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# Post-Harvest Fungi Associated with Cowpea (*Vigna unguiculata* L. Walp.) Seeds Produced in Burkina Faso

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## Abstract

Cowpea is a very popular foodstuff among people in sub-Saharan Africa. In Burkina Faso, it is the main food legume, especially in rural areas. Its production is facing difficulties including post-harvest losses caused by fungi. Therefore, the objective of this study was to isolate and identify fungal strains associated with cowpea seeds produced in Burkina Faso. Thus, a total of 108 seed samples were collected in the three agro-ecological zones of Burkina Faso. The sanitary analysis of the seeds was carried out using the direct contact method. The isolation and purification of the isolates were performed on Potato Dextrose Agar medium while their identification was done through macroscopic and microscopic phenotypical characterization using different culture media (Potato Dextrose Agar (PDA), Malt Extract Agar (MEA) and Czapeck Dox Agar (CZA)) and different identification keys. A total of 10 fungal species were isolated, with predominance of *Aspergillus flavus*, *Aspegillus niger*, *Macrophomina phaseolina*, *Fusarium oxysporium* and *Rhizopus* sp. whose infection rates were 70.8% to 100% of seed samples. In addition to being present in all three zones, the infection rates of *Aspergillus flavus* (56.55%), *Aspergillus niger* (20.35%) and *Rhizopus* (32.80%) were higher in the Sahelian zone. In the Sudano-Sahelian zone, *Macrophomina* (50.66%) and *Fusarium* (18.88%) presented the highest infection rates, while *Penicillium* sp. showed the highest infection rate (2.84%) in the Sudanian zone. This finding

demonstrated the necessity to improve post-harvest and conservation techniques of cowpea to limit crop losses and preserve the sanitary quality of this important foodstuff.

## Keywords

Cowpea, Seed-Borne Fungi, Agro-Ecological Zones, Burkina Faso

## 1. Introduction

Post-harvest pests affect agricultural production around the world, causing sometimes significant yield losses, and exacerbating food insecurity especially in developing countries like those located in the Sahelian region of sub-Saharan Africa. In order to consolidate the resilience of African populations to food and nutrition insecurity, crops diversification and the reduction of post-harvest losses are required [1]. Consequently, many studies have been conducted to improve the yield of different food crops, focusing on cereals and legumes, the staple foods in most African countries. In Burkina Faso, cowpea is the most important legume for many rural populations and urban people, especially during the dry season, lasting nine (9) months (October to June) every year [2]. In order to have their crops available throughout the year, rural farmers use several traditional storage methods. However, these storage methods do not provide the expected protection to seeds. Stored products are frequently damaged due to the action of many agents including insects and fungi [3]. Indeed, the storage and conservation of agricultural products are under serious threat due to the rapid multiplication of pests that create huge income losses for farmers [4] [5]. According to the United Nation Food and Agriculture Organization, about a quarter of world food production is spoiled and lost annually because of the uncontrolled development of fungi, corresponding to an economic decline of 5% to 10% [6]. These high rates of post-harvest losses contribute to insufficient food supply for the population and reduce agricultural incomes [7]. Fungi acidify, discolor, ferment and make food products unpleasant or even dangerous [8]. In fact, their development in seeds can generate low-dose toxic compounds such as mycotoxins, responsible of poisoning (chronic or acute) in vertebrates (humans and animals). Several fungi genera including *Aspergillus*, *Penicillium* and *Fusarium* are known to be contaminants of agricultural products and/or for their ability to produce secondary toxic metabolites [9]. In view of the huge economic losses and health risks related to post-harvest fungi, mitigation actions are required. The identification of post-harvest fungi incriminated in food spoilage and the production of mycotoxins are an essential step in achieving food security. Several studies have reported the presence of fungi in cowpea seeds. Makun [10] showed the presence of fungi such as *Aspergillus niger* (19.78%), *Fusarium verticilloides* (14.85%), *Mucor* spp. (5.95%), *Penicillium* spp. (4.95%) and *Rhi-*

*zopus* spp. (0.99%) in cowpea seeds from Nigeria. Gyasi [11] isolated seven (7) fungal species (*Aspergillus flavus*, *Aspergillus niger*, *Aspergillus tamari*, *Penicillium* sp., *Rhizopus stolonifer*, *Fusarium verticillioides* and *Colletotrichum* sp.) from 200 samples of cowpea seeds collected in Ghana. Khare [12] reported the presence of *Penicillium*, *Aspergillus* and *Fusarium* species in cowpea seeds from Botswana. In Burkina Faso, there is very little significant scientific data on the fungal species that contaminate crops such as cowpea seeds. To reduce post-harvest losses of cowpea, identification of the main fungal species contaminating its seeds is an essential step. This study was conducted to isolate and identified the diversity of post-harvest fungi associated with seeds of cowpea produced in Burkina Faso, to determine their infection rates according to agro-ecological zones and to assess their incidence.

