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Detection of Seed-Borne Fungal Pathogens Associated with Wheat (*Triticum aestivum* L.) Seeds Cultivated in Cameroon

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Abstract

Seed-borne fungi of wheat (*Triticum aestivum* L.) are responsible for numerous fungal diseases transmitted to plants through seeds. These fungal diseases result in enormous yield losses. The objective of this study was to carry out a study on seed-borne fungi of wheat. To achieve this objective, seeds from eight wheat varieties collected at the Institute of Agricultural Research for Development (IRAD) in Nkolbisson, Yaoundé, were collected and transported to the Life Science Laboratory at the University of Buea for detailed studies. This work was carried out using Potato Dextrose Agar (PDA) culture medium method and the Rolled Blotting Paper method. The results obtained from this work show that 21 fungal species are associated with wheat seeds. Twenty-one fungal species were identified by the PDA culture medium method and an additional 11 fungal species by the Rolled Blotting Paper method. With the PDA culture medium method, *Fusarium graminearum* (14.17%), *Colletotrichum graminicola* (9.89%) and *Curvularia lunata* (9%) were the most frequent and *Penicillium* sp. (1.83%) was the least frequent. Using the Rolled Blotting Paper method, species such as *Acremonium* sp., *Alternaria triticina*, *Bipolaris sorokiniana*, *Cladosporium herbarum*, *Epicoccum purpurascens*, *Fusarium culmorum*, *Mucor* sp., *Nigrospora* sp., *Ulocladium atrum* and *Zymoseptoria tritici* were not sampled. These results revealed a wide diversity of seed-borne fungi of wheat, hence the need to develop an effective means of controlling these seed-borne fungi.

Keywords

Wheat, Seed-Borne Fungi, Seeds, PDA, Rolled Blotting Paper

1. Introduction

Wheat (*Triticum aestivum* L.) is a herbaceous plant of the Poaceae family, native to the Near East [1] and cultivated in several regions of the world, mainly in temperate zones [2] for its seeds rich in carbohydrates, proteins, minerals, vitamins, and fiber, which facilitate intestinal transit [3]. It is one of the most widely consumed cereals in the world [4] and ranks third after maize and rice [5]. Wheat grains are one of the main raw materials in the baking industry, and the starch extracted from these grains is used as a raw material in the manufacture of several non-food compounds such as ethanol, bioplastic, adhesive, paper, and cosmetics. This crop is also used as animal feed [6].

In 2023, global wheat production was estimated at 799 million tons. China, India, and Russia were the three leading global producers, with outputs of 136.6, 110.6, and 91.5 million tons, respectively. Cameroon, with an average production of around 599.67 tons, is not among the world's leading wheat producers [7]. With such low wheat production, Cameroon therefore relies on imports to meet its wheat consumption. Hence, it is dependent on external sources for its wheat supply and imported approximately 859,000 tons, worth 154.27 billion CFA francs, in 2023 [8]. Consequently, the Cameroonian government has, since the Russian-Ukrainian war, been facing difficulties in importing wheat. To address these difficulties, the State of Cameroon has launched initiatives aimed at improving its wheat production. This revival of wheat production is being achieved through the distribution of improved seeds, fertilizers, pesticides, and agricultural tools to farmers, as well as through the organization of training sessions and the implementation of financial support programs.

As with any crop, the success of wheat cultivation depends on the use of good quality seeds, free from seed-borne fungi, and the application of good farming practices [9]. Despite these initiatives by the Cameroonian government, wheat production remains low and does not meet national demand. This low production is due to biotic and abiotic constraints. Among the biotic constraints, seed-borne fungi of wheat are the most feared. They are responsible for numerous fungal diseases transmitted to plants through seeds. These fungal diseases cause yield losses of up to 43% [10]. To support this evidence, several studies have reported numerous fungal species that cause diseases transmitted to plants through seeds. Thus, research conducted in Iran by [11] reported that fungi of the genera *Penicillium*, *Bipolaris*, *Tilletia*, *Aspergillus* and *Fusarium* were associated with wheat seeds. Similarly, work by [12] demonstrated that fungal species such as *Alternaria alternata*, *Fusarium moniliforme*, *Aspergillus flavus* and *Bipolaris sorokiniana* were associated with wheat seeds cultivated in Pakistan. Those by [13], conducted in Egypt, showed that wheat seeds harbour 44 fungal species belonging to 20 genera, the most frequent of which were *Alternaria alternata*, *Cladosporium* sp., *Epicoccum purpurascens*, *Fusarium verticillioides*, *Penicillium* sp., *Stemphylium* sp., and *Tilletia tritici*. These seed-borne fungi of wheat are responsible for numerous diseases, such as damping-off, which causes low germination rates [13]. Helmin-

