OPEN ACCESS Int. J. Phytopathol. 15 (01) 2026. 97-106 DOI: 10.33687/phytopath.015.01.6027

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Available Online at EScience Press
International Journal of Phytopathology
ISSN: 2312-9344 (Online), 2313-1241 (Print)
https://esciencepress.net/journals/phytopath

PHYTOPATHOLOGICAL CHARACTERIZATION OF MICROSCOPIC FUNGI ASSOCIATED WITH FIFTEEN SWEET-STEM SORGHUM GENOTYPES (*SORGHUM BICOLOR* L. MOENCH) IN BURKINA FASO

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ARTICLEINFO

Article History
Received: January 15, 2026
Revised: March 17, 2026
Accepted: April 23, 2026

Keywords
Sweet-stem sorghum
Sorghum bicolor
Fungal diversity
Seed-borne pathogens
Foliar diseases
Burkina Faso

ABSTRACT

Sweet-stem sorghum [*Sorghum bicolor* (L.) Moench] is subject to various fungal diseases that can adversely affect grain quality and reduce crop productivity. In order to contribute to improved valorisation of this crop in Burkina Faso, the present study aimed to identify and inventory fungal species associated with sweet-stem sorghum. The study was conducted at the Gampela experimental station from July to October 2023. Fungal identification was carried out on seeds and symptomatic leaves collected from fifteen sweet-stem sorghum genotypes, using the moist blotter technique, appropriate culture media and the identification key of Mathur and Kongsdal (2003), based on morphological, microscopic and cultural characteristics. Fungal isolates were obtained and purified from symptomatic leaves and from seeds incubated on blotter paper, followed by cultivation on SDA medium. Seed samples were infected by the genera *Alternaria*, *Aspergillus*, *Curvularia*, *Phoma*, *Fusarium* and *Bipolaris*. All these genera were also detected on leaf samples, with the additional presence of the genus *Colletotrichum*. In seeds, *Fusarium* sp. was the most frequently isolated genus, with an incidence of 68.53%. For leaf samples, *Phoma* sp. and *Curvularia lunata* showed equally high frequency of occurrence (82.22%). The findings of this study provide valuable baseline information that can be utilised in breeding programs and phytosanitary management strategies for sweet-stem sorghum in Burkina Faso.

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INTRODUCTION

Sorghum (*Sorghum bicolor* [L.] Moench) is a cereal crop domesticated in Africa, where it remains one of the species best adapted to semi-arid tropical regions due to its hardiness and moderate water requirements. Having spread beyond its continent of origin and gradually acclimatised to temperate zones, sorghum now occupies an important place in the agricultural systems of several emerging and developed countries, where it is mainly cultivated for animal feed (Chantereau *et al*., 2013). Among the different sorghum types, sweet-stem sorghum has attracted increasing interest because of its potential for biofuel production. It comprises a group of ecotypes characterized by the accumulation of large amounts of carbohydrates in their juicy stems (Ritter *et al*., 2008; Wang *et al*., 2013). The main sugars present in the stem juice are sucrose, glucose and fructose, occurring in variable proportions depending on the variety (Schaffer & Gourley, 1982; Murray *et al*., 2009). According to Gutjahr (2012), sweet sorghums are generally defined as tall plants producing large amounts of biomass. Sweet-stem sorghum is cultivated in several regions worldwide, notably in the

United States (Ali *et al*., 2007; Murray *et al*., 2009; Wang *et al*., 2009), Niger (Deu *et al*., 2008), China (Zhang *et al*., 2009; Pei *et al*., 2010), as well as in Guinea and Mali (Sagnard *et al*., 2011). In Africa, it is traditionally grown for direct consumption of the stems as snacks, while the grains are used for both human and animal nutrition. Nowadays, its industrial importance continues to increase, particularly for the production of syrup and bioethanol from the sweet stem juice. In Burkina Faso, although sweet-stem sorghums are cultivated under diverse agroecological conditions and exhibit moderate genetic diversity (Nebié *et al*., 2013), the available genetic resources remain insufficiently characterized and underutilized. Sweet-stem sorghum genotypes cultivated in Burkina Faso represent underexplored genetic resources with potential importance for biomass production, bioethanol development and adaptation to semi-arid environments. However, information regarding fungi associated with these genotypes remains limited. Moreover, like most crops, sweet-stem sorghum is exposed to various abiotic and biotic stresses that can affect its growth, productivity and product quality. Among biotic stresses, pests and pathogens, particularly fungi associated with sweet-stem sorghum, constitute a major constraint. These microorganisms can infect seeds, stems and leaves, leading to yield losses and deterioration of the technological and sanitary quality of the crop. In this context, the characterization of microscopic fungi associated with sweet-stem sorghum is essential for its conservation, valorisation and improvement. Accordingly, the general objective of the present study was to characterize the microscopic fungi associated with the seeds and leaves of fifteen sweet-stem sorghum genotypes.

