Characterization of *Fusarium* spp associated with Solanaceous seeds in Burkina Faso

ABSTRACT

The Solanaceae family, comprising tomato, pepper, chili, and eggplant, plays a pivotal role in agriculture in Burkina Faso. However, these crops face significant biotic challenges, notably from fungi of the *Fusarium* genus, which are responsible for severe diseases and yield losses that can reach up to 80%. Beyond their devastating effects on both the quantity and quality of harvests, these pathogens also produce mycotoxins that compromise food safety. This study aims to enhance the production of Solanaceous crops by characterizing the *Fusarium* fungal agents associated with their seeds in Burkina Faso. A sanitary analysis of 400 seeds per seed type (both disinfected and non-disinfected) identified 11 fungal genera, including *Fusarium*, *Colletotrichum*, *Curvularia*, *Alternaria*, *Aspergillus*, *Didymella*, *Epicoccum*, *Melanospora*, *Spirodactylon*, *Rhizopus*, and *Stachybotrys*, with varying proportions depending on the genus. Among these, *Fusarium* spp. predominated, even after disinfection, though fungal diversity and contamination rates were lower. Microscopic and molecular analyses revealed several *Fusarium* species, including *F. solani*, *F. proliferatum*, *F. incarnatum*, and *F. equiseti*. Pathogenicity tests showed a high incidence of Fusarium wilt on seedlings, with variability based on the isolates and crops. Specifically, isolates T2F, P3F, and Pi1F exhibited particularly aggressive behavior on tomato, pepper, and chili, respectively. These findings underscore the urgent need for effective seed management to limit the spread of these pathogens and safeguard agricultural productivity in Burkina Faso.

Keywords: Solanaceae, Seeds, Sanitary analysis, Pathogenicity, Virulence, Burkina Faso

1. INTRODUCTION

Market gardening, introduced to Burkina Faso between 1920 and 1930 by missionaries and government officials(1), has since become a central component of the country's agricultural sector. Among the market crops, Solanaceae species such as tomato (*Solanum lycopersicum*), pepper (*Capsicum annuum*), chili (*Capsicum frutescens*), and eggplant (*Solanum melongena*) are particularly significant for their nutritional, economic, and cultural contributions.

Tomato is a cornerstone crop, providing substantial income for farmers and playing a pivotal role in reducing poverty and seasonal unemployment in various regions (2,3). Beyond its economic value, it is crucial for food security, offering a rich source of carbohydrates, proteins, vitamins, and minerals. Its regular consumption is associated with improved public health, reducing the risks of cardiovascular diseases, cancers, and diabetes (2).

Chilies, especially *Capsicum annuum*, can yield up to 4.8 tons per hectare under optimal conditions (4). Their cultivation, well-suited to the local climate, generates significant income and contributes to alleviating rural poverty (5). Rich in antioxidants and phenolic compounds, chilies enhance the nutritional value of local diets and play a key role in food security (6). Additionally, their production creates seasonal employment opportunities, particularly for vulnerable populations in both rural and urban areas (7).
Pepper is another lucrative crop that bolsters farmers' financial stability while diversifying the local diet (2).
Eggplant plays an essential role in combating malnutrition, especially among women and children, due to its high content of vitamins and minerals (8). This crop contributes to the economic stability of rural communities and supports agricultural biodiversity, enhancing food security and resilience to climate change (9). Its genetic diversity allows for better adaptation to local conditions, supporting sustainable production (10).
The Solanaceous crops, valued for their nutritional and economic benefits, serve as a strategic pillar for the socio-economic development of Burkina Faso. However, their production faces both abiotic and biotic challenges, particularly diseases caused by *Fusarium* fungi. These pathogens induce vascular wilt, root and collar rot, and produce mycotoxins, which compromise both the quality and safety of agricultural products (11). Seven major *Fusarium* species have been identified in Burkina Faso, including *F. oxysporum*, *F. solani*, and *F. proliferatum* (11,12). These fungi are often transmitted through soil, seeds, and environmental conditions, and can also be introduced via the importation of contaminated seeds, which exacerbates the risks to local production systems (13).
In this context, early detection of pathogens on seeds and the management of their spread are essential to ensuring the sustainability of market gardening production. The current study, titled "Characterization of *Fusarium* spp. associated with Solanaceous Seeds in Burkina Faso," aims to inventory the fungal species linked to Solanaceous seeds, characterize the *Fusarium* spp. found on these seeds, and evaluate the pathogenicity of these fungi on seeds of the four most widely cultivated Solanaceous crops in Burkina Faso.