thosporiosis caused by *Bipolaris sorokiniana* is a disease transmitted to plants through seeds, characterised by brown to black spots on the leaves and blackening of the grains [12].

In Cameroon, information on fungal diseases transmitted to plants by fungi associated with wheat seeds is virtually non-existent. The objective of this work was to carry out a study on the fungi associated with wheat seeds in order to demonstrate their existence and develop a means of controlling these wheat pathogens.

2. Materials and Methods

2.1. Collection of Wheat Seeds

Eight varieties of wheat seeds (11 SATYNDR, 12 HZWYT, 9 WYCYT, 20 HTWYT, 16 STEMRRN, IRAD 2, 42 ESWYT, and TCHAD) collected from the Institute of Agricultural Research for Development (IRAD) in Nkolbisson, Yaoundé, Cameroon, were used to carry out this work. These seeds were brought to the Life Science laboratory of the University of Buea separately in appropriate bags and labeled. The seed samples were placed in a refrigerator at a temperature of 4˚C until the tests were carried out.

2.2. Detection of Fungi Associated with Wheat Seeds

Wheat seed varieties were subjected to seed health testing following the standard rolled blotting paper method [14] and the agar plate method [15].

2.3. Disinfection of Wheat Seeds

Wheat seeds were surface disinfected in a 5% sodium hypochlorite solution for 5 minutes, then rinsed three times successively with distilled water to remove traces of the disinfectant and placed on hydrophilic paper to absorb excess water [16].

2.4. Rolled Blotting Paper Method

Four hundred seeds of each variety were placed aseptically between two layers of blotter paper soaked with 10 mL of sterile distilled water. These seeds were arranged in rows of 4 × 4 (16 seeds per sheet of paper). The whole thing was rolled up and placed in labelled plastic trays. These trays were incubated at 24˚C ± 2˚C under alternating cycles of 12 hours of darkness and 12 hours of light provided by a white fluorescent tube.

After 7 days, incubated seeds were examined visually under a stereo binocular microscope for the associated mycoflora [17]. Identification was done based on their morphological characters and microscopic examination of spores under a microscope [18]-[21].

The frequency (F) of each fungus was determined from the percentage of the colonies of all the fungi developed as the following formula:

$$\mathrm{F}(\%) = \frac{\text{Number of specific isolated species}}{\text{Total number of isolated fungal species}} \times 100$$

2.5. Agar Plate Method

Surface-disinfected wheat seeds were placed at ten seeds per Petri plate containing 20 ml of PDA and incubated for 7 days [22]. The fungi growing out from the seeds were examined based on colony appearance, and identification of spores was done as described under the rolled blotting method.

The laboratory experiment was conducted following a completely randomized design (CRD) with four replications.

2.6. Data Analysis

The recorded data on fungal frequency were statistically analyzed using the SPSS statistical package version 22.

3. Results

All the seeds tested were found to be infected by fungi, but at different levels. Overall, 4785 isolates belonging to 21 fungal species were identified from the different varieties of wheat seeds, including 2402 isolates for 21 species through the PDA culture medium method and 2383 isolates for 11 species using the rolled blotting paper method (Table 1). Among these different fungal species, the most frequent were species of *Fusarium* genus, followed by *Colletotrichum* and *Curvularia*. *Penicillium* was the least frequent species. With the agar plate method, five fungal species were in the genus *Fusarium* and two species in the genera *Aspergillus* and *Colletotrichum* were detected. The same observation (with the exception of the *Fusarium* genus) was made by the rolled blotting paper method, where four fungal species were identified.