## 2. Material and Methods

### 2.1. Seed Sampling

In order to have a global view of the situation across the country, a total of 108 seed samples of cowpea were collected in the three agro-ecological zones (Sahelian zone, Sudan-Sahalian and Sudanian zone) of Burkina Faso. These sampling sites were selected based essentially on the importance of the crop in each agro-ecological zone. Therefore, 21 samples were collected from 9 locations in the sahelian zone; 33 samples from 22 locations in the Sudano-Sahalian zone and 54 samples from 16 locations in the Sudanian zone. Each sample (1000 to 2000 g) was placed in a sterile plastic bag on which was recorded its main identification information (sample number, collection site, name of the crop, year of production, date of collection) and transported to the laboratory for analysis. In the laboratory, each seed sample was divided into two equal quantities; the first portion was used for the study and the remaining portion was stored in a freezer.

### 2.2. Seed Health Testing

The direct plating method for examining seed-borne fungi was adopted as described by Perrone [13]. Fifty seeds were randomly chosen from each cowpea sample and surface-sterilized in 1.5% sodium hypochlorite for 2 min. The sterilized seeds were rinsed in three changes of sterile distilled water and blotted dry on a sterile paper towel. After drying, 10 seeds were placed on a plate of sabouraud chloramphenicol agar. The plates were incubated at 28˚C for 3 to 5 days and observed daily for fungal development. Then, the individual seeds were examined for the presence of fungi under a stereo-microscope (MOTIC SMZ-140-N2LED, Spain). Preliminary identification of each fungus developed on the seeds was made by examining the mycelium and/or conidia under a compound microscope (MOTIC SFC-18, Hong Kong, Asia) and the different strains present on each seed were recorded. Then, the infection rate of each fungus and the percentage of infected samples were computed using the following formula [14]:

$$\text{Infection rate}(\%) = \frac{\text{Number of seeds infected with a fungal strain}}{\text{Tota number of seeds}} \times 100 \quad (1)$$

$$\text{Percentage of infected sample} = \frac{\text{Number of samples contaminated with a fungal strain}}{\text{Total number of samples}} \times 100 \quad (2)$$

### 2.3. Isolation and Purification of Fungal Strains

Each visible mycelial growth on the seeds was isolated by collecting a fragment of the mycelium using a sterilized needle that was placed in the center of a Petri dish containing PDA medium for growth. During the collection, precautions were taken to avoid contact with other neighboring mycelia from the same seed; then, successive subcultures were performed on PDA medium in order to purify the isolated fungus. The subculturing was carried out by placing a fragment of the mycelium in the center of a new Petri dish using a sterilized loop. The purified strains obtained were kept on PDA at 4˚C.

### 2.4. Identification

Three culture media (Potato Dextrose Agar, Malt Extract Agar, and Czapeck Dox Agar) were used for morphological identification. Macroscopic features of the isolates including colony growth, color, texture, spores, and reverse color were observed after 7 days of inoculation [15] [16]. For microscopic evaluation, a fragment of mycelium was collected from the fungus and placed on a slide; a drop of methylene blue was added before covering with a coverslip. Microscopic features such as conidiophores, vesicles, metules, phialides, shape, and texture of spores were observed under a microscope (MOTIC SFC-18, Hong Kong, Asia) at 10, 40, and 100 magnifications. Several identification keys were used including those described by Klich [15], Samson [17], Samson [18], and Samson [16].

### 2.5. Data Analyses

Seed-borne fungi of cowpea and infection rates were determined using Equations (1) and (2). The distribution of fungal strains on the samples and between agro-ecological zones was compared by the Analyses of Variance (ANOVA); it was done with the Duncan's Multiple Range (DMR) test at the significance level of $p < 0.05$ using Statistical Analysis System, version 8.