MATERIALS AND METHODS

Experimental Site

The field experiment was carried out at the experimental station of the Institute for Rural Development (IDR) located in Gampèla (Burkina Faso) at geographical coordinates 1°21'9,6'' west longitude and 12°24'29'' north latitude during the 2023 rainy season. The soils are predominantly silty-sandy in texture and low in carbon and organic matter, with high levels of phosphorus and potassium, a pH ranging 5 to 6.3, and traces of mineral salts. These soils present several constraints, including low organic matter content and low water-holding capacity (BUNASOLS, 1988; Kiébré, 2016). The climate of the Centre Region is a dry tropical climate of the northern Sudanian type. The experiment was conducted under rainfed conditions.

Experimental Design

The experimental design was a completely randomized α-lattice with three replications spaced 1 m apart (Figure 1). The fifteen sweet-stem sorghum genotypes were evaluated in elementary plots consisting of single rows measuring 2.8 m in length. The spacing within the experimental area was 0.80 m between rows and 1 m between adjacent blocks, with an intra-row spacing of 0.40 m, allowing for eight planting stations per row. The experiment was conducted on a plot measuring 59.6 m in length and 18 m in width, corresponding to a total area of 1072.8 m^2.

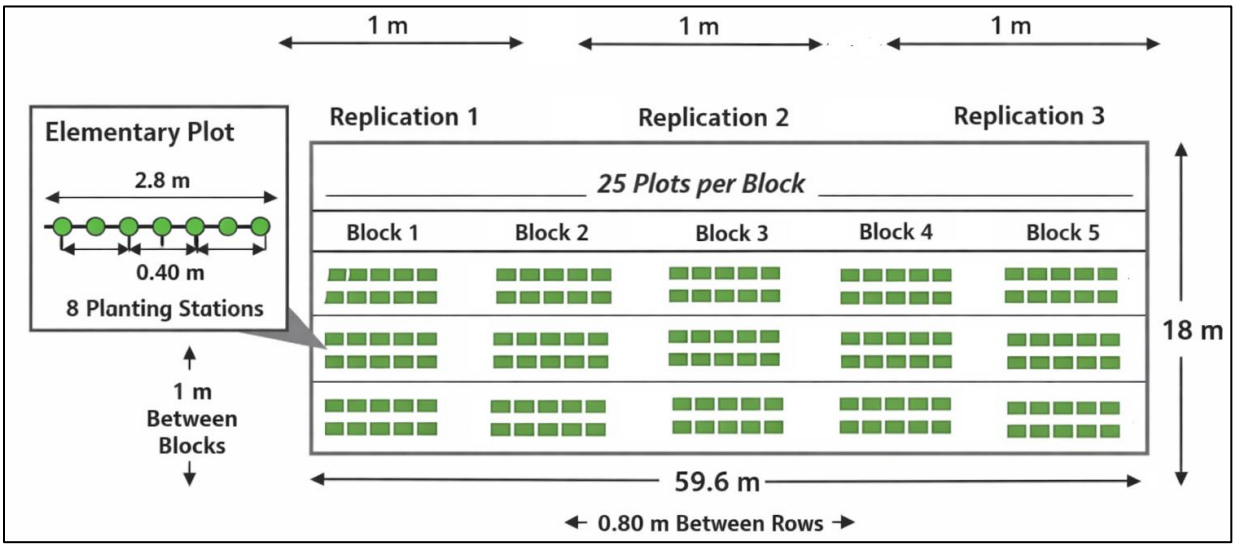

Figure 1. α-lattice experimental design.