2. MATERIAL AND METHODS

2.1 Seed Sampling

The plant material studied includes the main Solanaceae varieties cultivated in Burkina Faso, sourced from two origins: improved seeds from commercial companies and local farmer seeds collected from local producers. This selection reflects the common agricultural practices, which vary between seed reuse and the purchase of improved varieties. The varieties studied are:

- **Tomato Cobra F1**: an early maturing variety, tolerant to TYLCV and bacterial wilt.
- **Pepper Simbad F1**: a very early variety, resistant to multiple pathogens.
- **Yellow Chili from Burkina**: a late maturing, highly pungent variety with bright yellow fruits.
- **Eggplant Safari Cobra F1**: a long-cycle variety with purple fruits and a strong flavor.
- **Farmer seeds**, collected from local market gardeners.

2.2 Sanitary seed analysis

A total of 400 seeds per seed type were analyzed, divided into two groups: 200 disinfected seeds (treated with 70% alcohol, 1% bleach, and rinsed with sterile water) and 200 non-

disinfected seeds. These seeds were incubated in sterile Petri dishes at 25°C, with regular observations over one month to monitor fungal contamination. Fungal colonies were analyzed under a microscope to examine their morphological structures. Species identification was carried out using the key from (14), and the data were recorded on a registration sheet. *Fusarium* spp. colonies were isolated and transferred onto PDA medium, then incubated at 27°C with 12 hours of UV light. Successive subcultures were performed to obtain pure cultures for further analysis.

2.3 Microscopic Characterization

To characterize the fungi microscopically, a conidial suspension was prepared by adding 10 ml of sterile distilled water to a seven-day-old culture. A drop of this suspension was placed on a slide for observation under an optical microscope. The microscopic analysis was performed using a system consisting of a microscope connected to a camera and computer. Using Image Focus Plus V2 software, the length and width of 50 conidia per isolate were measured, and the number of septa was recorded. These parameters provided essential information for species identification.

2.4 Molecular Characterization

DNA extraction was carried out using a modified CTAB method (Permingeat et al., 1998). Monospore isolates of *Fusarium* spp*.* grown on PDA were sampled to obtain 40 mg of mycelium. The mycelium was homogenized with 600 µl of CTAB solution and ground at 30 Hz for 2 minutes. After incubation at 65°C for 15 minutes, 1 mL of chloroform-isoamyl alcohol was added, followed by centrifugation at 14,000 rpm. DNA was precipitated with 450 µl of cold isopropanol, washed twice with 70% ethanol, dried, and resuspended in 100 µl of sterile distilled water before being stored at -20°C. The extracted DNA was quantified and amplified by PCR. Two specific primer pairs targeted *F. solani* and *F. oxysporum*, while a universal primer pair (ITS1-F/ITS4) was used to assess genetic diversity (Table 1). Each PCR reaction (25 µl) included 5 µl of DNA, 2.5 µl of each primer (10 mM), 5 µl of Fire Hot Mix, and 10 µl of distilled water. Negative controls were included to prevent contamination. Amplification was performed on a thermocycler with programs tailored for the specific primers.

The PCR products were separated on a 1% agarose gel prepared with TAE buffer, containing 0.5 µg/ml of BET and 3 µl of GelRed™. After solidification, 4 µl of each amplified product was loaded into the wells, and electrophoresis was conducted at 100 V for 30 minutes. A 100 bp molecular marker was used to estimate the size of the DNA fragments. DNA bands were visualized using a gel imager. Isolates showing amplicons of the expected size (targeted by the ITS1-F/ITS4 primers) were sent to Genewiz for sequencing. The obtained sequences were analyzed and assembled into consensus sequences using BioEdit software, and then compared with the NCBI database via BLAST for identification.