## 3. Results

### 3.1. Mycoflora of Cowpea

A total of 10 fungal strains belonging to 08 genera were isolated from the cowpea seeds tested: *Macrophomina*, *Aspergillus*, *Fusarium*, *Rhizopus*, *Penicillium*, *Cladosporium*, *Rhizoctonia*, *Emericella* (**Table 1**). The health testing of the seeds revealed that all of the tested seed samples were infected by at least two fungal strains. Fungi such as *A. flavus* and *A. niger* in addition to their presence in all samples, were found at very high infection rates reaching 100% in some samples.

**Table 1.** Fungal isolates and infection rates of cowpea seeds.

| Fungal isolate | Infected sample (%) | Range of infection rate (%) |
|---|---|---|
| *Aspergillus flavus* | 100 | 2.00 - 100.00 |
| *Aspergillus niger* | 89.7 | 2.00 - 68.00 |
| *Macrophomina phaseolina* | 93.7 | 2.00 - 100.00 |
| *Fusarium oxysporium* | 77.1 | 2.00 - 68.00 |
| *Rhizopus* sp. | 70.8 | 2.00 - 100.00 |
| *Penicillium notatum* | 22.00 | 2.00 - 38.00 |
| *Rhizoctonia Solani* | 12.5 | 2.00 - 16.00 |
| *Cladosporium sphaerospermum* | 7.00 | 2.00 - 4.00 |
| *Aspergillus* sp. | 3.11 | 2.00 - 8.00 |
| *Emericella nidulans* | 1.57 | 2.00 - 4.00 |

The species *Fusarium oxysporium* was found in 77.1% of the samples analyzed with infection rates ranging from 2% to 68%. *Rhizopus* sp. and *Macrophomina phaseolina* recorded infection rates ranging from 2% to 100% and infected 70.8% and 93.7% of analyzed samples, respectively. *Penicillium notatum* was found in 22% of the samples with infection rates ranging from 2% to 38%. *Rhizoctonia solani*, *Cladosporium sphaerospermum*, *Emericella nidulans* contaminated 12.5%, 7% and 1.5% of the samples respectively at infection rates of 2% - 16%, 2% - 4% and 2% - 4%; respectively.

### 3.2. Distribution of Fungi in the Three Agro-Ecological Zones of Burkina Faso

Statistical analysis (ANOVA at 5% level) performed on the averages of seed infection rates revealed a positive effect of the agro-ecological zones on the distribution of fungi species such as *A. flavus*, *A. niger*, *M. phaseolina*, *F. oxysporium*, *Rhizopus* sp. and *C. sphaerospermum* (Table 2). All fungi detected were present in all climatic zones except *C. sphaerospermum* and *E. nidulans* which are absent in the Sahelian and Sudanian zones, respectively (Table 2).

Comparing the three climatic zones, there are significant differences between the average infection rates of some fungi. The average infection rates of fungi species such as *A. flavus*, *A. niger* and *Rhizopus* sp. are higher in the Sahelian zone (56.55%, 20.35% and 32.80%, respectively) than in the other two zones. They were found in 100%, 100%, and 75% of the samples from this zone, respectively (Table 2).

In the Sudan-Sahelian zone, *M. phaseolina* and *F. oxysporium* recorded relatively higher infection rates (50.66% and 18.88%, respectively) than in the other two zones. They were found in 94.44% and 85.18% of the analyzed samples collected in this zone, respectively.

**Table 2.** Distribution of post-harvest fungi of cowpea in the three agro-ecological zones of Burkina Faso.

| Infection rates (%) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Agro-ecological zones | Af | An | Mp | Fo | Rsp | Pn | Rs | Cs | Asp | En |
| Sahelian zone | 56.55a | 20.35a | 30.66b | 9.850b | 32.80a | 1.85a | 0.06a | 0.00a | 0.05a | 0.01a |
| Sudano-sahelian zone | 25.33b | 17.1ab | 50.66a | 18.88a | 19.87b | 1.59a | 0.03a | 0.37a | 0.22a | 0.003a |
| Sudanian zone | 24.12b | 14.48b | 51.45a | 15.03b | 17.70b | 2.84a | 0.14a | 0.24ab | 0.06a | 0.00a |
| Average | 34.85 | 17.44 | 44.55 | 59.91 | 23.02 | 2.00 | 0.72 | 0.22 | 0.12 | 0.04 |
| **Infected samples (%)** | | | | | | | | | | |
| Agro-ecological zones | Af | An | Mp | Fo | Rsp | Pn | Rs | Cs | Asp | En |
| Sahelian zone | 100 | 100 | 93.5 | 57.50 | 75 | 17.5 | 7.50 | 00 | 2.50 | 2.50 |
| Sudano-Sahelian zone | 100 | 94.44 | 94.44 | 85.18 | 59.25 | 16.66 | 12.96 | 12.96 | 3.70 | 1.8 |
| Sudanian zone | 100 | 93.93 | 87.87 | 84.87 | 72.72 | 36.36 | 18.18 | 6.06 | 3.03 | 00 |