The frequency of *F. graminearum*, *C. graminicola*, and *Curvularia lunata* was 14.17, 9.89, and 9% respectively for the agar plate method, and 23.84, 14.55, and 11.05% respectively for the rolled blotting paper method. The frequency of *Penicillium* sp. (the least frequent species) was 1.83 and 3.28% for the agar plate method and the rolled blotting paper method, respectively.

Table 1. Frequency (%) of different seed-borne fungi associated with wheat seeds.

Fungal species	Agar plate method	Rolled blotting paper method
Acremonium sp.	3.54 (85)*	
Alternaria triticina	4.25 (102)	
Aspergillus flavus	2.36 (57)	4.12 (98)
Aspergillus niger	3.37 (81)	8.54 (203)
Bipolaris sorokiniana	6.6 (159)	
Cladosporium herbarum	3 (72)	
Colletotrichum graminicola	9.89 (238)	14.55 (347)
Colletotrichum sp.	3.46 (83)	5.83 (139)
Curvularia lunata	9 (216)	11.05 (263)
Epicoccum purpurascens	4.49 (108)	

Continued

Fusarium avenaceum	6 (144)	10.26 (244)
Fusarium culmorum	3.68 (88)	
Fusarium graminearum	14.17 (340)	23.84 (568)
Fusarium oxysporum	4.19 (101)	7.18 (171)
Fusarium solani	2.42 (58)	3.98 (95)
Mucor sp.	2.74 (66)	
Nigrospora sp.	1.93 (46)	
Penicillium sp.	1.83 (44)	3.28 (78)
Trichoderma hamatum	3.38 (81)	7.37 (176)
Ulocladium atrum	2.56 (62)	
Zymoseptoria tritici	7.12 (171)	
Total	100 (2402)	100 (2383)

*The numbers in brackets represent the number of occurrences of the fungal species, and/means that the fungal species was not observed.

Using the agar plate method, *Fusarium graminearum* was the most frequent on all wheat seed varieties except for variety 20 HTWYT, where *Curvularia lunata* was the most frequent (12.38%) (Table 2). On the IRAD 2 variety, *F. graminearum* (14.72%), *Colletotrichum graminicola* (13.56%), and *Curvularia lunata* (9.68%) were the most frequent, and *Penicillium* sp. (1.16%) was the least frequent. With variety 20 HTWYT, *C. lunata* (12.38%) was the most frequent, followed by *F. graminearum* (10.99%) and *Zymoseptoria tritici* (10.31%). *Mucor* sp. was the least frequent (1.71%). *C. graminicola* and *C. lunata* had a frequency of 7.85% on variety 11 SATYNDR. On this same variety, species like *Aspergillus*, *Nigrospora*, and *Acremonium* were not detected. Similarly, *Cladosporium herbarum* was not observed in varieties 12 HZWYT and 16 STEMRRSN.

Table 2. Frequency (%) of different seed-borne fungi associated with wheat seeds by the agar plate method.

Fungal species	IRAD 2	9 WYCYT	11 SATYNDR	12 HZWYT	16 STEMRRSN	TCHAD	20 HTWYT	42 ESWYT
Acremonium sp.	3.48 (10)*	5.61 (17)			4.25 (13)	5.54 (17)	3.09 (9)	6.31 (19)
Alternaria triticina	4.65 (14)	3.63 (11)	5.37 (16)	4.27 (13)	4.64 (14)	4.34 (13)	3.78 (11)	3.32 (10)
Aspergillus flavus	1.93 (6)	3.3 (10)		2.74 (8)	3.09 (9)	2.41 (7)	2.74 (8)	2.65 (8)
Aspergillus niger		5.94 (18)		5.18 (16)	5.79 (18)	5.06 (15)		4.98 (15)
Cladosporium herbarum	4.26 (13)	4.29 (13)	4.95 (15)			4.09 (12)	4.47 (13)	1.99 (6)
Bipolaris sorokiniana	5.42 (16)	7.26 (22)	7.85 (24)	6.09 (18)	7.35 (22	6.02 (18)	5.84 (18)	6.97 (21)
Colletotrichum graminicola	13.56 (41)	10.3 (31)	7.85 (24)	10.97 (33)	8.12 (25)	10.85 (32)	5.84 (18)	11.62 (35)
Colletotrichum sp.	4.26 (13)	2.31 (7)	4.13 (12)	2.43 (7)	4.63 (14)	3.13 (9)	3.78 (11)	2.99 (9)
Curvularia lunata	9.68 (29)	8.58 (26)	7.85 (24)	9.75 (29)	7.35 (22)	7.14 (21)	12.38 (37)	9.3 (28)

