarium moniliforme*, *Phoma sorghina* and *Bipolaris* sp.

Diversity of common parasitic fungi identified at different stages

From the different analyses carried out, it appears that three species of pathogenic fungi were reported on pre-sowing seeds, symptomatic leaves (vegetative stage) and on doughy grains in pre-harvest in sweet grain sorghum. These are *Fusarium moniliforme*, *Curvularia lunata* and *Phoma sorghina* (Table 3).

Fig. 1 (a-d): Sampling and storage of symptomatic leaves. **a:** Leaf showing fungal symptoms, **b**: Collection of infected leaves, **c:** Labelling and bagging of collected leaves, **d:** Storage of leaves in a cooler with ice.

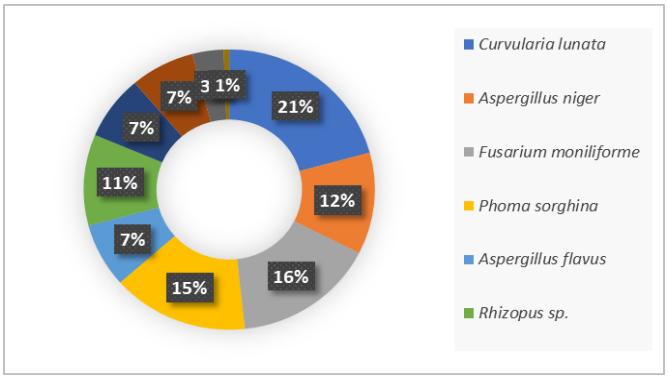

Fig. 2: Abundance rate of fungi identified in pre-planted sweet grain sorghum seeds.

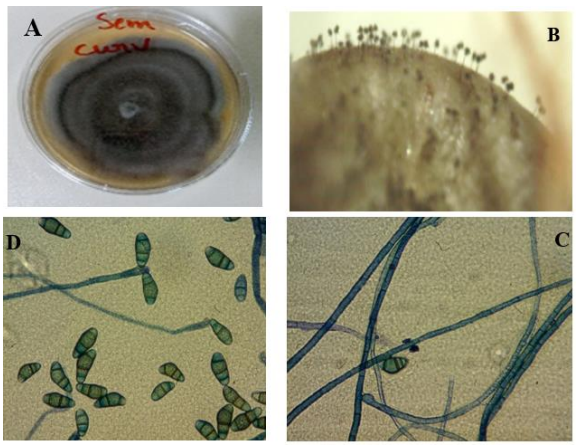

Fig. 3 (A-D): Different morphological aspects of *Curvularia lunata.* **A:** Magnified sporangia on a seed (G 10 ×40); **B:** Mycelial development on PDA medium; **C:** Partitioned conidiophores (G 10 ×40); **D:** Curved conidia (G 10 ×40)

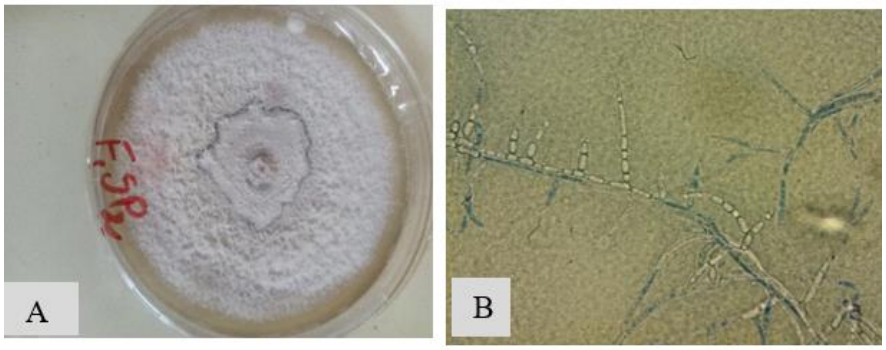

Fig. 4: Different morphological aspects of *Fusarium moniliforme.* **A:** Mycelial development on PDA medium; **B:** Conidiophore and conidia formation (G 10×40).