For a given fungus, infection rates with different letters are significantly different. Af: *Aspergillus flavus*; An: *Aspergillus niger*; Mp: *Macrophomina phaseolina*; Fo: *Fusarium oxysporium*; Rsp: *Rhizopus* sp; Pn: *Penicillium notatum*; Rs: *Rhizoctonia solani*; Cs: *Cladosporium sphaerospermum*; Asp: *Aspergillus* sp; En: *Emericella nidulans.*

### 3.3. Characteristics of the Isolated Strains

The macroscopic (colony appearance on PDA, CZA, MEA media), microscopic (mycelium morphology: presence/absence of septa, color, differentiation, etc.), and spore morphology (shape, color, wall texture etc.)) characteristics of the isolated fungal strains are presented in Figures 1-7.

The strain of *A. flavus* is yellowish to greenish on the PDA medium. The reverse side is white. The colony has a green and white surface color, a velvety radiating texture at the edge, and a white underside on CZA medium. On the MEA medium, the colonies are green-yellow, relatively flat with a white margin. The undersides are colorless to yellowish. Microscopically, the numerous single-celled spores are globose to ovoid (Figure 1).

The color of *M. phaseolina* colonies in culture varies from white to brown or grey and darkens with age. It grows very rapidly. On PDA medium, the colonies are black on the surface and on the reverse side. On the CZA medium, the colonies are whitish black with a filamentous texture. The reverse side is also whitish black. On MEA medium the colonies are black with a brown pigment. The reverse side is black.

Microscopic observation shows microsclerotia that are black in color and appear smooth and round to oblong (Figure 2).

*Fusarium oxysporium* growth is generally slow on all three media used, the colony formed with white aerial mycelia which then produce a dark purple pigment on the PDA medium. The reverse side is white-yellow. On CZA medium, the colonies are white with aerial filaments both for all side. On MEA medium, the colonies are white with a slightly brown pigment at the bottom. The reverse side is yellow. Microscopic observation shows slightly fusiform conidia, more or less curved (Figure 3).

