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Identification and Characterization of Fungi Species Associated of Pearl Millet (*Pennisetum glaucum* R. Br.) to Seed, Leaves and Physiological Maturity Stage in Burkina Faso

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A B S T R A C T

Crops seed are sometimes susceptible to parasitic diseases related storage conditions. In response to this situation, for all material vegetal intended for introduction into a program breeding, it is important to characterized fungal diseases associated. Pearl millet from ICRISAT-Niger gene bank and Biosciences laboratory in Burkina Faso. A block Fisher with 3 repetitions is been carried in experimental station in Burkina Faso during the period July to October 2024, led to the Phytopathology Laboratory of Joseph KI-ZERBO University. Mathur & Kongsdal (2003) methods have been used to characterized the different fungi. A total of 14 fungal isolates were obtained for all countries with *Curvularia sp*. as specie predominating. Furthermore, 10 fugal isolates on seeds, 10 isolates fungal on leaves, and 8 isolates fungal on mush seeds. However, significant differences were been identified between countries. *Aspergillus flavus, Aspergillus niger, Bipolaris* sp., *Curvularia* sp., *Fusarium sp.4, Fusarium* sp.5, and *Phoma sorghina* are species common to all three countries. As for the other species, *Fusarium* sp.3, *Fusarium* sp.6, *Penicillium* sp., and *Rhizopus sp*. were found alone in pearl millet accessions from Togo. The species *Fusarium* sp.2 was found alone in pearl millet from Burkina Faso and India. *Curvularia* sp. (84.84%) and *Bipolaris* sp. (37.18%) found on the seeds were also found on the leaves and mushy seeds from these three countries. This study reported that *Curvularia* is the main disease dominant in collection.

Introduction

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is one of the staple important cereals in sub-Saharan Africa. In world, pearl millet ranks as the sixth most widely cultivated, following wheat barley maize, rice, and sorghum (Mbodj *et al.,* 2023). Global production increases from 28.33 million tons in 2021 to 30.80 million tons in 2023 (FAO, 2023). The principal pearl millet producing countries are Burkina Faso, India, Niger, Nigeria, Mali, Senegal and Tchad. Sub-Saharan Africa contributes around of 46% in pearl millet world production (Mbodj *et al.,* 2023) In Burkina Faso, pearl millet ranks the second most producing after sorghum

(Bamba *et al.,* 2016). Pearl millet is distinguished others cereals due remarkable adaptability to diverse climatic conditions and soil types. Khajuria *et al.,* (2018) reveals, pearl millet growing in hot regions with temperature of 20-30°c unsuitable for crops like maize and sorghum. In recent times, pearl millet represents among promising agricultural products due to their nutritional properties (Adebiyi *et al.,* 2017 and 2018). Bashir *et al.,* (2014) showed a wide range of nutriments of pearl millet evaluated for Fe (37.4-45.4 mg kg−1) and Zn (35.5-38.8 mg kg−1).

Despite its adaptability to difficult conditions, pearl millet is now facing challenges related to biotic stress, mainly diseases caused by phytopathogens. According to Twarużek *et al.,* (2021), Penicillium, Aspergillus, Cladosporium, Fusarium, Rhizopus, and Mucor are the common mould genera contaminating extruded samples during storage. Others studies revealed that sorghum, maize and pearl millet are subject to fungal diseases such as stalk rots, ear rots and grain mold, which may cause great economic losses (Vismer *et al.,* 2019). These fungal spread through seeds, wind, and rainwater splashes (Boua, 2022). The studies of Yuvashree *et al.,* (2025) revealed in India a total of 14 pure fungal isolates of Fusarium species associated with sheath and stem blight on pearl millet. These results reported the new disease, sheath and stem blight on pearl millet which was caused by *Fusarium pseudonygamai* in India. It is important to mention the impact of fungi reduce both the yield and quality of pearl millet crops. For growing pearl millet enormous benefit in production, it is fundamental for developing effective management strategies to mitigate the impact of these diseases on pearl millet crops in Burkina Faso. Therefore, the present study was designed (i) to identify and (ii) to characterized the fungi on three stages of pearl millet.