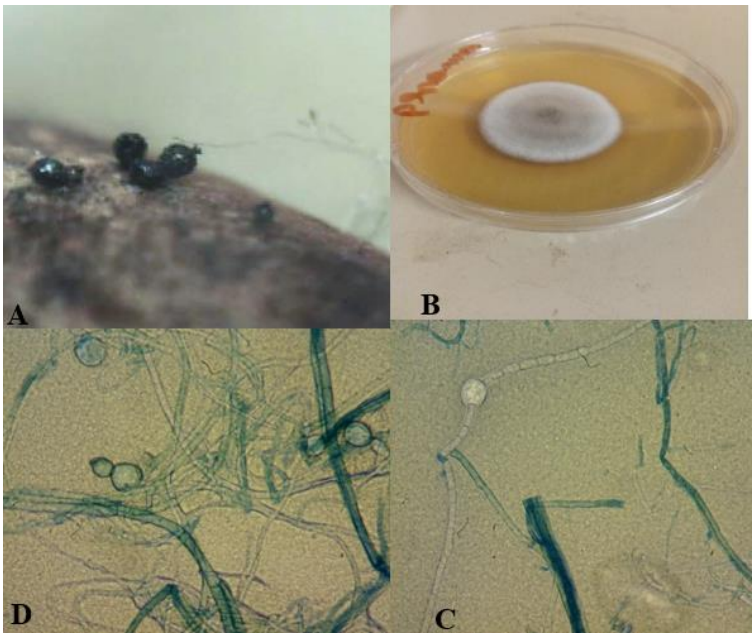

Fig. 5: Different morphological aspects of *Phoma sorghina.* **A:** Pycnidia observed with a magnifying glass on a seed (G 10 ×40), **B:** Mycelial development in the medium (PDA), C&D: Hyphae partitioned with chlamydospores in formation (G 10 ×40).

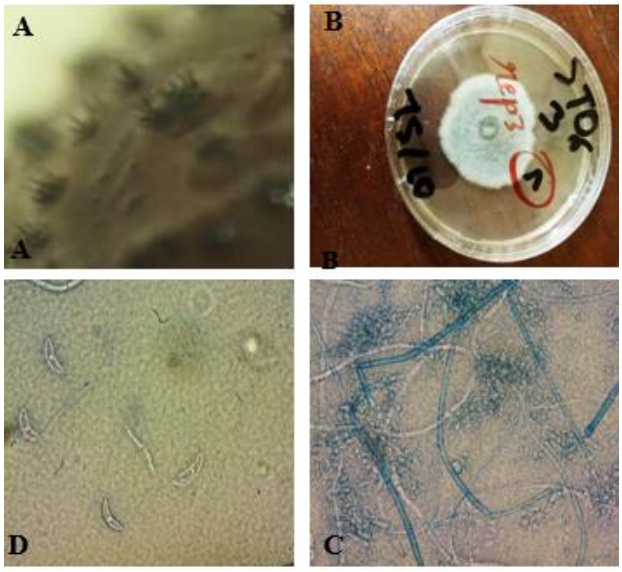

Fig. 6: Different morphological aspects of *Colletotrichum graminicola*. **A:** Acervulus seen under magnifying glass, **B:** Mycelial development in medium (PDA); C&D Conidiophores with sickle-shaped conidia (G 10 ×40).

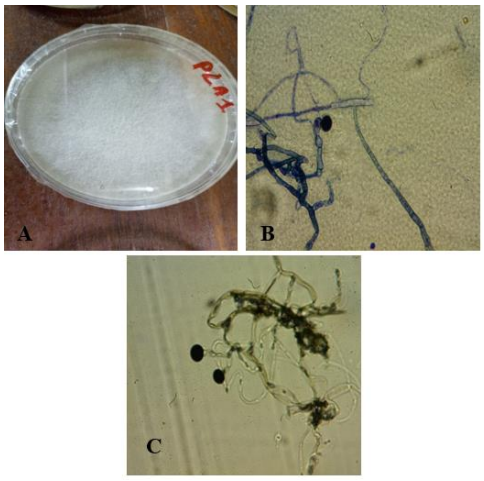

Fig. 7: Different morphological aspects of *Nigrospora oryzae*. **A:** mycelial development in medium (PDA); **B-C:** conidiophore bearing black conidia (G 10 ×40).