Table 1. Characteristics of the primers used

Primers	Codes	Sequences	Authors	Hybridation temperature
Fusarium solani	TER-Fs4r	GGC-GTC-TGT-TGA-TTG-TTA-G	**Moine, 2013**	58°C
	FO-F	AGG-GTC-ATG-CTC-TGA-AGC-AGA-G		58°C
Fusarium oxysporum	FO-R	GAA-CTG-TTT-AAT-GTT-CTG-AAT-TTC-A	**Yada *et al*., 2017**	58°C
	FO-F	AGG-GTC-ATG-CTC-TGA-AGC-AGA-G		58°C

ITS	ITS1-F	CTTGGTCATTTAGAGGAAGTAA	**Gardes *et al*., 1993 ; White *et al*., 1990**	52°C
	ITS4	TCCTCCGCTTATTGATATGC		52°C

2.5 Pathogenicity Testing

Four *Fusarium* spp. isolates (T2F, Pi1F, P3F, and A5F), identified through molecular assays and originating from tomato, pepper, chili, and eggplant seeds, were selected for pathogenicity testing. The seeds were disinfected with 95% ethanol for 1 minute, rinsed with sterile water, and subsequently exposed to 7-day-old mycelium for 12 hours at room temperature. The control seeds were incubated on PDA medium without mycelium. After the incubation period, the seeds were sown in alveolar plates filled with sterilized potting soil, and regular watering was performed every two days with distilled water. The experiment included 12 repetitions for each seed type (tomato, pepper, and chili), resulting in a total of three plates with 72 wells each.

2.6 Data Analysis

Three primary parameters were assessed from the collected data :

- **Fusarium Incidence:** Measured on the 40th day after sowing (DAS) by counting plants exhibiting necrosis, chlorosis, or mortality. The average incidence for each treatment group was determined using the formula outlined by Cooke *et al.* (2006) :

$$\boldsymbol{I} = \sum (\frac{n}{N}).100$$

  I represents the incidence, **n** is the number of diseased plants, and **N** is the total number of plants observed (N = 12).

- **Fusarium Severity:** Evaluated on the 40th DAS using the five-class scale by (Abawi and Oastor-Corales, 1990):
  1 : No visible symptoms.
  3 : 1 to 3 leaves (10% of the foliage) wilted and chlorotic.
  5 : About 25% of the leaves and branches wilted and chlorotic.
  7 : About 50% of the leaves and branches wilted and chlorotic.
  9 : About 75% of the leaves and branches wilted, chlorotic, or defoliated, with possible plant death.

The severity index (**Is**) was calculated according to the formula by (16):

$$\boldsymbol{IS} = \sum (\frac{Xi.ni}{N.Z}).100$$

Is is the severity index; **Xi** is the severity of class i; **ni** is the number of plants affected in this class; N is the total number of plants observed (N = 12), and **Z** is the maximum severity class (Z = 9).

The collected data were entered and organized using Microsoft Excel 2019, which was also used to generate graphs. Statistical analyses, including analysis of variance (ANOVA) and mean comparisons, were conducted using XLSTAT software (version 2016), applying Fisher's test at a 5% significance level.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1. Fungal identification on ieeds from both origins analyzed

Fusarium, *Colletotrichum*, *Curvularia*, *Alternaria*, *Aspergillus*, *Didymella*, *Epicoccum*, *Melanospora*, *Spirodactylon*, *Rhizopus*, and *Stachybotrys* (Figure 1). Prior to disinfection, the farmer-saved seeds from the various crops exhibited high levels of fungal contamination, predominantly caused by *Fusarium* spp. along with species specific to each crop. Tomato seeds were infected exclusively by *Fusarium* spp. (24%), which remained the dominant fungus even after disinfection, despite the emergence of *Colletotrichum* spp. and *Curvularia* spp. In chili seeds, *Fusarium* spp. (21%) predominated before disinfection, followed by *Epicoccum* spp. (4%), with only *Fusarium* spp. (6%) and *Melanospora* spp. (12%) persisting after disinfection. Pepper seeds showed a high incidence of *Fusarium* spp. (41%) and a minor presence of *Aspergillus flavus* (2.5%) before disinfection. After treatment, *Fusarium* spp. (6.5%) and *Spirodactylon* spp. (20%) were the predominant fungi. Finally, eggplant seeds were primarily contaminated by *Aspergillus niger* (14.5%) and *Fusarium* spp. (12.5%), but post-disinfection, the contamination levels dropped to 2.87% for *Fusarium* spp. and 3.5% for *Spirodactylon* spp. These findings demonstrate that disinfection significantly reduces both the fungal diversity and infestation levels, although *Fusarium* spp. remains the most prevalent species.