Continued

Epicoccum purpurascens	4.65 (14)	4.29 (13)	6.61 (20)	5.18 (16)	3.86 (12)	2.89 (9)	4.8 (14)	3.65 (11)
Fusarium avenaceum	7.75 (23)	4.62 (14)	3.71 (11)	5.18 (16)	5.79 (18)	6.02 (18)	7.9 (24)	6.97 (21)
Fusarium culmorum	4.65 (14)	3.63 (11)	3.3 (10)	2.74 (8)	5.41 (16)	2.89 (9)	3.78 (11)	2.99 (9)
Fusarium graminearum	14.72 (44)	14.19 (43)	16.94 (51)	15.85 (48)	13.53 (41)	13.51 (40)	10.99 (33)	13.62 (41)
Fusarium oxysporum	3.48 (10)	3.3 (10)	4.54 (14)	4.57 (14)	3.48 (11)	4.33 (13)	5.15 (16)	4.66 (14)
Fusarium solani	1.93 (6)	1.32 (4)	2.89 (9)	1.52 (5)	4.25 (13)	1.69 (5)	3.09 (9)	2.65 (8)
Mucor sp.	1.55 (5)	2.64 (8)	3.71 (11)	3.35 (10)	3.48 (11)	3.13 (9)	1.71 (5)	2.32 (7)
Nigrospora sp.	1.55 (5)	2.64 (8)		2.13 (6)	3.48 (11)	2.65 (8)		2.99 (9)
Penicillium sp.	1.16 (3)		2.89 (9)	3.65 (11)		1.93 (6)	2.74 (8)	2.32 (7)
Trichoderma hamatum	3.87 (12)		7.02 (21)	4.57 (14)	3.48 (11)	4.33 (13)	3.78 (11)	
Ulocladium atrum	1.55 (5)	2.64 (8)	4.13 (12)	3.04 (9)	1.54 (5)	2.17 (6)	3.78 (11)	1.66 (5)
Zymoseptoria tritici	5.81 (17)	9.57 (29)	6.17 (19)	6.7 (20)	6.4 (19)	6.02 (18)	10.31 (31)	5.98 (18)
Total	100 (300)	100 (300)	100 (300)	100 (300)	100 (303)	100 (299)	100 (301)	100 (300)

*The numbers in brackets represent the number of occurrences of the fungal species, and/means that the fungal species was not observed.

With the rolled blotting paper method, the frequency of seed-borne fungi of wheat was present depending on the variety and fungal species involved (Table 3). *Fusarium graminearum* was the most frequent on all wheat seed varieties except for variety 42 ESWYT, where *Colletotrichum graminicola* was the most frequent (24.3%). For variety 20 HTWYT, *Curvularia lunata*, *F. graminearum*, and *F. avenaceum* were the most frequent with respective frequencies of 19.73, 18.06, and 13.04%. Species such as *Aspergillus flavus* and *Penicillim* sp., with frequencies of 4.34% each, were the least frequent. *C. graminicola* (24.3%), *F. graminearum* (23.95%), and *F. avenaceum* (14.58%) were the most frequent in the 42 ESWYT variety. *A. flavus* and *F. solani* were the least frequent (5.55% each). *Trichoderma hamatum* was not observed in this wheat variety. In variety 12 HZWYT, *F. graminearum* was the most frequent (28.66%), followed by *C. lunata* (17.66%), and *F. solani* was the least common (2.66%). *C. graminicola* was not among the species identified on this wheat variety.

Table 3. Frequency (%) of different seed-borne fungi associated with wheat seeds by the rolled blotting paper method.