Samples and Sampling Procedure

Between July 16 and October 23, 2023, diseased samples were collected from leaves and grains in sorghum fields and transported to the *Phytopathology et Tropical Mycology* (*PM_Trop*) Laboratory at Joseph Ki-Zerbo University. A total of fifteen (15) sorghum genotypes obtained from the Biosciences Laboratory in Burkina Faso were used in this study (Table 1). Leaf samples were collected approximately two months after sowing, at the onset of visible disease symptoms. Grain samples were collected at the physiological maturity of the panicles.

Table 1. Different genotypes profile.

Code	Origin	
	Province	Village
KSM2	Kossi	Mourdjié
SNK5	Sanmatenga	Korsimoro
SNK13	Sanmatenga	Korsimoro
KB12	Kossi	Gninanou
KB16	Kossi	Gninanou
KB17	Kossi	Tira
KDU2	Kossi	Mounakoro
KNO5	Kossi	Koussiri
KNO6	Kossi	Kolonkoura
KNO9	Kossi	Nouna
MBO1	Mouhoun	Syn-Bekuy
MDE8	Mouhoun	Kari
MTC4	Mouhoun	Bissanderou
NDA4	Namentenga	Boulmiougou-mossi
GBO9	Gnagna	Gnimpiendi

Isolation of Fungal Pathogen

Fungal isolation was performed from seeds and from leaves exhibiting typical symptoms of fungal infection. Fungal isolates were cultured on Sabouraud Dextrose Agar (SDA) (Figure 2), a selective medium composed mainly of dextrose, peptone and tryptone, commonly used for fungal isolation and cultivation. Originally developed by Sabouraud in 1892, SDA promotes fungal growth while inhibiting the development of most bacteria due to its acidic pH. Leaf and seed samples were initially washed with tap water to remove adhering soil particles and debris. Surface disinfection was carried out by immersion in 3% sodium hypochlorite for 2–3 min, followed by three rinses with sterile distilled water to remove residual disinfectant. The samples were then air-dried on sterile blotting paper. This step was intended to eliminate epiphytic microorganisms while preserving internal fungal structures. Samples were incubated at 25 °C under alternating cycles of 12 h light and 12 h darkness. After three days of incubation, individual fungal colonies emerging from the samples were aseptically transferred to fresh Petri dishes containing SDA medium. Distinct colonies were subcultured repeatedly on agar media until pure isolates were obtained. Preliminary identification was based on macroscopic colony characteristics, followed by detailed microscopic examination using a stereomicroscope and compound microscope after staining with methyl blue. Identification was based on morphological, microscopic and cultural characteristics using standard taxonomic keys and descriptions provided by Mathur and Kongsdal (2003), Blancard et al. (2009) and Crous et al. (2014). Molecular confirmation using ITS and TEF-1α sequencing will be necessary for accurate species delimitation.

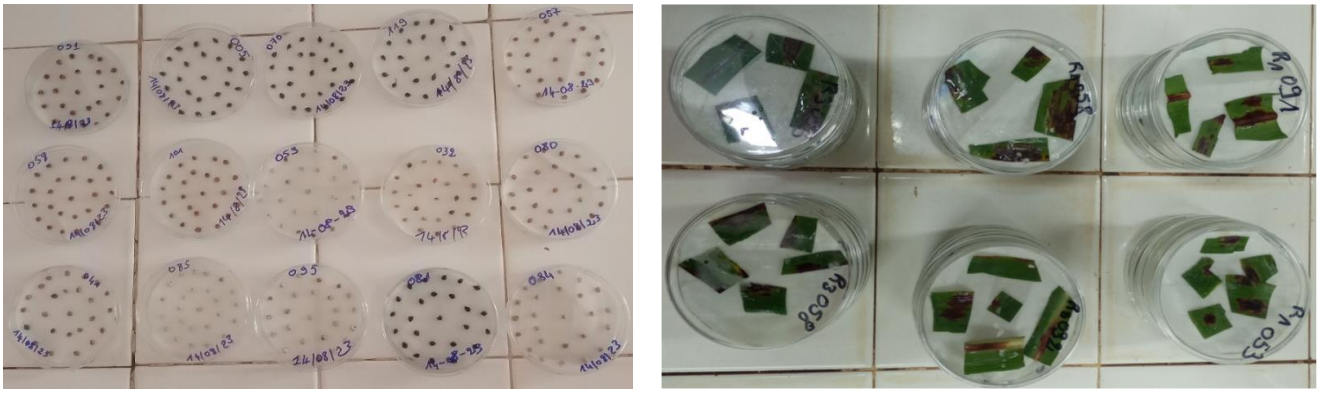

Figure 2. Grains (left) and leaves (right) incubated in Petri dishes using the blotter test method for fungal isolation.