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 12˚1' west longitude and 12˚24' north latitude during the raining season 2023-2024. Block Fisher with three repetitions has been used. 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 4 to 6.4, and traces of mineral salts.

Samples and sampling procedure

Between September 27, 2024, and October 15, 2024, diseases samples were collected on leaves and grain in crops field and delivered to Laboratory “Phytopathologie et Mycologie Tropicale (PMTrop)” of University Joseph KI-ZERBO. A total of thirty (30) genotypes from ICRISAT-Niger genes bank and laboratory Biosciences in Burkina Faso is used (Table 1). Leaves samples were collected two months after sowing the emergence of diseased. The grains were collected coincidence with the maturity physiological of panicles.

Isolation of fungal pathogen

Isolation is carried out from leaves showing the characteristic symptoms of a fungal disease and out the seeds. The standard blotter method and SCDA medium described by Mathur and Kongsdal (2003) with slight modifications, was used to detect fungi growth from seeds and leaves in the presence of humidity.

Indeed, leaves and seeds are first cleaned with simple water to remove sand and other debris on the leaves and seeds. They are then disinfected by soaking in 3% bleach for 2-3 minutes, rinsed in distilled water to remove traces of bleach, and dried on lotus paper. Disinfection is carried out to remove exogenous microflora.

The samples were incubated under alternating 12 hours of light and 12 hours of darkness at 25°C. After three days of incubation, each fungal colony was transferred individually to a new box containing SDCA. Based on macroscopic observation, the various colonies were collected and inoculated onto agar until pure colonies were obtained. Each purified colony has been observed at a magnifying glass and microscopic observation after staining with methyl blue. The features were compared with identification keys of Mathur & Kongsdal (2003), Blancard *et al.,* (2009), Crous *et al.,* (2014), and Jones *et al.,* (2014).

Statistical analysis

Excel software was used to determine the infestation rates on leaves and grains seed. Correlation tests were realized between isolates found on leaves, seeds and

mushy seeds with R 4.5.1 software.

Results and Discussion

Fungal occurrence distribution on seeds

A total of ten pure fungal isolates were obtained on pearl millet seeds (Table 1). Morphological examination revealed five genera characteristic of *Curvularia* sp., *Aspergillus* sp. *Fusarium* sp. *Bipolaris* sp. and *Phoma* sp. on pearl millet providing in Burkina Faso.

In Togo, nine species of fungal separated into six genera, *Curvularia* sp., *Phoma* sp., *Bipolaris sp*., *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp.), although five genera, *Curvularia s*p., *Aspergillus* sp., *Fusarium* sp., *Bipolaris* sp. and *Phoma* sp. in India have been revealed. *Curvularia* sp. was specie dominant on pearl millet.

Fungal occurrence distribution on leaves

A total of seven pure fungal isolates were obtained on pearl millet leaves (Table 2). Morphological examination revealed five genera characteristic of *Curvularia* sp., *Aspergillus* sp. *Fusarium* sp. *Bipolaris* sp. and *Phoma* sp. on pearl millet providing in Burkina Faso. Pathogens such as *Curvularia* sp., *Phoma* sp., *Bipolaris* sp., *Aspergillus* sp., *Fusarium* sp., *Rhizopus* sp. have been identified in Togo. However, four genera such as *Curvularia* sp., *Bipolaris* sp., *Fusarium* sp. and *Aspergillus* sp. have been identified on pearl millet India. Among the fungal, *Curvularia* sp. was specie dominant.