Fungal species	IRAD 2	9 WYCYT	11 SATYNDR	12 HZWYT	16 STEMRRSN	TCHAD	20 HTWYT	42 ESWYT
Aspergillus flavus	3 (9)*	6.33 (19)		5 (15)	5.05 (15)	4 (12)	4.34 (13)	5.55 (16)
Aspergillus niger	8 (24)	11.33 (34)	6.66 (20)	9.33 (28)	9.42 (28)	8.33 (25)	5.01 (15)	10.41 (30)

Continued

Colletotrichum graminicola	20 (60)	19.33 (58)	12.66 (38)		13.13 (39)	18 (54)	9.69 (29)	24.3 (70)
Colletotrichum sp.	6.33 (19)	4.33 (13)	6.66 (20)	4.33 (13)	7.4 (22)	5.33 (16)	6.35 (19)	6.25 (18)
Curvularia lunata	14.33 (43)		12.66 (38)	17.66 (53)	11.78 (35)	12 (36)	19.73 (59)	
Fusarium avenaceum	11.33 (34)	8.66 (26)	6 (18)	9.33 (28)	9.42 (28)	10 (30)	13.04 (39)	14.58 (42)
Fusarium graminearum	21.66 (65)	27 (81)	27.33 (82)	28.66 (86)	21.54 (64)	22.33 (67)	18.06 (54)	23.95 (69)
Fusarium Oxysporum	5 (15)	6.33 (19)	7.33 (22)	8.33 (25)	5.72 (17)	7.33 (22)	8.36 (25)	9.72 (28)
Fusarium solani	3 (9)	2.66 (8)	4.66 (14)	2.66 (8)	5.72 (17)	2.66 (8)	5.01 (15)	5.55 (16)
Penicillium sp.	1.66 (5)		4.66 (14)	6.66 (20)	5.72 (17)	3.33 (10)	4.34 (13)	
Trichoderma harzianum	5.67 (17)	14.33 (43)	11.33 (34)	8.33 (25)	5.72 (17)	7.33 (22)	6.35 (19)	
Total	100 (300)	100 (300)	100 (300)	100 (300)	100 (297)	100 (300)	100 (299)	100 (288)

*The numbers in brackets represent the number of occurrences of the fungal species, and/means that the fungal species was not observed.

Description of the Different Fungal Species Associated with Wheat Seeds

***Acremonium* sp.**: aged 7 days, presented a cottony, circular, greyish mycelium, which gradually developed a pinkish pigmentation, localized differently in places and most especially in the center. The mycelium appeared dense in the center and finer at the edges. Under the microscope, simple, hyaline conidiophores were observed, bearing rounded to ovoid conidia with thick walls.

***Alternaria triticina*:** on PDA culture medium, 7-day-old *A. triticina* exhibited circular, cottony-looking, and greyish mycelium. Under the microscope, the species showed dark brown, elliptical conidia with a tapered base. These conidia were multi-septate, with transverse and longitudinal partitions (Figure 1(a)).

***Aspergillus flavus*:** on PDA medium, 7-day-old A. flavus initially had yellow mycelium, which turned olive green with age, with a powdery texture and irregular contours. Under the microscope, long hyaline conidiophores were observed, ending in a vesicle covered with phialides bearing spherical conidia arranged in chains.

***Aspergillus niger*:** on PDA medium, 7-day-old *A. niger* formed black mycelium with a granular appearance, flat and with regular edges. Under the microscope, the species had long, straight, hyaline conidiophores ending in large vesicles covered with phialides. These produced numerous spherical conidia arranged in chains.

***Bipolaris sorokiniana*:** on PDA medium, aged 7 days, presented a circular cottony mycelium of brownish colour with shades of white. Under the microscope, ellipsoidal conidia were observed, dark brown, straight but slightly thickened in the middle and thinner at the ends, with several transverse septa.

***Cladosporium herbarum*:** on PDA culture, after 7 days of growth, presented a

regular, olive-coloured mycelium with a velvety texture. Under the microscope, the conidiophores were elongated and bore cladospores arranged in chains.

Colletotrichum graminicola: on PDA culture medium, aged 7 days, had a concave, cottony mycelium, purple in the center and white at the ends. Under the microscope, the conidia were falciform, with tapered ends, hyaline, and non-septate (Figure 1(b)).