RESULTS

Diversity of Fungal Flora on Seeds

Table 2 highlights a relatively high fungal diversity associated with the seeds of the fifteen sweet-stem sorghum genotypes analysed. Seven fungal species belonging to six genera were identified, indicating widespread

contamination of seeds by fungi associated with sweet-stem sorghum. *Fusarium* sp. exhibited by far the highest frequency of occurrence (68.53%), suggesting its predominant role as a soil-borne and potentially seed-borne pathogen in sweet-stem sorghum. The genera *Bipolaris* sp. (27.73%) and *Curvularia lunata* (14.13%) also showed notable infection levels, confirming their importance within the seed-associated mycoflora. The presence of *Aspergillus* species (*A. flavus* and *A. niger*) at moderate frequencies likely reflects secondary contamination related to storage conditions or surrounding organic matter. In contrast, *Alternaria alternata*, detected at a very low frequency (1.06%), appears to be an occasional species. The simultaneous presence of several fungal taxa on seeds and leaves suggests a possible epidemiological link between both organs.

Table 2. Fungal flora isolated from seeds of 15 sampled genotypes.

Genus	Species	Percentage (%)	Genotypes
Alternaria	*Alternaria alternata*	1.06	MDE8
Aspergillus	*Aspergillus flavus*	9.33	KSM2, SNK13, KB12, KB16, KB17
Aspergillus	*Aspergillus niger*	6.13	MBO1, KNO9, KNO6, KB17, SNK13
Bipolaris	*Bipolaris* sp.	27.73	MDE8, MTC4, NDA4, GBO9, MBO1
Curvularia	*Curvularia lunata*	14.13	KSM2, SNK5, SNK13, KB12
Fusarium	*Fusarium* sp.	68.53	KB12, KB16, KB17, KDU2
Phoma	*Phoma* sp.	11.73	KDU2, MBO1

Diversity of Fungal Flora on Leaves

Table 3 reveals a high fungal diversity at the foliar level, with six species belonging to six distinct genera. The highest frequencies of occurrence were recorded for *Curvularia lunata* and *Phoma* sp. (82.22% each), indicating their strong affinity for leaf tissues and their likely direct involvement in the development of the symptoms observed in the field. *Fusarium* sp. also showed a relatively high frequency (46.66%), suggesting both soil-borne and systemic behaviour. The detection of *Colletotrichum* sp. (33.33%), which was absent from seeds, points to leaf-specific contamination, probably resulting from dissemination via wind, water or plant residues. Although *Aspergillus* and *Bipolaris* were present, their relatively low frequencies of occurrence suggest a secondary role in foliar pathology. Collectively, these findings confirm that leaves represent an organ highly exposed to fungal infections, with a dominance of pathogenic species responsible for leaf spots and necrotic lesions.

Table 3. Fungal flora isolated from infected leaves of 15 genotypes.

Genus	Species	Percentage (%)	Genotypes
Aspergillus	*Aspergillus flavus*	13.33	KB12, KB17, KB16, KNO6
Bipolaris	*Bipolaris* sp.	6.66	SNK13, KDU2
Colletotrichum	*Colletotrichum* sp.	33.33	SNK5, MBO1, KNO5, KNO6
Curvularia	*Curvularia lunata*	82.22	KNO9, KSM2, MTC4, SNK5
Fusarium	*Fusarium* sp.	46.66	KDU2, KNO9, KSM2, NDA4
Phoma	*Phoma* sp.	82.22	KNO6, KB16

Diversity of Fungal Flora Present on Both Seeds and Leaves

Table 4 demonstrates a marked overlap of certain fungal species between seeds and leaves. Five species belonging to the genera *Aspergillus*, *Bipolaris*, *Curvularia*, *Fusarium* and *Phoma* were detected in both organs, suggesting epidemiological continuity between seeds and aerial plant parts. Among these species, *Fusarium* sp. (57.59%) and *Curvularia lunata* (48.17%) exhibited the highest frequencies, confirming their ability to persist and spread within the plant. This dual presence indicates that seeds may act as a primary source of inoculum, facilitating the establishment and persistence of foliar infections. These results emphasise the key role of contaminated seeds in the epidemiology of fungal diseases of sweet-stem sorghum and highlight the importance of integrated phytosanitary management, including strict control of seed health quality.