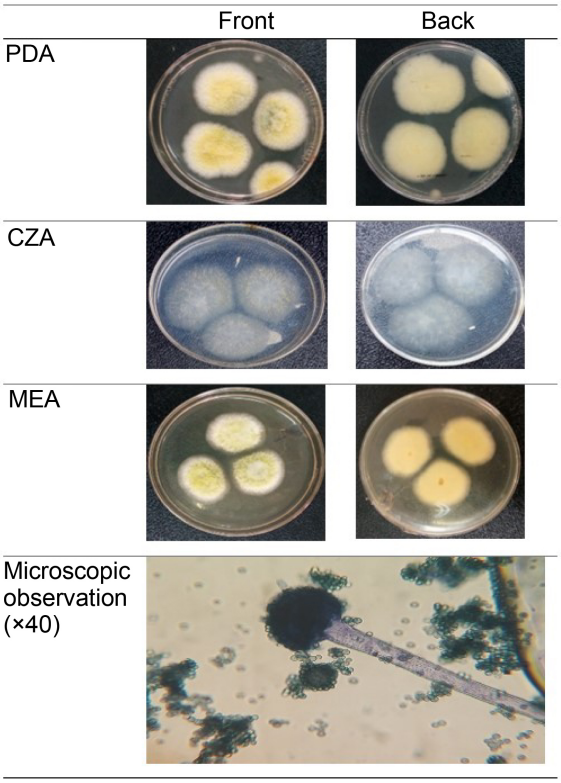


**Figure 1.** Macroscopic and microscopic characteristics of *Aspergillus favus.*

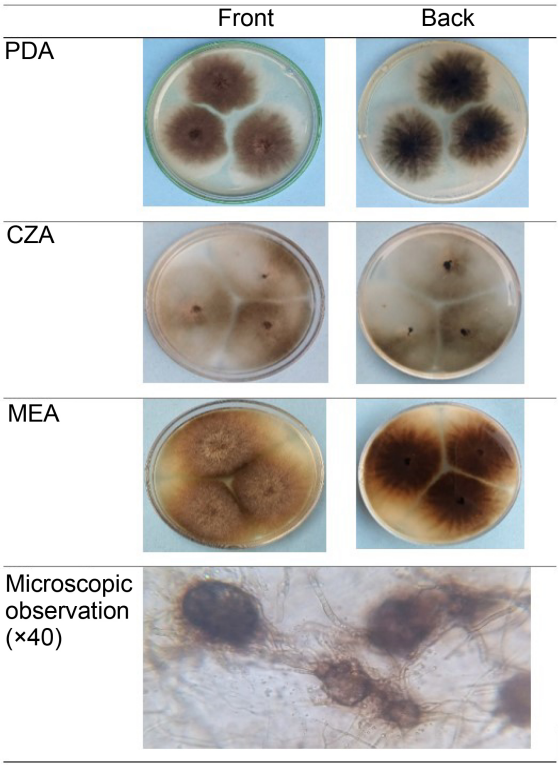


**Figure 2.** Macroscopic and microscopic characteristics of *Macrophomina phaseolina.*

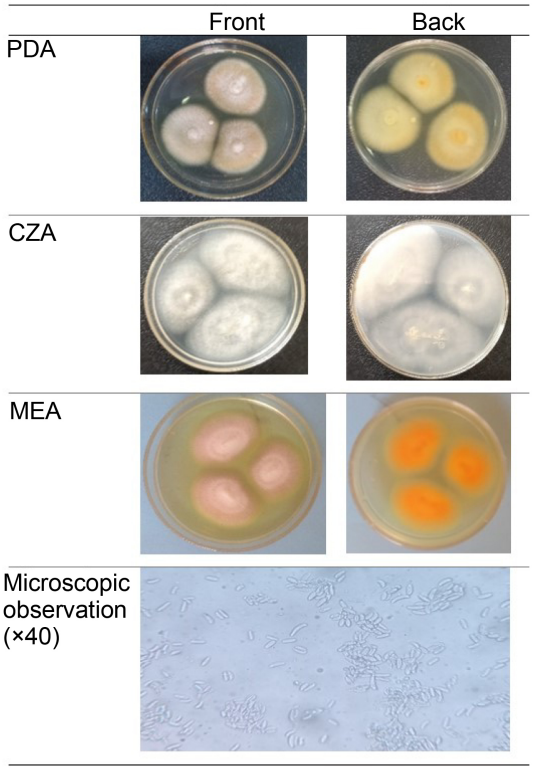


**Figure 3.** Macroscopic and microscopic characteristics of *Fusarium oxysporium.*

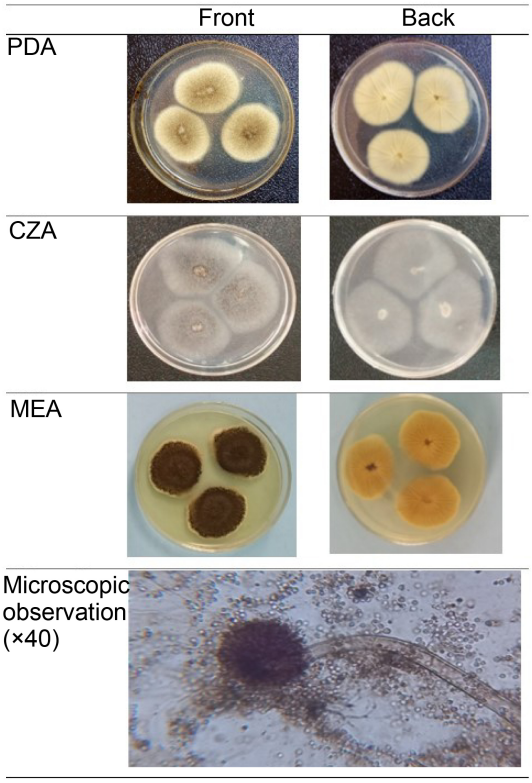


**Figure 4.** Macroscopic and microscopic characteristics of *Apergillus niger.*

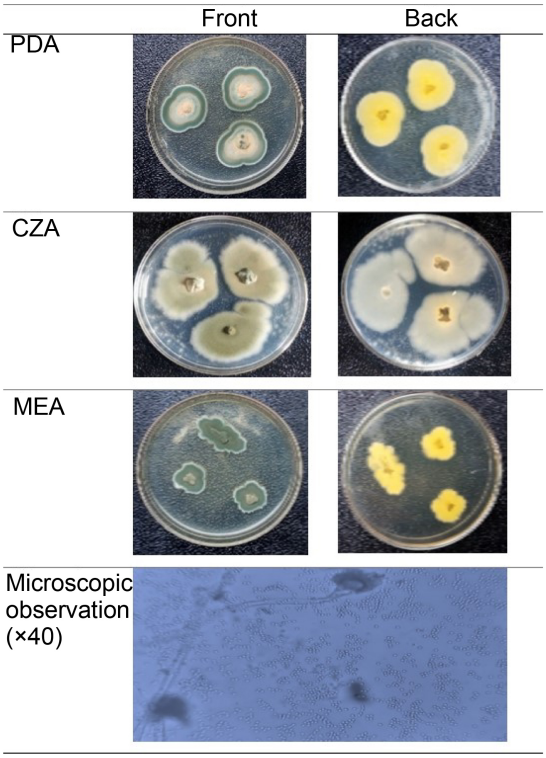


**Figure 5.** Macroscopic and microscopic characteristics of *Penicillium notatum.*

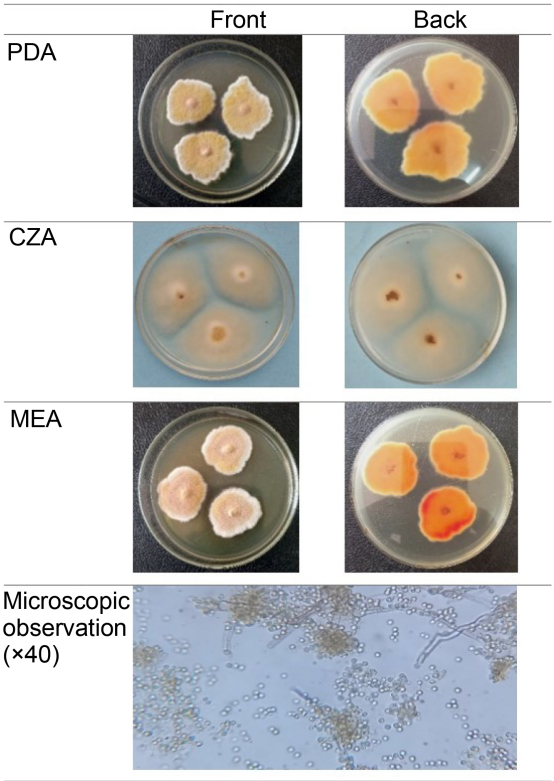