***Colletotrichum* sp.:** aged 7 days had a circular, white mycelium with a cottony aspect. Under the microscope, the conidia observed were solitary, oval, and non-septate (Figure 1(c)).

Curvularia lunata: on PDA medium, aged 7 days, presented a circular, flat, smooth, and black mycelium. Under the microscope, straight to slightly curved conidiophores were observed with oval, multicellular conidia formed of 3 to 4 transverse septa. These solitary, brown conidia were straight to curved; large and dark in the center, small and light at the ends (Figure 1(d)).

Epicoccum purpurascens: on PDA medium, aged 7 days, had a circular, white mycelium, dotted with dense rust-coloured granules, with growth greater than that of the mycelium. Under the microscope, numerous round, warty conidia were observed.

Fusarium avenaceum: on PDA medium, aged 7 days, exhibited a filamentous mycelium with a cottony texture and cream colour, dense in the center and lighter at the edges. Under the microscope, one- to two-celled, fusiform microconidia and falciform macroconidia with 3 to 7 transverse septa were observed (Figure 1(e)).

Fusarium culmorum: on PDA medium, aged 7 days, presented irregular, cottony, yellowish mycelium. Under the microscope, hyaline, thin-walled, fusiform macroconidia with 3 to 5 transverse septa were observed (Figure 1(f)).

Fusarium graminearum: on PDA medium, aged 7 days, presented an irregular, cottony mycelium, whitish to purplish in colour. Under the microscope, macroconidia with 3 to 7 transverse septa, elliptical to fusiform in shape, were observed (Figure 1(g)).

Fusarium oxysporum: on PDA medium, aged 7 days, presented a circular, cottony mycelium, pink in colour with grey tones. Under the microscope, unicellular, oval falciform microconidia were observed, as well as abundant, sickle-shaped macroconidia with 3 to 5 transverse septa (Figure 1(h)).

Fusarium solani: on PDA medium, aged 7 days, *F. solani* exhibited rhizoid mycelium, velvety in appearance and white in colour. Under the microscope, sickle-shaped macroconidia of varying sizes with 3 to 5 transverse septa were observed.

***Mucor* sp.:** aged 7 days showed a lobed, cottony, white mycelium, turning grey in the center with age. Under the microscope, spherical spores were observed, single or grouped.

***Nigrospora* sp.:** on PDA medium, aged 7 days, showed a cottony mycelium, initially white, whose center turned black with age. Under the microscope, hyaline, septate, and branched conidiophores were observed, bearing spherical, black conidia.

***Penicillium* sp.:** aged 7 days showed a circular, pinkish mycelium with a velvety texture. Under the microscope, septate, hyaline hyphae were observed, bearing simple conidiophores grouped in a bush, with the conidia arranged in bundles at the ends.

***Trichoderma hamatum*:** on PDA medium, aged 7 days, *T. hamatum* presented a regular mycelium, initially white, which turned powdery green with age. Under the microscope, hyaline, branched conidiophores were observed, bearing several spherical conidia in clusters, greenish in color.

***Ulocladium atrum*:** on PDA medium, aged 7 days, exhibited irregular, cushion-shaped, blackish, velvety mycelium. Under the microscope, hyaline, simple conidiophores were observed, bearing brownish, ovoid to cylindrical conidia with 0 to 3 septa.

***Zymoseptoria tritici*:** on PDA medium, aged 7 days, had a velvety mycelium, black in colour with pinkish and whitish shades. Under the microscope, spindle-shaped conidia were observed.

Fig 1a: *Alternaria triticina* : (i) Pure culture and (ii) conidia (x 400)

Fig 1b: *Colletotrichum graminicola*: (i) Pure culture and (ii) conidia (x 400)

Fig 1c: *Colletotrichum* sp: (i) Pure culture and (ii) conidia (x 400)

Fig 1d: *Curvularia lunata* : (i) Pure culture and (ii) conidia (x 400)

Fig 1e: *Fusarium avanaceum*: (i) Pure culture and (ii) micro and macroconidia (x 400)

Fig 1f: *Fusarium culmorum*: (i) Pure culture and (ii) micro and macroconidia (x 400)

Fig 1g: *Fusarium graminearum* : (i) Pure culture and (ii) micro and macroconidia (x 400)

Fig 1h: *Fusarium oxyporum* : (i) Pure culture and (ii) micro and macroconidia (x 400)

Figure 1. Morphology of some fungi identified on wheat seeds.