Table 4. Fungal flora common to seeds and leaves.

Genus	Species	Percentage (%)	Genus	Species	Percentage (%)
Aspergillus	*Aspergillus flavus*	11.33	*Fusarium*	*Fusarium* sp.	57.59
Bipolaris	*Bipolaris* sp.	17.19	*Phoma*	*Phoma* sp.	46.97
Curvularia	*Curvularia lunata*	48.17			

Cultural and Microscopic Characteristics of Identified Fungal Species

Plates 1 to 8 describe the different species identified in this study. ss

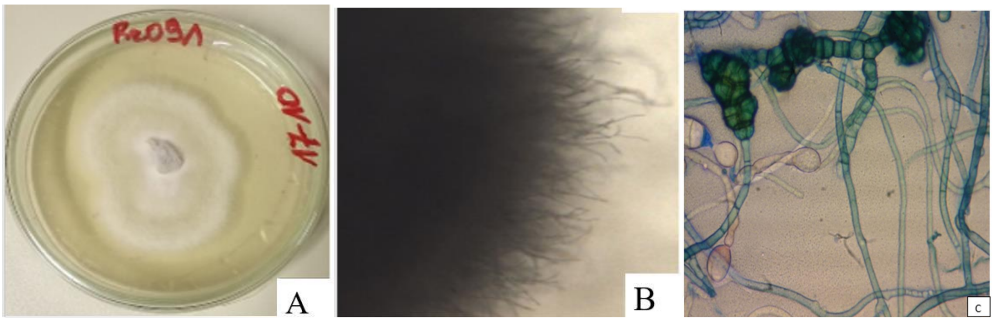

Plate 1 A-C. Different forms of *Alternaria alternata***.** A: pure mycelial colony; B: sporangia observed under a stereomicroscope on seeds; C: conidia and hyphae observed under a light microscope (×400).

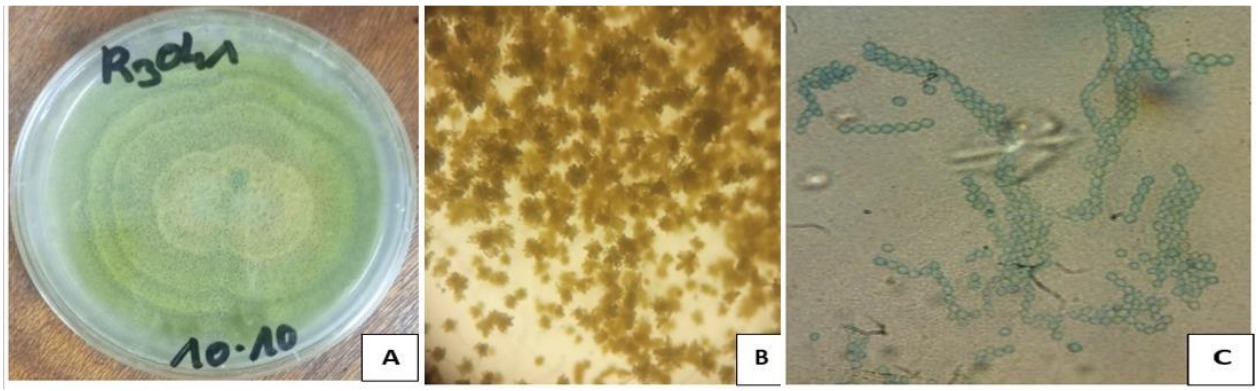

Plate 2 A-C. Morphological characteristics of *Aspergillus flavus*. A: pure mycelial colony; B: sporangia observed under a stereomicroscope; C: spores observed under a light microscope (×400).