Fungal occurrence distribution on mushy seeds

A total of seven pure fungal isolates were obtained on pearl millet leaves (Table 3). Five fungal species separated into four genera such as *Curvularia* sp., *Bipolaris* sp., *Fusarium* sp. and *Phoma* sp. were identified on mushy seeds from Burkina Faso. Morphological examination revealed six fungal species corresponding to five genera characteristic of *Curvularia* sp., *Aspergillus* sp. *Fusarium* sp. *Rhizopus* sp. and *Bipolaris* sp. on pearl millet providing in Togo. Pathogens such as *Curvularia* sp., *Bipolaris* sp., *Aspergillus* sp., *Fusarium* sp., *Phoma* sp. have been identified.

Relationship between infestation rates of organs

The analysis reveals a relationship between the infestation rates of seeds, leaves, and mushy seeds (Figure 1). Analysis of the correlation circle shows a positive correlation between seeds, leaves, and mushy seeds. Fungal species are generally negatively correlated with the type of infection (Figure 2).

Diversity of different forms of fungal parasitic

Morphological examination in vitro revealed the presence of various species affecting pearl millet (Photos 1 to 14). Mainly diseases caused by *Bipolaris* sp. and *Curvularia* sp. revealed less forms on pearl millet (Photos 1 and 2). Numerous Fusarium species showed the forms characteristic of *Fusarium* sp. 1 *Fusarium* sp. 2, *Fusarium* sp. 3, *Fusarium* sp. 4, *Fusarium* sp. 5, *Fusarium* sp. 6, and *Fusarium* sp. 7 (Photos, 3, 4, 5, 6, 7, 8 and 9). Two species, characteristic of *Aspergillus flavus* and *Aspergillus niger* have been identified with different forms on seeds and mushy seeds (Photos 11 and 12) and characterized the pathogen responsible for seeds and leaves in pearl millet of genotypes studied. Various species of *Rhizopus* sp., *Phoma Sorghina* and *Penicillium* sp. presented different features on pearl millet (Photos 10, 13 and 14).

Pearl millet faces diversity diseases challenges, primarily from diseases caused by various phytopathogens. In this study, a total of seven fungal genera were identified, *Bipolaris, Curvularia, Fusarium, Penicillium, Phoma, Aspergillus, and Rhizopus*. The emergence of these diseases on seeds and leaves impact pearl millet in Burkina Faso. Similar results were found on sorghum in Burkina Faso by Ganou (2023), as major genera, particularly *Aspergillus, Bipolaris, Curvularia, Fusarium,* and *Phoma*. However, *Alternaria* and *Colletotrichum* is associated with sorghum (Ganou, 2023). Globally, various species of Fusarium (Nectriaceae, Hypocreales, Ascomycetes) have been reported to cause blight diseases independently (Yuvashree *et al.,* 2025). In India, a total of 14 pure fungal isolates was obtained and designated as FPM1 to FPM14 (Yuvashree *et al.,* 2025).

Table.1a Profile of genotypes material

Origin	Code	Number
Togo (ICRISAT-Niger)	ISP 230, ISP 283, ISP 211, ISP 167, ISP 202, ISP 227, ISP 282, ISP 219, ISP 292, ISP 277, ISP 184, ISP 181, ISP 313, ISP 222.	14
Burkina Faso (Biosciences Laboratory)	MCN-20, MC13-14, MS11-1, MCN2, MO10-3	05
Inde (ICRISAT-Niger)	ISP1985, ISP 1819, ISP 1906, ISP 1894, ISP 2004, ISP 1963, ISP 1807, ISP 1991, ISP 1936, ISP 1924, ISP 1900.	11