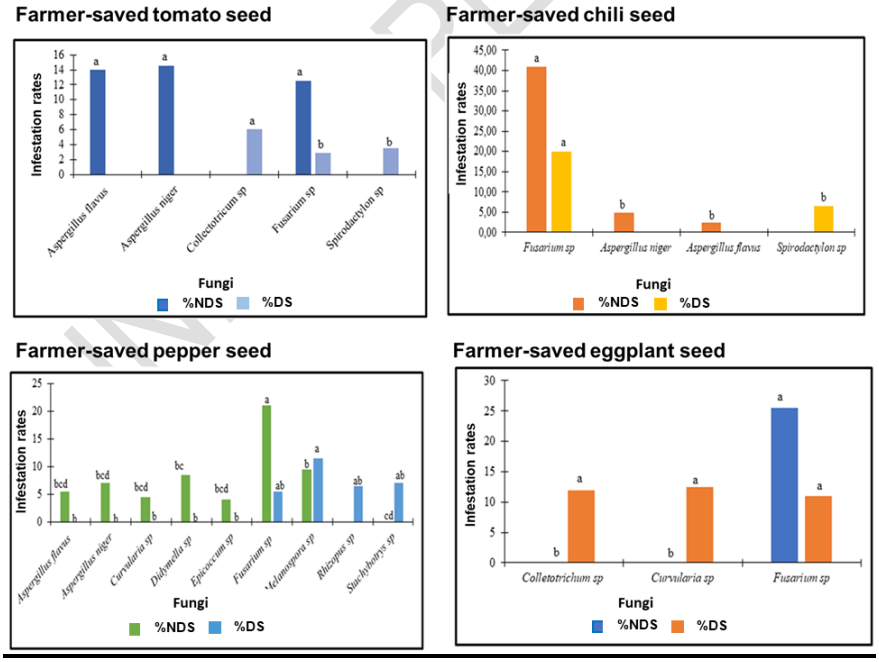


Fig. 1. Fungal Species Infestation Rates on Farmer-Saved Seeds

NDS: No-disinfected seed ; **DS:** Disinfected seed

3.1.2 Improved Varieties Seeds

The improved seeds of tomato, chili, pepper, and eggplant showed two genera of fungi: *Fusarium* spp. and *Alternaria* spp. *Fusarium* spp. reached a maximum infestation rate of 22% on tomato, while *Alternaria* spp. was less frequent (1.5% on chili). *Fusarium* spp. significantly dominates all the crops (Figure 2).

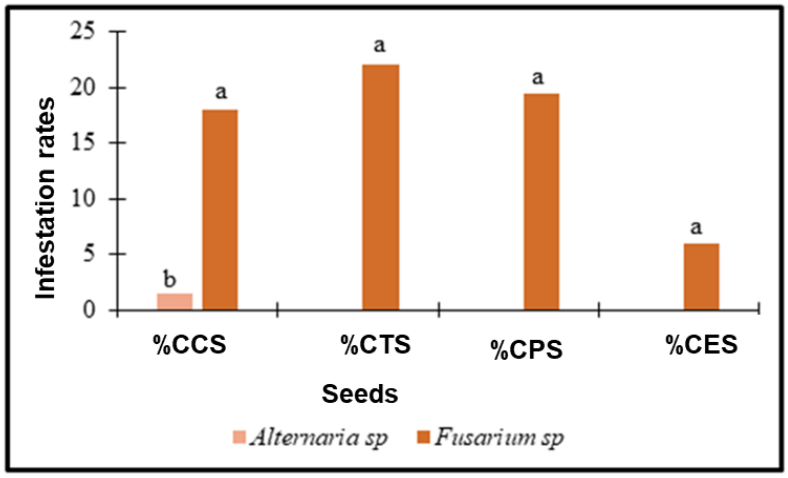


Fig. 2. Infestation rates of fungal species on improved seeds

CCS : Commercial Chili Seed, **CTS :** Commercial Tomato Seed, **CPS :** Commercial Pepper Seed, **CES :** Commercial Eggplant Seed.