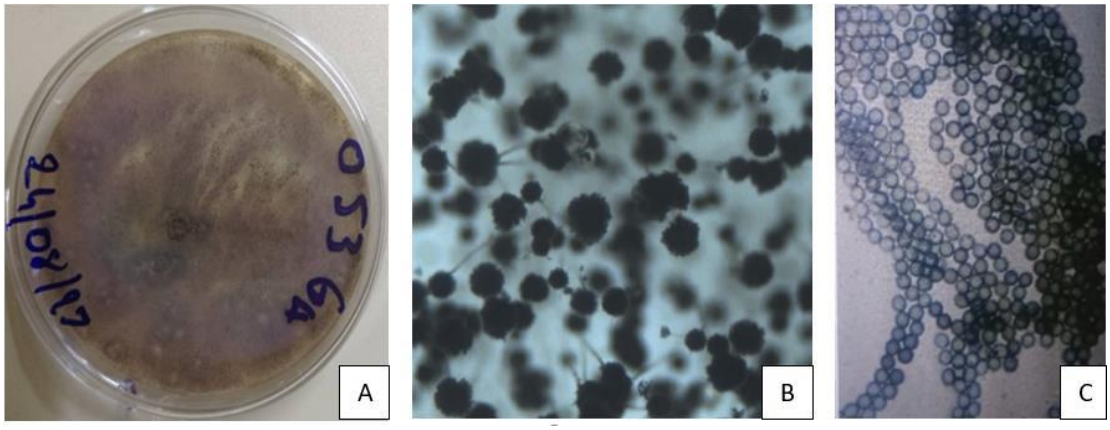

Plate 3 A-C. Morphological characteristics of *Aspergillus niger*. A: pure colony observed with the naked eye; B: sporangia observed under a stereomicroscope; C: spores observed under a light microscope (×600).

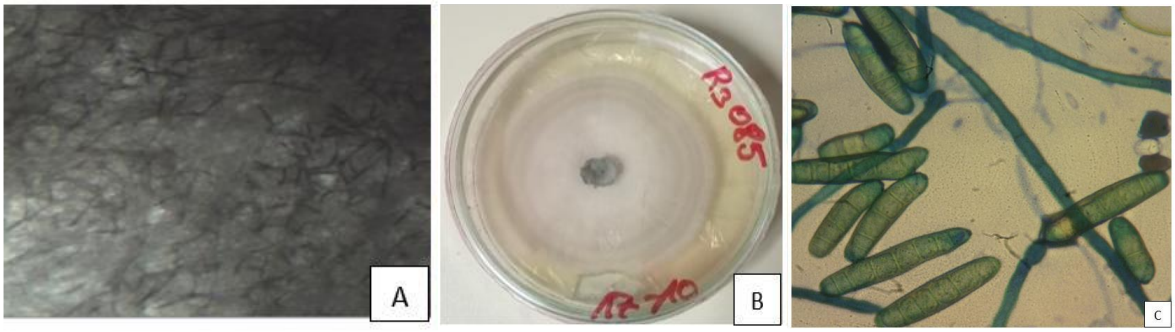

Plate 4 A-C. Morphological characteristics of *Bipolaris* sp**.** A: sporangia observed under a stereomicroscope on seeds; B: pure colony observed with the naked eye; C: conidia and hyphae observed under a light microscope (×400).

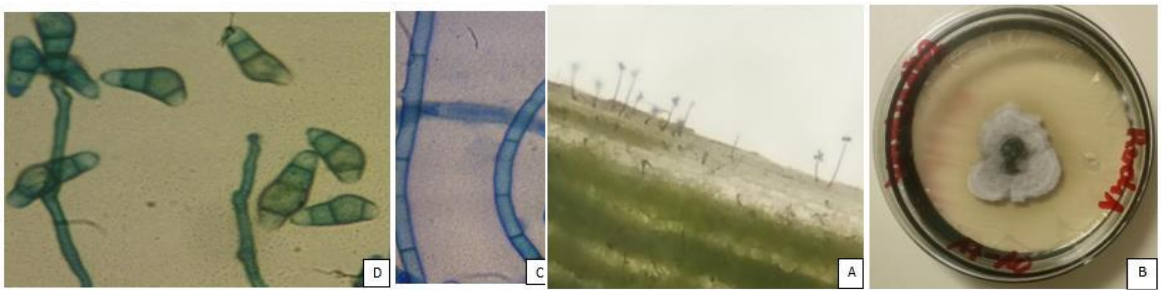

Plate 5 A-D. Morphological characteristics of *Curvularia lunata*. A: sporangia observed under a stereomicroscope on a leaf fragment; B: pure colony observed with the naked eye; C: hyphae observed under a light microscope (×400); D: conidia observed under a light microscope (×400).