Table.1b Frequency distribution of the isolates on seeds per country

Country	Genera	Species	Isolates number	Pourcentage (%)
Burkina Faso	*Curvularia*	*Curvularia* sp.	5	100
	Aspergillus	*Aspergillus flavus*	3	60
	Fusarium	*Fusarium* sp.5	2	40
	Bipolaris	*Bipolaris* sp.	1	20
	Phoma	*Phoma sorghina*	1	20
Togo	*Curvularia*	*Curvularia* sp.	14	100
	Phoma	*Phoma sorghina*	11	78.57
	Bipolaris	*Bipolaris* sp.	9	64.28
	Aspergillus	*Aspergillus flavus*	5	35.71
	Fusarium	*Fusarium* sp.5	2	14.28
	Fusarium	*Fusarium* sp. 3	1	7.14
	Fusarium	*Fusarium* sp.6	1	7.14
	Fusarium	*Fusarium* sp.7	1	7.14
	Penicillium	*Penicillium* sp.	1	7.14
India	*Curvularia*	*Curvularia* sp.	6	54.54
	Aspergillus	*Aspergillus flavus*	5	45.45
	Fusarium	*Fusarium* sp.5	5	45.45
	Bipolaris	*Bipolaris* sp.	3	27.27
	Fusarium	*Fusarium* sp.7	2	18.18
	Fusarium	*Fusarium* sp.1	1	9.09
	Fusarium	Fusarium sp.2	1	9.09
	Phoma	*Phoma sorghina*	1	9.09

Table.2 Frequency distribution of the isolates on leaves per country

Country	Genera	Species	Isolates number	Pourcentage (%)
Burkina Faso	*Curvularia*	*Curvularia* sp.	4	80
	Phoma	*Phoma sorghina*	2	40
	Aspergillus	*Aspergillus flavus*	2	40
	Bipolaris	*Bipolaris* sp.	2	40
	Aspergillus	*Aspergillus niger*	1	20
	Fusarium	*Fusarium* sp. 2	1	20
Togo	*Curvularia*	*Curvularia* sp.	7	87,5
	Bipolaris	*Bipolaris* sp.	5	62.5
	Aspergillus	*Aspergillus flavus*	3	37.5
	Rhizopus	*Rhizopus* sp.	2	25
	Aspergillus	*Aspergillus niger*	1	12.5
	Fusarium	*Fusarium* sp.1	1	12.5
	Fusarium	*Fusarium* sp.3	1	12.5
	Phoma	*Phoma sorghina*	1	12.5
India	*Curvularia*	*Curvularia* sp.	4	100
	Bipolaris	*Bipolaris* sp.	3	75
	Fusarium	*Fusarium* sp.1	3	75
	Aspergillus	*Aspergillus niger*	1	25
	Fusarium	*Fusarium* sp.4	1	25

Table.3 Frequency distribution of the isolates on mushy seeds per country

Country	Genera	Species	Isolates number	Percentage (%)
Burkina Faso	***Curvularia***	*Curvularia* sp.	2	66,66
	Bipolaris	*Bipolaris* sp.	1	33,33
	Fusarium	*Fusarium* sp.*2*	1	33,33
	Fusarium	*Fusarium* sp.4	1	33,33
	Phoma	*Phoma sorghina*	1	33,33
Togo	***Bipolaris***	*Bipolaris* sp.	5	62,5
	Curvularia	*Curvularia* sp.	4	50
	Fusarium	*Fusarium* sp.1	4	50
	Aspergillus	*Aspergillus flavus*	2	25
	Fusarium	*Fusarium* sp. 4	2	25
	Rhizopus	*Rhizopus* sp.	1	12,5
India	***Bipolaris***	*Bipolaris* sp.	2	40
	Curvularia	*Curvularia* sp.	2	40
	Fusarium	*Fusarium* sp. 1	1	20
	Fusarium	*Fusarium* sp. 2	1	20
	Phoma	*Phoma sorghina*	1	20
	Aspergillus	*Aspergillus flavus*	1	20

Figure.1 Correlation matrix between the infection rate of seeds, leaves and mushy seeds