**Figure 6.** Macroscopic and microscopic characteristics of *Cladosporium sphaerospermum.*

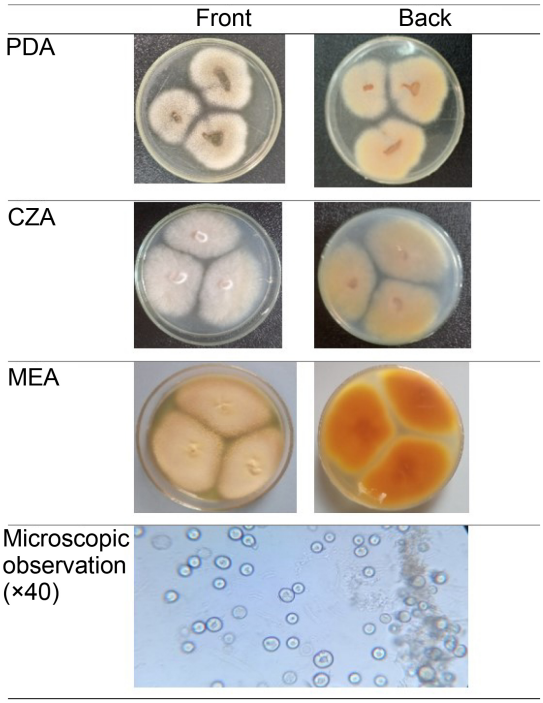


**Figure 7.** Macroscopic and microscopic characteristics of *Emericella nidulans*.

*Aspergillus niger* colonies have a powdery appearance and a black color (spore coating) on PDA medium. On CZA medium, the colonies invade the available space, sometimes with a black color on a slightly white background. On MEA medium, the fungus is black, fast growing and has a granular appearance. Under the microscope, the spores are more or less globose to ovoid (**Figure 4**).