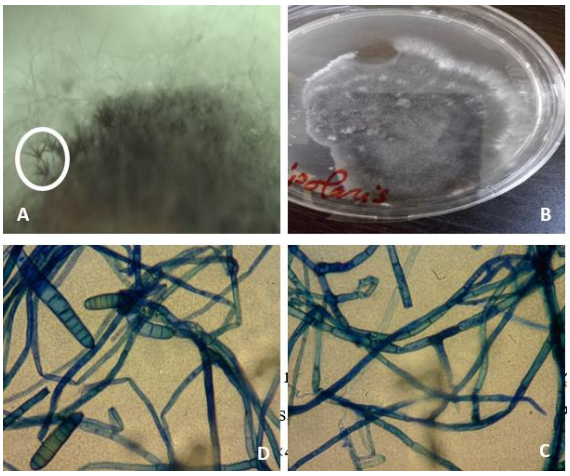

Fig. 8: Different morphological aspects of *Bipolaris* sp. **A:** Acervulus seen with a magnifying glass, **B:** Mycelial development on PDA; **C & D:** Coniophores with fusiform conidia (G 10 ×40).

Table 1: Percentage of contamination of sweet grain sorghum leaves by fungi.

Fungi	Genotypes												
	BKB1	**PGO3**	**PBO4**	**KBZ4**	**LOU10**	**SKA2**	**SKA3**	**MBO8**	**PLA1**	**SBR5**	**STO6**	**Total**	**(%)**
Aspergillus niger	X	X	X				X			X		**05**	**45,45**
Curvularia lunata	X	X	X	X	X	X	X	X	X	X	X	**11**	**100**
Phoma sorghina		X		X	X					X		**04**	**36,36**
Colletotrichum graminicola		X	X	X			X	X		X	X	**07**	**63,64**
Fusarium moniliforme				X	X	X	X	X		X		**06**	**54,54**
Nigrospora oryzae									X			**01**	**09,10**
Fusarium sp. 1			X									**01**	**09,10**
Total	**02**	**04**	**04**	**04**	**03**	**02**	**04**	**03**	**02**	**05**	**02**		

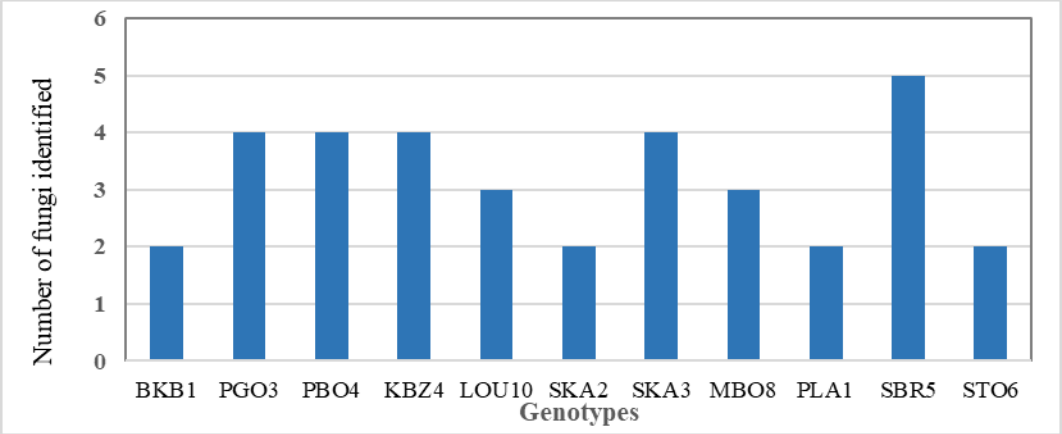

Fig. 9: Number of fungi identified on symptomatic leaves of sweet grain sorghum according to genotype.