Plate 6 A-D. Morphological characteristics of *Colletotrichum* sp. A: sporangia observed under a stereomicroscope on a leaf fragment; B: pure colony observed with the naked eye; C: hyphae observed under a light microscope (×400); D: conidia observed under a light microscope (×400).

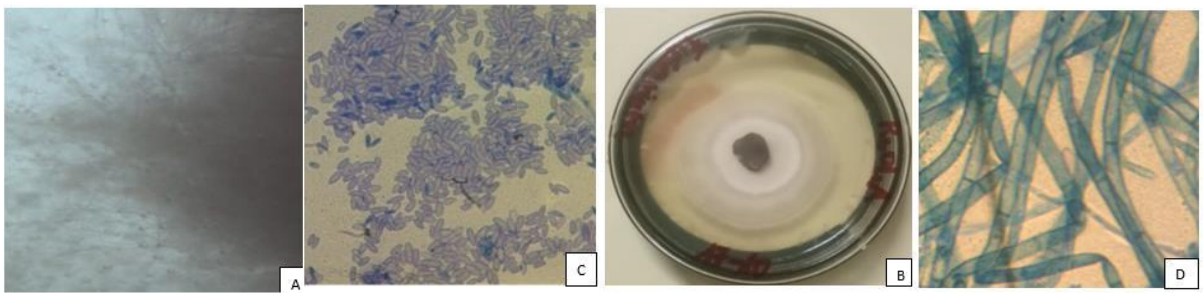

Plate 7 A-D. Morphological characteristics of *Fusarium* sp. A: sporangia observed under a stereomicroscope; B: pure colony observed with the naked eye; C: conidia observed under a light microscope (×400); D: hyphae observed under a light microscope (×400).

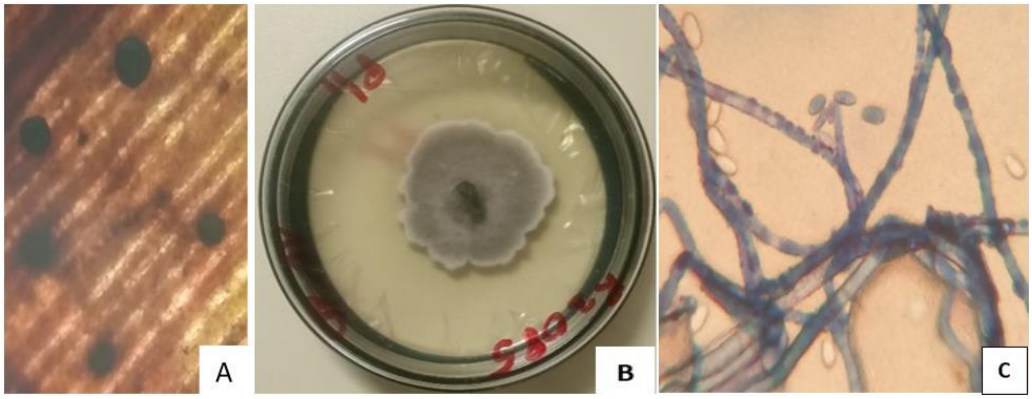

Plate 8 A-C. Morphological characteristics of *Phoma* sp. A: sporangia observed under a stereomicroscope on a leaf fragment; B: pure colony observed with the naked eye; C: conidia and hyphae observed under a light microscope (×400).

DISCUSSION

Microscopic and macroscopic analyses carried out after incubation of sweet-stem sorghum seeds and leaves revealed a diversity of pathogenic and/or saprophytic fungal species.

Identification results showed that most seed samples were infected by *Aspergillus flavus*, *Aspergillus niger*, *Curvularia lunata*, *Fusarium* sp., *Phoma* sp. and *Bipolaris* sp. Previous studies have reported the presence of these fungi in sorghum seed samples in several countries, notably in Burkina Faso (Zida *et al*., 2008a) and in the Philippines (Yago *et al*., 2011). Seeds may be contaminated in the field or during storage by mites, which act as vectors of fungal spores. This could explain the diversity of fungi observed on seeds. Nguyen (2007) reported that cereals infested by weevils generally harbour a high fungal population during storage.