On PDA medium, *Penicillium notatum* colonies are flat, formed by short aerial filaments which are green in the periphery and white in the center. The underside of the colonies is yellow. On the CZA medium, they have a velvety texture. The mycelium is green-white in the periphery. The underside is white-green. On the MEA medium, the colony is dense, with a smooth texture and a green color. The border is irregular. The underside is yellow. Under the microscope, the conidia are unicellular and globose (**Figure 5**).

*Cladosporium* sp colonies have a velvety texture, a yellow-green colour on the PDA medium and a white periphery. The underside is orange. On the CZA they are white-yellow and transparent. The underside is also yellow-white. On the middle, they are velvety, white with a green-yellow background. The underside is yellow-orange. Microscopic observation showed systematically septate, branched hyphae. The conidia were spherical, with a greenish color (**Figure 6**).

*Emericella nidulans* colonies are white-brown with a powdery appearance on PDA. The underside is yellow-orange. It has a filamentous appearance on CZA with white color. The reverse side is yellow-orange. On MEA medium, the colonies have a golden color, a powdery texture. The reverse side is yellow-orange. Microscopically, they showed conidia with granules inside (**Figure 7**).

## 4. Discussion

In this study, we isolated a several fungi belonging to genera categorized as field and storage moulds. In addition to being present in all samples, fungi strains like *A. flavus* and *A. niger* also have very high infection rates (2% - 100%) compared to the others. Our results are similar to those of Gyasi [11], who reported infection rates of *A. flavus* ranging from 3.0% to 57.0% and a percentage of infected samples of 54.5% on cowpea seeds produced in Ghana. In the same line, Afolabi [19] have reported 52.5% infection rates of *Aspergillus* strains from cowpea seed samples collected from markets in Nigeria. Moreover, Theses two species (*A. niger* and *A. flavus)* were found to be associated with cowpea seed infection (with rates of 23.57% and 16.42%, respectively) in India by Zanjare [20]. Contamination from field, during post-harvest operations, or storage could explain the presence of *A. flavus* and *A. niger* strains in the tested seeds. According to Degraeve [21] and Baddi [22], inappropriate harvesting, drying, and storage practices contribute to the development of fungi, mainly the genus *Aspergillus*. Numerous plant and agricultural product illnesses, ranging from harvest to processing transformation, are caused by *Aspergillus*. Ours *Aspergillus* strains presented similar morphological characteristic to those isolated by Abdallah [23], Okayo [24], and Ono [25] with ability to produce aflatoxins and ochratoxin A (OTA), two toxins that could be the cause of liver cancer [26]. The ability of these fungi to adapt to a wide temperature range may be the reason for their prevalence in all three agro-ecological zones. *Aspergillus* is widely spread geographically but is more frequently found in areas with warm temperature [13], [27]. The majority of *Aspergillus* species prefer temperatures between 25˚C and 40˚C for optimum growth. For this reason, they grow very well in the so-called "dry" food products like cowpea. Thus, precautions must be taken during post-harvest activities and storage to avoid contamination of cowpea crops by these ubiquitous moulds.

The relatively high infection rate (2.00% - 68.00%) of seeds by *Fusarium* could be explained by late harvesting. Indeed, *Fusarium* is a field fungus, and the long stay of cowpea pods in the field and their contact to the soil favors their contamination by this fungus. Khare [12] also isolated *Fusarium* species from cowpea seeds grown in Botswana at 5% infection rates. Shahnaz [28] obtained 3.5% infection rate of *F. oxysporium* on cowpea samples grown in Pakistan. In addition, *Fusarium* species have been found on seeds of other crops such as Bambara groundnut and rice in Burkina Faso [29] [30], and millet in Tunisia [31]. *Fusarium* species are cosmopolitan. They are found in all regions of the world, their ideal growth temperature is between 22˚C and 37˚C [32]. *Fusarium oxysporum* is the causal agent of head blight in cowpea and is one of the major diseases threatening cowpea production worldwide [33]. Several species of this fungus are saprophytic but can be parasites or plant pathogens by infecting fruits, vegetables, grains, and seeds. These include *F. oxysporium*, *F. solani*, *F. proliferatum*… etc. [34] [35]. Some species such as *F. graminearum*, *F. culmorum*, *F.*

*equiseti* can produce several types of toxins of which the best known are zearalenone, fumonisin, moniliformin and trichotchenes [9]-[35]. *Fusarium* mycotoxins have a toxic effect in humans and animals. They can cause birth defects, abortions and even cancers [35].