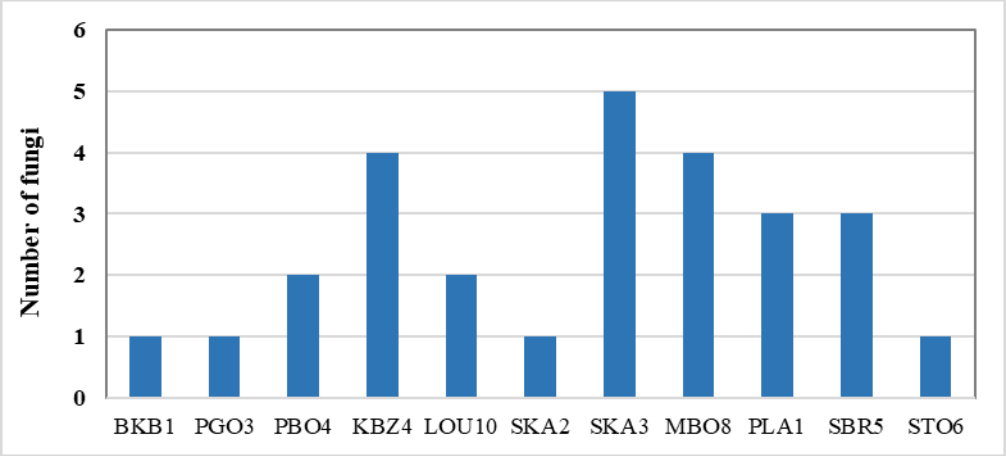

Fig. 10. Number of fungi identified on doughy grains of sweet grain sorghum according to genotypes.

Table 2: Percentage of contamination of doughy grains by fungi.

Fungi	Genotypes												
	BKB 1	**PGO 3**	**PBO 4**	**KBZ 4**	**LOU 10**	**SKA 2**	**SKA 3**	**MB O8**	**PLA 1**	**SBR 5**	**STO 6**	**Total**	**(%)**
Aspergillus niger										x		1	9.09
Fusarium moniliforme	x			x	x			x	x	x	x	7	63.63
Rhizopus sp.			x	x		x	x					4	36.36
Curvularia lunata		x			x		x	x	x			5	45.45
Phoma sorghina							x	x	x	x		4	36.36
Bipolaris sp.							x					1	9.09
Penicillium sp.			x	x			x	x				4	36.36
Total	1	1	2	3	2	1	5	4	3	3	1		

Table 3: Fungal pathogens common to the three developmental stages of sweet grain sorghum.

Pathogenic fungi	Seeds (pre-sowing)	Symptomatic leaves	Pasty grains
Fusarium moniliforme	x	x	x
Bipolaris sp.	x		x
Curvularia lunata	x	x	x
Colletotrichum graminicola	x	x	
Phoma sorghina	x	x	x
Nigrospora oryzae	x	x	
Fusarium sp.1		x	

Discussion

All the analyses carried out show that three species of pathogenic fungi are common to the three stages of development concerned in this study: pre-sowing seeds, symptomatic leaves and doughy grains. These are the following fungal species: *Fusarium moniliforme*, *Curvularia lunata* and *Phoma sorghina*. According to Frederiksen and Odvody (2000), because of its acclimatization to a wide range of environments, sorghum in cultivation has many pests of all types: viruses, bacteria, fungi, nematodes, insects, parasitic plants, birds. However, in some situations, additional factors exacerbate the impact of fungi. This can be the case for crops planted on unsuitable soils that weaken the plants, or inappropriate cultivation practices that favour the maintenance and multiplication of fungi, such as the absence of rotation or the proximity of varieties with different cycles. In addition, climatic accidents in terms of temperature or rainfall are also factors that aggravate fungal attacks. Chantereau *et al*. (2013) add that seed moulds constitute a major phytosanitary problem for sorghum. For them, this is due to the susceptibility of seeds to microorganism attack when crop ripening occurs in rainy and hot conditions. In contrast, Turner, 2013, explains this presence of mould on cereal seeds by a deposition of mould spores on the floral parts that come into contact with the ovary. These germinate and their mycelium first invades the internal tissues (albumen and embryo) and eventually the external tissues of the seed. In addition, other fungal species may be involved in the process, with opportunities for contamination increasing with high humidity and the presence of panicle insects. In this panicle microclimate, the two types of organisms interact to favour the installation and development of the first fungal complex, *Pythium* sp. and secondarily species of the genera *Fusarium*, *Aspergillus*, *Rhizoctonia*. Depending on the region, fungi of the Fusarium genus or species such as *Curvularia lunata*, *Colletotrichum graminicola*, *Phoma sorghina* are associated with the damage observed. In addition, mycotoxins associated moulds can cause health problems when sweet grain sorghum crops are consumed as a paste. Mycotoxins are defined as non-protein, low molecular weight, thermostable chemical compounds that are not easily destroyed by physical or chemical treatments. They are products of the secondary metabolism of moulds belonging mainly to the genera *Aspergillus*, *Penicillium* and *Fusarium*. Among the families of mycotoxins that can be present in cereals, five of them are particularly well known. These are fumonisins, trichothecenes, zearalenones, ochratoxins and aflatoxins.