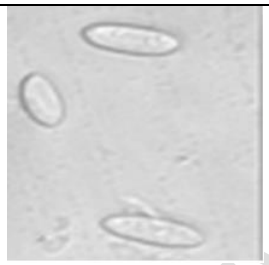
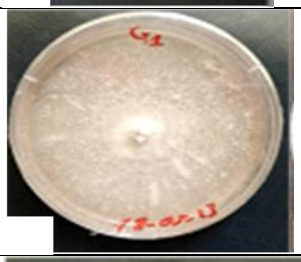
Given the significance of *Fusarium* on all the analyzed seeds, even after disinfection, and its negative impact on the resulting plant health, a thorough characterization of this pathogen was carried out.

3.1.3 Morphological Characteristics

The analysis of morphological characteristics, based on macroscopic and microscopic parameters, revealed the distinction of four groups of isolates (Table 2).

Table 2. Morphological characteristics of the isolates

Groups	Mycelium on PDA	Conidia under the microscope	Morphological characteristics

Group 1 : P1F ; P3F ; P4F			**Colony**: Whitish on both the surface and underside of the Petri dish with a regular outline. The mycelium has a cotton-like appearance. The fungus contained fusiform or ellipsoidal macroconidia, septate (1 to 3 septa), with an average size of 10.65 - 14.62 µm x 3.44 - 3.61 µm.
Group 2 : T1F ; T2F			The mycelium is whitish with a fluffy appearance. The macroconidia were ellipsoidal with an average size of 6.54 - 13.34 µm x 1.85 - 4.62 µm.
Group 3 ; T3F ; T4F			The mycelium had a granular appearance with moderately fast growth. Fusiform and septate macroconidia (1 to 3 septa) and microconidia were present. The macroconidia had an average size of 15.41 - 28.18 µm x 3.61 - 3.65 µm.
Group 4 : Pi_1F ; Pi2F			The mycelium was brown in the center and white at the periphery on the surface, with a light yellow color on the underside of the Petri dish. It had a cotton-like appearance. The macroconidia were ellipsoidal in shape and septate (1 to 3 septa). The average size was 16.31 - 29.25 µm x 2.80 - 4.13 µm.

3.1.4 Molecular Characteristics

PCR analyses with universal primers specific to *Fusarium* spp. allowed the detection of amplicons of approximately 500 bp in 14 isolates : 3 from tomato (T2F, T3F, T4F), 4 from chili (Pi1F, Pi2F, Pi3F, Pi4F), 2 from pepper (P2F, P3F), and 5 from eggplant (A1F, A2F, A3F, A5F, A6F) (Figure 3A).

Tests conducted with primers specific to *Fusarium solani* (500-600 bp) confirmed the presence of this pathogen in 7 isolates : 3 from tomato (T2F, T3F, T4F), 2 from chili (Pi1F, Pi3F), 1 from pepper (P3F), and 1 from eggplant (A5F) (Figure 3B).

However, no amplification was obtained with primers specific to *Fusarium oxysporum*.