Species of the genus *Macrophomina* and *Rhizopus* are highly present in the soil, and contact of the pods with their spores during harvesting could explain their presence in the analyzed samples. In addition, there is a lack of good dehulling, drying, and seed storage practices by the farmers. Shahnaz [28] also isolated species of the genus *Macrophomina* and *Rhizopus* from cowpea seeds produced in Pakistan with infection rates of 1% and 30.8% respectively. *Macrophomina* is a phytopatogenic ascomycete fungus causing charcoal rot on cowpea roots or stems. It causes seeds rot and complete wilting of the plant [19] [36] [37]. Fungi of the genus *Rhizopus* are classified in the order Mucorales. They rapidly colonize decaying plants and fruits where they develop as filaments.

The specie *P. notatum* is a storage fungus, its presence in the analyzed cowpea seed samples could be explained by inadequate storage techniques. According to Kpatinvoh [38], this fungus proliferates mainly during storage. Khare [12], Kpatinvoh [38], Afolabi [19] and Jyoshna and Neeti [39] have also isolated *Penicillium* species from cowpea seeds produced in Botswana, Benin, Nigeria and India, respectively. Several toxins are produced by a variety of *Penicillium* species during food transport and storage operations. These include cyclopiazonic acid (*P. chrysogenum*), penicillic acid (*P. cyclopium*), patulin or clavacin (*P. expansum*, *P. griseofulvum*), citrinin (*P. expansum*), ochratoxin A (*P. verrucosum*) [40].

The species of *Rhizoctonia* found in our seed samples are similar to those isolated in several studies. Indeed, Jyoshna and Neeti [39] have found the genus *Rhizoctonia* on cowpea seeds produced in India. Thies [41] reported that *R. solani* was one of the most important pathogens of cowpea in the USA, causing roots rot, especially in cold weather.

*Cladosporium* species are saprophytic, phytopathogenic fungi and are pathogenic to humans. *Cladosporium* spores are known to cause allergic reactions. The toxins produced by *Cladosporium* can cause eye, nose, and throat irritation. Gamal [42] showed that *Cladosporium* is pathogenic through the appearance of lesions on inoculated leaves of *Vicia faba* (faba bean). The existence of this fungus in pods can cause capillary spread of internal tissues, leading to the formation of white felted spots extending into the pod cavity [42].

*Emericella nidulans* is not particularly common in foods and has not been implicated in actual spoilage, but has been isolated from a wide variety of food matrices. The most common reports are from cereals and cereal products (wheat, flour and bread, barley, rice, maize, and sorghum), nuts (peanuts, hazelnuts), dried beans, and spices [43].

The occurrence of these fungi on cowpea seeds can affect agricultural production as well as the health of consumers. In order to reduce seed infestation and

mitigate the impact of fungi on cowpea production, it is necessary to improve harvesting and storage practices.

## 5. Conclusion

Ten fungal species belonging to eight genera were identified from 108 cowpea seed samples in this study. *Aspergillus flavus*, *Aspergillus niger*, *Penicillium notatum*, and *Fusarium oxysporium* are the species that were not only often observed but also had high seed infection rates reaching 100% in certain cases. These fungal species are mycotoxigenic, hence their abundance in cowpea seed samples could pose a health risk. Additionally, a range of harmful fungi are present in the cowpea seeds grown in Burkina Faso. These include *R. solani*, *M. phaseolina*, *Rhizopus* sp., *Cladosporium* sp., and *E. nidulans*. The nutritive, organoleptic, and germination value of seeds may be diminished as a result of the frequency and abundance of these fungi in samples. It is therefore important to develop methods to control the growth of these fungi in cowpea seeds and crops in general to contribute to the preservation of consumer health and the reduction of food insecurity.

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## Conflicts of Interest

The authors declare that there are no conflicts of interest.

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