Seed-borne pathogens belonging to the genera *Bipolaris*, *Alternaria*, *Fusarium* and *Curvularia* identified in this study were also reported by Berber *et al.* (2008). Similarly, studies by Bonzi (2013) identified pathogenic species such as *Fusarium moniliforme*, *Curvularia lunata* and *Phoma sorghina*, with a frequency of occurrence of 99.5%. in common grain sorghum seeds and other Poaceae. Furthermore, *Fusarium moniliforme*, *Curvularia lunata* and *Phoma sorghina* are responsible for seed rot, which subsequently leads to seedling damping-off (Chantereau *et al*., 2013), potentially explaining the lack of emergence observed in certain genotypes.

The presence of the genus *Aspergillus* on both seeds and leaves may also be explained by the application of manure in sweet-stem sorghum fields. Castegnaro and Pfohl-Leszkowicz (2002) showed that *Aspergillus* species commonly develop on decomposing organic matter, soil, compost and food products, and are more frequently associated with warm climatic regions.

The genus *Fusarium* showed the highest contamination rate on seeds. According to Burgess *et al.* (1994), it can persist in soil for several years in the form of chlamydospores or through hyphal development. Variations in contamination rates among species may be related to differences in humidity and temperature conditions. Anahosur (1992) reported that species frequency depends on environmental conditions favourable to fungal development.

During this study, several characteristic fungal symptoms were observed on leaves, including reddish lesions, small circular to elliptical leaf spots and necrotic leaves. Similar observations were reported by Chantereau *et al.* (2013) in Grenoble and by Prom *et al.* (2020) in Niger.

Fungal isolation from the foliar parts of sweet-stem sorghum yielded fungi associated with sweet-stem sorghum belonging to the genera *Aspergillus*, *Bipolaris*, *Curvularia*, *Phoma*, *Fusarium* and *Colletotrichum*. Except for *Colletotrichum*, these fungi were also detected on seeds and may have been transmitted to leaves. Previous studies have identified *Curvularia*, *Fusarium* and *Colletotrichum* on sorghum leaves (Zida *et al*., 2014). Several authors have reported the presence of *Colletotrichum graminicola* on sorghum leaves, associated with anthracnose disease (Chantereau *et al*., 2013). Moreover, certain phytopathogenic species of *Bipolaris* and *Fusarium* are known to cause leaf deterioration (Berber *et al*., 2008).

The presence of these fungi on leaves may be explained by their ability to develop under diverse climatic conditions. They may be disseminated via compost or manure, water, wind or seeds, and may originate from the soil, where

they are considered soil-borne fungi. These species cause similar lesions on the same plant organs. Chantereau *et al.* (2013) and Prom *et al.* (2020) also highlighted the role of seed-borne inoculum, wind and water in fungal dissemination.

Williams *et al.* (1978), Williams and Frederiksen (1986), Chantereau *et al.* (2013) and Prom *et al.* (2020) reported that *Colletotrichum graminicola* causes circular to elliptical lesions approximately 5 cm in diameter, with a straw-coloured centre and purple, red or tan margins depending on the variety.

Castegnaro and Pfohl-Leszkowicz (2002) showed that *Aspergillus* species commonly develop on decomposing organic matter, soil, compost and food products. Fungi such as *Aspergillus flavus*, *Bipolaris* sp., *Curvularia lunata*, *Fusarium* sp. and *Phoma* sp. were identified on both seeds and leaves. This may be explained by the fact that these fungi are soil-borne, systemic, and able to persist on both leaves and seeds, as well as on plant debris and infected crop residues. According to Emechebe and McDonald (1979) and Emechebe (1981b), pathogens survive during the dry season through acervuli in contaminated seeds, soil and plant debris, which constitute primary sources of inoculum. Soil therefore acts as a reservoir of inoculum for these pathogenic agents.

CONCLUSION

This study revealed a high diversity of fungi associated with sweet-stem sorghum seeds and leaves in Burkina Faso, with *Fusarium* sp., *Curvularia lunata* and *Phoma* sp. being among the most frequently isolated taxa. The findings suggest a possible epidemiological relationship between seed- and leaf-associated fungi and provide baseline information for future studies on disease management, molecular identification and breeding programs.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHORS' CONTRIBUTION

Ganou K. Jennifer D-Gracias, the primary investigator and interested party in the work, set up and monitored the trials, overseeing data collection and processing. Dabiré Kounbo, assisted with the macroscopic and microscopic analyses in the laboratory and the supervision on crops. Bakiono Bénovana, assisted with data analyses and writing. Elise Sanon contributed to the reading and correction of the manuscript, ensuring accuracy in both form and content.

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