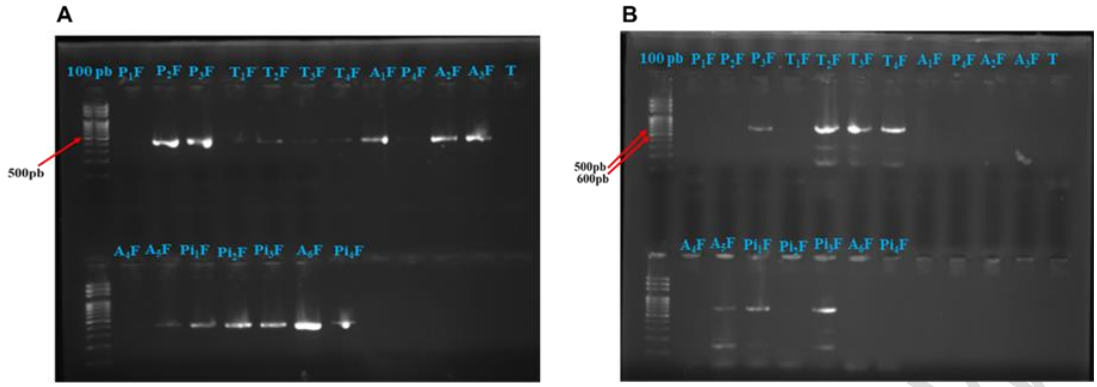


Fig. 3. **Migration profile on agarose gel for the detection of *Fusarium* spp. and *Fusarium solani***

A : *Fusari*um spp. detection gel*;* **B :** gel *Fusarium solani* detection gel

3.1.5 Sequence Analysis

The sequence analysis of *Fusarium* spp. isolates from Burkina Faso revealed their close relation to four species: *F. solani* (P1F, T3F, T4F, Pi1F), *F. proliferatum* (A2F), *F. incarnatum* (A3F), and *F. equiseti* (A5F, Pi4F) (Table 3).

Table 3. Blast of consensus sequences of isolates from different *Fusarium* species on www.ncbi.nlm.nih.gov

Isolate Code	Seed	Species	Size (pb)	Accession	Similarity (%)
T3F	Tomato	*F. solani*	574	ON377361.1	99,09
T4F	Tomato	*F. solani*	574	ON377361.1	100
Pi1F	Chilli	*F. solani*	551	MN602849.1	94,92
Pi4F	Chilli	*F. equiseti*	536	KX196808.1	99,23
P1F	Pepper	*F. solani*	568	KU382502.1	99,40
A2F	Eggplant	*F. proliferatum*	535	KX196810.1	98,85
A3F	Eggplant	*F. incarnatum*	525	OQ422695.1	99,48
A5F	Eggplant	*F. equiseti*	543	OL998441.1	99,43

3.1.6 Pathogenicity of the Isolates

3.1.6.1 Fusariosis incidence

The incidence of fusariosis in tomato, pepper, and chili plants derived from infested seeds varied depending on the crop and the isolates at the 40 th day after sowing (DAS). Statistical analysis revealed a highly significant difference ($p < 0.001$) among the isolates across all crops

at the 5% threshold (Table 4). The isolate T2F caused a high incidence of 41.67% in tomato seedlings, while the isolate P3F resulted in high incidences of 16.67% and 75.00% in pepper and chili seedlings, respectively (Table 4).

Table 4. **Incidence of four *Fusarium* spp. isolates on tomato, pepper, and chili plants**

	Incidence (%)		
Isolates	Tomato	Pepper	Chili
Pi1F	16,67d	00c	33,34c
T2F	41,67a	8,34b	41,67b
P3F	33,34b	16,67a	75,00a
A5F	25,00c	8,34b	41,67b
Control	00[e]	00c	0,00d
Standard Deviation	15,28	6,05	25,67
Probability	< 0,001	< 0,0001	< 0,0001
Significance	THS	THS	THS

The values followed by different alphabetical letters are significantly different at the 5% threshold according to Fisher's test; THS: Very Highly Significant. Pi1F : Isolate 1 of Fusarium from chili seed ; T2F : Isolate 2 of Fusarium from tomato seed ; P3F : Isolate 3 of Fusarium from pepper seed ; A5F : Isolate 5 of Fusarium from eggplant seed.

3.1.6.2 Fusariosis Severity

The results revealed variability in disease severity depending on the crops. For tomato, only the isolate T2F caused a significantly higher severity (20.41%) compared to the control. In contrast, for pepper, no significant difference was observed ($p > 0.05$). For chili, the severity of fusariosis was similar in plants derived from seeds infested with the four Fusarium isolates, and this severity was higher than that observed in the control (Table 5).