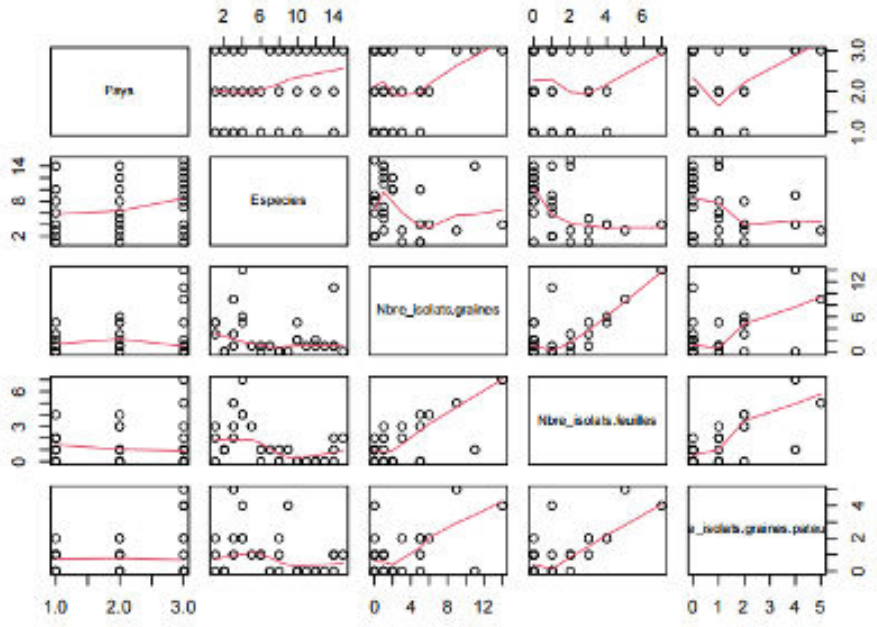
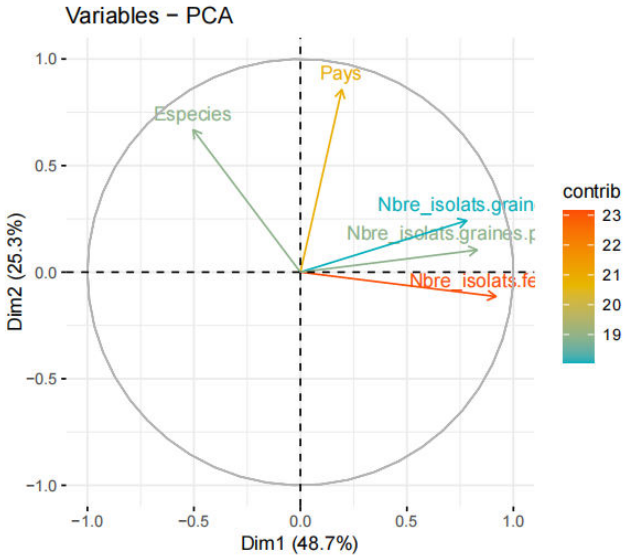
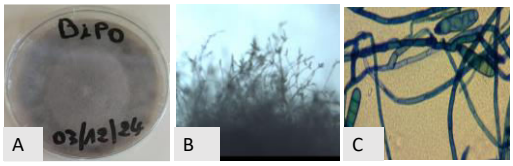
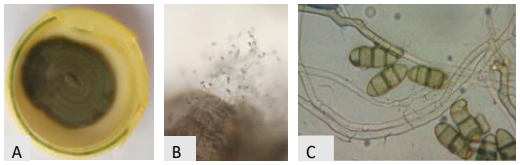


Figure.2 Correlation circle from PCA of fungal infections on millet showing the distribution of fungal isolates according to infected organs, countries, and species