4. Discussion

Twenty-one fungal species were detected in all varieties of wheat seeds. Their presence could be explained by the fact that wheat provides a favorable substrate for their development. The number of fungal species identified depended on the study method used. On PDA culture medium, 21 fungal species were sampled, while with the rolled blotting paper method, 11 fungal species were sampled. The presence of a greater number of fungal species on PDA culture medium is thought to be due to the fact that it is a favorable medium for the development of fungi, as it provides the nutrients necessary for their growth, whereas blotting paper is not a culture medium as it does not provide any nutrients. These results corroborate those of [12] [23], who showed that PDA culture medium yielded a greater number of fungi compared to blotting paper.

The species such as: *Alternaria triticina*, *Aspergillus flavus*, *A. niger*, *Bipolaris sorokiniana*, *Epicoccum purpurascens*, *Colletotrichum graminicola*, *Colletotrichum* sp., *Curvularia lunata*, *Fusarium culmorum*, *F. graminearum*, *F. oxysporum*, *F. avenaceum*, *F. solani*, *Mucor* sp., *Penicillium* sp., and *Zymoseptoria tritici* isolated in this study have been reported in the works of [24]-[26].

The work of [13] carried out in Egypt reported that wheat seeds harbour 44 fungal species, whereas the present study reported 21 fungal species. The low diversity of seed-borne fungi of wheat observed in this study could be explained by the quality of the seeds used. Indeed, these seeds came from IRAD and are considered to be of good quality. IRAD implements rigorous practices during the harvesting, processing, and storage of seeds, which greatly limits fungal contamination and helps maintain healthy seeds. In addition, agroclimatic conditions, duration, and storage conditions strongly influence the appearance of certain fungal species [12] [27].

F. graminearum, *C. graminicola*, and *C. lunata* were the most common species, reflecting their pathogenic nature and ability to colonize wheat seeds. These fungal species, therefore, pose a threat to seed germination, the growth and development of wheat plants, and yield [28]. Furthermore, their predominance could also be due to environmental conditions favorable to their development, such as moderate to warm temperature and high humidity, which promote spore germination and infection of seeds [29].

Among the most frequent were *F. graminearum* and *C. lunata* species. These results align with those of [11] [25], whose studies show that *F. graminearum* was less frequent on wheat seeds grown in Canada. The higher frequency observed in our study could be related to local environmental conditions, including warm temperatures and prolonged humidity periods, which favor the proliferation of *F. graminearum*. Other factors, such as differences in working or storage conditions for wheat seeds. *Penicillium* sp. was the least frequent. This low frequency of *Penicillium* could be explained by the fact that it is an opportunistic fungal species. In addition, the disinfection of wheat seeds in a sodium hypochlorite solution may have contributed to the destruction of all *Penicillium* sp. associated with wheat

seeds.

The work of [30] has shown that *F. graminearum* causes significant yield losses and secretes mycotoxins that are harmful to human and animal health. *Colletotrichum graminicola* negatively affects seed viability and wheat plant vigour [31]. *Curvularia lunata* causes leaf necrosis in wheat plants [32].

5. Conclusion

This study highlighted the diversity of seed-borne fungi of wheat cultivated in Cameroon. These results suggest the development of an effective strategy to combat these fungi in order to improve wheat production. In this regard, the findings highlight the need to screen wheat varieties for resistance, evaluate targeted seed treatment options, and strengthen routine seed health monitoring programs.

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Contribution of Authors

N.N.N. and S.S.G. manipulated and drafted the main text of the manuscript. E.K.K., I.N. and G.T.N. coordinated the manipulations and also approved the final version of the manuscript. All authors carefully proofread the manuscript.

Conflicts of Interest

Authors have declared that there is no potential conflict of interest.

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