Table 5. Severity Index of Four *Fusarium* spp. strains on Tomato, Pepper, and Chili Plants

	Severity (%)		
Isolates	Tomato	Pepper	Chili
Pi1F	0,83b	00	27,50a
T2F	20,41a	13,33	31,25a
P3F	13,33ab	8,75	50,83a
A5F	7,91ab	7,91	32,91a
Control	00b	00	00b
Standard Deviation	*2,45*	*2,06*	*3,79*
Probability	*< 0,05*	*> 0,05*	*< 0,01*
Significance	*S*	*NS*	*HS*

The values followed by different alphabetic letters are significantly different at the 5% level according to Fisher's test; **S**: Significant; **NS**: Not Significant; **HS**: Highly Significant. **Pi1F**: Fusarium isolate 1 from chili seeds; **T2F**: Fusarium isolate 2 from tomato seeds; **P3F**: Fusarium isolate 3 from pepper seeds; **A5F**: Fusarium isolate 5 from eggplant seeds.

3.2 Discussion

Seed and plant quality is a key factor in agricultural production. The sanitary analysis of seeds from the four most widely cultivated Solanaceae species in Burkina Faso revealed a high diversity of associated fungi, with 11 genera identified. Similar studies have shown comparable fungal diversity transmitted by seeds. For example, Gyasi *et al.* (2020) detected nine genera and 11 fungal species associated with pepper seeds in Ghana. Research conducted in Pakistan (18) and India (19) also identified numerous fungal species, including genera like Colletotrichum, Curvularia, and Fusarium on tomato seeds. (20), in Bangladesh, observed 11 fungal species from seven vegetables, including eggplant and tomato.

Seed infestation rates varied depending on their origin. Indeed, farmer-saved seeds were contaminated by 11 genera of fungi, while commercial seeds were only contaminated by *Fusarium* spp. and *Alternaria* spp. These results indicate that local seeds have a higher contamination rate than commercial ones. This can be explained by the lack of sanitary control over farmer-saved seeds before use, unlike commercial seeds, which are treated, selected, and protected. According to Gyasi *et al.* (2020), over 70% of farmers in developing countries use their own untreated seeds, which may favor the spread of pathogens and the increase in seed-borne diseases.

The vulnerability of seeds to fungal attacks, whether saprophytic or parasitic, is well documented (21). Most of the fungi identified in this study, such as Fusarium, Aspergillus, and Colletotrichum, can negatively affect seed germination and seedling development. Diseases caused by Colletotrichum lead to significant yield losses in various parts of the world, including Malaysia for tomato (22) and Indonesia for eggplant (23). Additionally, *A. flavus* and *A. niger* are concerning due to their ability to produce aflatoxins, toxic metabolites that affect human health and the quality of fruits and seeds (24). These results confirm the crucial role of seeds as disease vectors, emphasizing the need for control measures, including preventive treatments to reduce the spread of pathogens.

Molecular characterization results confirmed the presence of four Fusarium species among the isolates analyzed. These findings highlight the importance of molecular characterization for precise identification, as some morphologically similar species can only be distinguished using molecular methods. Molecular analyses also revealed the presence of *F. solani* in seeds from all four Solanaceae species studied, a pathogen responsible for root and seedling rot. These results align with studies by Hami *et al.* (2021), which also identified *F. solani* as the main pathogen of chili.

F. solani was identified as the most frequently associated species with the seeds of the studied Solanaceae and was shown to be pathogenic in cross-inoculation tests. Managing seeds, especially through appropriate fungicidal treatments, could reduce the spread of this disease. The results of this study reinforce the importance of molecular characterization for reliable pathogen identification and highlight the risks that infected seeds pose to agricultural production. Finally, particular attention should be paid to the quality of seeds used, especially in developing countries where untreated seeds are commonly used.

4. CONCLUSION

This study revealed the fungal diversity associated with Solanaceae seeds grown in Burkina Faso, identifying 11 fungal genera, including pathogens such as *Fusarium* , *Colletotrichum*, and *Aspergillus*. Farmer-saved seeds, often untreated, exhibited a higher contamination rate than commercial seeds, emphasizing the importance of rigorous sanitary control. Fusarium

solani was identified as a major pathogen, calling for proactive seed management, including fungicidal treatments. The study also highlights the importance of molecular characterization for precise pathogen identification and the need to strengthen seed management, particularly in developing countries. For sustainable agricultural production, tailored prevention strategies and strict sanitary control are essential.

CONSENT

Not applicable

ETHICAL APPROVAL

Not applicable

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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