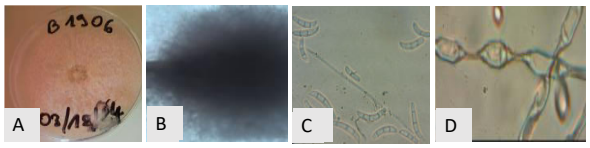




Photos.1 *Bipolaris* sp. different forms

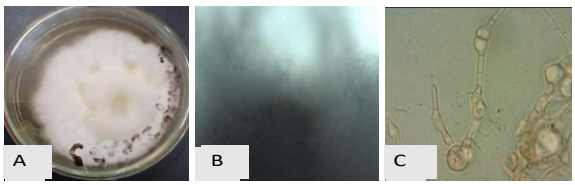


Photos.2 *Curvularia* sp. different forms

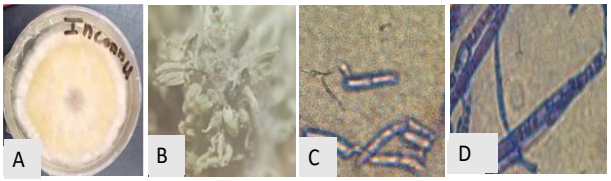


Photos.3 *Fusarium* sp.1 different forms

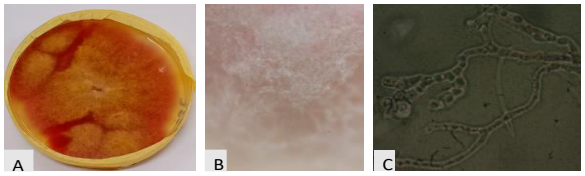
Photos.4 *Fusarium sp*. 2 different forms



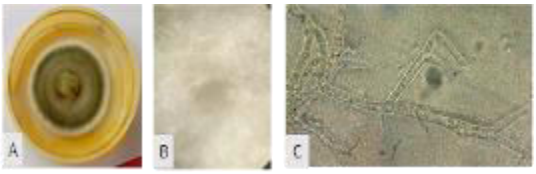
Photos.5 *Fusarium sp*. 3 different forms



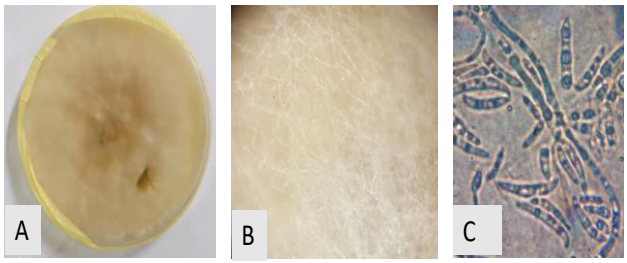
Photos.6 *Fusarium sp*.4 different forms



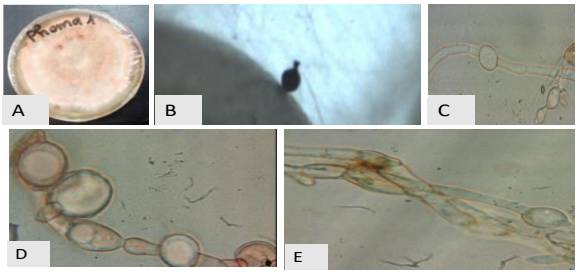
Photos.7 *Fusarium* sp.5 different



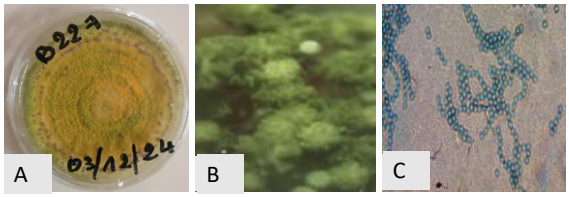
Photos.8 *Fusarium* sp.6 different forms



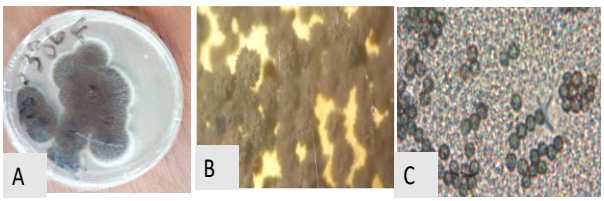
Photos.9 *Fusarium* sp.7 different forms