The results of our analyses confirm the presence of *F. moniliforme* on the doughy grains of 7 of our genotypes out of 11, those of *C. lunata* (5 genotypes out of 11) and *Phoma sorghina* (4 genotypes out of 11). According to Braly (2003), the toxicity of mycotoxins produced by these fungi depends on the nature and character of the molecule, the risk of exposure, the quantity absorbed and the nature of the receptor of these mycotoxins in humans and animals. Previously, occurrence of *C. lunata* has been reported on banana (Khan and Javaid, 2020), and that of *F. moniliforme* on rice (Javaid *et al.*, 2018).

According to Cahagnier (1993), moulds of the genus *Fusarium* produce several types of mycotoxins grouped under the term fusariotoxins: zearalenone, fumonisins, trichothecenes, moniliformin, beauvericin, fusarin C and fusaric acid. According to Kuiper-Goodman *et al*. (1987), numerous studies have shown that zearalenone binds to estrogen receptors and induces an estrogen-like effect. It is metabolized in several tissues, but particularly in the liver where it is transformed into two metabolites: α-zearalenol and β-zearalenol. Zearalenone is not highly toxic. However, α-zearalenol has a higher affinity for estrogen receptors and induces a higher toxicity. Zearalenone is further metabolised by glucuronoconjugation. The significance of the effects observed on sensitive animal species is therefore a function of the rate of conversion to α-zearalenol and the glucuronoconjugation capabilities of these animal species. The question of the possible effects of zearalenone in humans arises in the longer term because the sensitivity is low as are the ingested doses. In overview, zearalenone can be genotoxic and immunotoxic at high doses. However, it is not yet clearly established that zearalenone is carcinogenic, even though the International Agency for Research on Cancer classifies this molecule as "probably not carcinogenic to humans" (Le Bars and Le Bars, 1996). As for the trichothecenes, they are sesquiterpene epoxides. They all have a tricyclic ring and an epoxide function in CI2-CI3, which is essential for their toxicity. They are divided into four categories or types (A, B, C, D). Group A and B trichothecenes are widely present on cereals and foodstuffs, whereas types C and D are rarely found (Gledhill *et al*., 1991). Toxicity studies have shown that trichothecenes act as inhibitors of eukaryotic protein synthesis. Trichothecenes bind to one of the ribosomal subunits and interact with peptidyltransferase. This interaction leads to the inhibition of peptide formation (Cundlife and Davies, 1977). Trichothecenes are active on all rapidly dividing cells and their ingestion often causes digestive disorders followed by bloody diarrhoea or intestinal haemorrhage (Cahagnier, 1997). Fumonisins, identified by Bezuidenhout et al. (1988) are common contaminants of plants, particularly maize. They are synthesised by condensation of alanine with a precursor of an acetate derivative. Fumonisins are classified as probable human carcinogens by the International Agency for Research on Cancer. They are known to be hepatotoxic and immunotoxic (Bramham and

Plattner, 1993). Finally, fusariotoxins in food (mainly in sweet grain sorghum) pose public and animal health problems. The chronic toxicity of fusariotoxins has not yet been sufficiently studied to determine whether significant long-term effects can occur following the ingestion of regular small amounts of low-contaminated foodstuffs. Foods suspected of being contaminated should therefore be subject to regular monitoring and further study. With regard to the consumption of sweet sorghum doughy grains, hygiene measures should be strengthened to better protect the rural populations of sub-Saharan Africa for whom these grains are a staple food.

Conclusion

Sweet grain sorghum was found contaminated with both parasitic and saprotrophic fungi during three developmental stages. With the possibility that these fungi produce mycotoxins, which can be a threat to humans, it is therefore necessary that hygienic measures be taken to protect the rural population of sub-Saharan Africa, for whom sweet grain sorghum is a staple food.

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