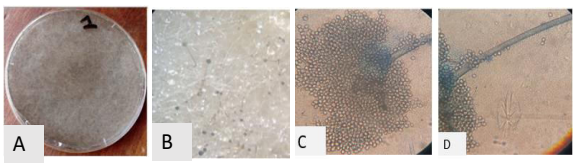
Photos.10 *Phoma Sorghina* different forms



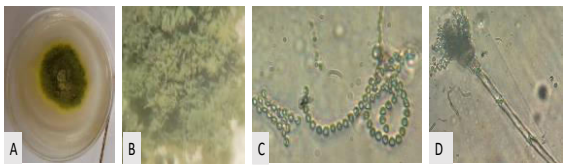
Photos.11 *Aspergillus flavus different forms*



Photos.12 *Aspergillus niger different forms*



Photos.13 *Rhizopus* sp. different forms



Photos.14 *Penicillium* sp. different forms

Species as *Bipolaris, Curvularia, Fusarium* and *Phoma* have been found, particularly in grains seeds. Bonzi (2013) identified the same species in *Sorghum bicolor* grains seeds. *Curvularia* frequency is higher in seeds for

all countries. Several studies revealed that *Curvularia* is common genus among fungal species associated with pearl millet pathogen (Odeph *et al.,* 2021). Leaves diseases are associated with *Bipolaris, Curvularia*, *Fusarium*, and *Phoma,* although *Bipolaris, Curvularia, and Fusarium* is common to all countries. Similarly, there are known similitude in the *Sorghum bicolor* that can be found from diseases leaves (Boua, 2022). Furthermore, *Curvularia* has been identified as an emergence agent as reported by Khatal *et al.,* (2019). There are two possible hypotheses regarding *Curvularia* specie. One is that particularly result *Curvularia sp.* can be causal agent of grains and leaves or *Curvularia sp*. has a strong ability to adapt to hot climatic conditions. Others studies in China showed that post-flowering stalk rot in maize is associated with *Fusarium acutatum*, *Fusarium verticillioides*, and *Fusarium andiyazi* (Yu *et al.,* 2017). In this study, there is a continuity in the infestation process justifying the correlation of grains seeds, leaves, and mushy seeds diseases.

Many species have been identified in India and Togo genotypes showing, although *Phoma* alone is absent in Togo on mush seeds. According to Twarużek *et al.,* (2021), *Penicillium, Aspergillus*, *Cladosporium*, *Fusarium*, *Rhizopus*, and *Mucor* are the common mould genera contaminating extruded samples during storage. Environmental conditions such temperature, time, and relative humidity are factors influencing the growth of phytopathogens.

In conclusion, this study has particularly identified and characterized the fungal responsible for seeds and leaves in pearl millet with genotypes providing of ICRISAT-Niger gene bank and Biosciences laboratory. A total of fourteen species were identified, with *Curvularia* sp. predominating. Among these fungal species, *Aspergillus flavus, Aspergillus niger, Bipolaris* sp., *Curvularia* sp., *Fusarium* sp.4, *Fusarium* sp.5, and *Phoma sorghina* where the fungal species are the common genera in Burkina Faso, Togo, and India.

In addition, *Fusarium* sp.3*, Fusarium* sp.6, *Penicillium* sp., and *Rhizopus* sp. were found alone in Togo. As these diseases have been identified, it is essential to conduct comprehensive studies on the morphological and molecular characteristics to identify and differentiate its.

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Author Contributions

Bougma Lardia Ali: Conceived the computational framework and analysed and designed the model and wrote the manuscript. Acquisition the material vegetal. Kounbo Dabire: Assistant in laboratory, analysed and interpretation and validated data primary. Bado Adèle: Made laboratory work and revised the paper. Elise Sanon: Conceived the original idea and validated data, Funding Acquisition.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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