Parasitic fungi of some cowpea varieties (*Vigna unguiculata* (L.) Walp.) from the Saria research station in the centre-west of Burkina Faso

KOUNBO DABIRE^{1,2*}, BENOVANA BAKIONO², ELISE SANON², HAMADOU SIDIBE³, ANDJIÈRÈYIR KUSIELE SOMDA², KIBSA JEAN EDOUARD SEDEGO², PHILIPPE SANKARA⁴

¹Tenkodogo University Center, 12 BP 417 Ouagadougou 12, BURKINA FASO ²Department of Plant Biology and Physiology, Joseph KI-ZERBO University, 03 BP 7021 Ouagadougou 03, BURKINA FASO

³Institute of Environment and Agricultural Research (INERA/DRREA-C) of Saria National Centre for Scientific and Technological Research (CNRST) 03 BP 7047 Ouagadougou 03, BURKINA FASO

⁴Science and Technology Training and Research Unit (UFR/ST), Agronomy Division New Dawn University, 06 BP: 9283 Ouagadougou, BURKINA FASO

Abstract-Cowpea is the main food legume in tropical Africa. Its production plays an important role against food insecurity, malnutrition and poverty because it is a great source of protein and provides cash in income to producers. However, like and all other crops, its production is confronted with numerous abiotic and especially biotic constraints, including parasitic fungi, the most important of which are seeds and foliar. The objective of study was to make an inventory of leaf and seed parasitic fungi of cowpea at the Saria research station. In total, seven (7) varieties were studied. To achieve this, we first carried out a sanitary test of the various seeds. Symptomatic leaves were then taken from the seed production plot of the Saria research station for laboratory analyzes.

The isolation and purification of the fungal strains were performed on Potato Dextrose Agar medium while their identification was done through macroscopic and microscopic phenotypical characterization using an identification keys. Isolation of the pathogen was performed from seeds and symptomatic leaves. A total of six (6) isolates were identified as *Fusarium* sp., *Macrophomina* sp., *Rhizopus* sp., *Phoma* sp., *Fusarium avenaceum* and *Fusarium decemcellulare* from leaves including *Fusarium* sp. is the predominant species while five isolates have been identified as *Aspergillus flavus*, *Aspergillus niger*, *Phoma* sp., *Macrophomina* sp. and *Fusarium* sp. from seeds of which *Aspergillus flavus* is the predominant species. These results reveal that the seeds and symptomatic leaves of cowpea harbor a diversity of fungal species.

Key-Words: Cowpea, Symptomatic leaves, Sanitary test, Isolates strains, Saria.

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1 Introduction

Burkina Faso is an agropastoral country in West Africa. According to Oudet [1], more than 85% of the population is involved in breeding and agriculture, and their products account for 86.6% of exports. By Kabore [2], agricultural production is dominated by food crops, mainly cereals and legumes. Legumes have a high atmospheric nitrogen-fixing capacity, which contributes to soil enrichment, agricultural sustainability, and the availability of food for everyone. Cowpea, *Vigna unguiculata* (L.) Walp., is one of the most important seed legumes grown in the world according to Neya [3]. This crop is a plant of hot climates that can tolerate long periods of drought. According to FAOSTAT [4], more than 7,233,408 tons of cowpea were produced in the world, 81% of which were produced in West Africa. The Burkina Faso agricultural region as a whole is well suited to cowpea cultivation and is ranked among the world's major producers after Nigeria and Niger. Cowpea is the major leguminous crop in Burkina Faso according to Kabore [2] and holds an important place in the diet of many populations. Kabore et al. [16] mentioned that cowpea is important because of his protein content, which varies from 20.5 to 31.7%, and its vitamins and minerals values compared to herbaceous pastures that are poor in the dry season.

However, despite cowpea's ability to adapt to some constraints, its productivity remains very low due to biotic and abiotic constraints. Insect pests, diseases (bacterial, viral and fungal), weeds, drought, poor soils and the low potential of some local varieties are the main constraints in cowpea production. Biotic constraints, in particular micromycetes parasitic, cause significant damage to cowpea cultivation ranging from low production to total loss of production.

The current research activity in this context aims to study the diversity of parasitic fungi of some cowpea varieties at the Saria research station. Specifically, it consisted in: (i) Collecting leaves showing symptoms of fungal origin at the level of experimental plots, (ii) Saria's Making morphological characterizations of hyphae and spores under magnifying glass and optical microscope thanks to pure fungal strains obtained by isolation on Potato Dextrose Agar (PDA) medium and blotting paper of the samples and (iii) Identifying fungi associated with diseases of cowpea leaves and seeds using an identification key.

2 Materials and methods

2.1 Collecting site

The sampling was carried out at the research station of the Institut of Environment and Agricultural Research (INERA), located in the province of Boulkiemdé, in the Centre-West region and in the village of Saria (figure 1). Covering an area of 400 ha, its climate is of the Sudan-Sahelian type, and it is characterised by a dry season from October to April and a short rainy season from June to September. The average annual rainfall is about 800 mm and the average annual temperature is 28°C. Geographical coordinates are 12° 16' north latitude, 2° 09' west longitude and 300 m altitude. The data collected was analysed at the BIOSCIENCES laboratory, within the Phytopathology and Tropical Mycology Team located within the Life and Earth Sciences Training and Research Unit (UFR/SVT) of the Joseph KI-ZERBO University of Ouagadougou.

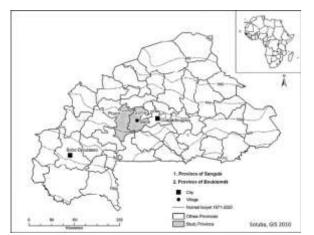


Figure 1. Geographical location of the experimental site of the Saria research station in Burkina Faso.

2. 2 Plant material

The study plant material used in this study consists of leaves and seeds of seven (7) cowpea varieties. These varieties are 'Gourgou', 'Teeksongo', 'Komcallé', 'Neerwaya', 'KVx745-11P', 'Yiipoussi' of Burkina Faso origin and 'Yisyandé' of Nigerian origin. The types of varieties are listed in table 1 below.

Table 1. Cowpe	a varieties used
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Varieties	Origins	Type of variety
Gourgou	Burkina Faso	Improved
Teeksongo	Burkina Faso	Improved
Komcallé	Burkina Faso	Improved
Neerwaya	Burkina Faso	Improved
KVx745-11P	Burkina Faso	Improved
Yiipoussi	Burkina Faso	Improved
Yisyandé		Improved
	Nigéria	

2. 3 Field and laboratory equipment

Technical equipment was used for the collection operation.

Sterile razor blades were used to cut the symptomatic leaves; 70° alcohol was used for disinfection of hands and slides after each sample taken; sterile plastic bags were used to collect the samples taken and a cooler containing ice was used to store the samples for transport to the laboratory for the various examinations. A number of equipment was used.

These included Petri dishes for culturing the fungi; blotting paper lined in the Petri dishes and on which the samples were placed for incubation; Potato Dextrose Agar (PDA), a culture medium used as a nutrient substrate and for the development of the various fungi; a fume hood with a benzene + butane gas burner that was used as a culture chamber to avoid contamination; an autoclave to sterilise the culture medium; a binocular optical microscope equipped with a computer and a binocular magnifying glass to observe the constituent elements of the different fungi; sterile forceps, slides, a test tube, distilled water, tape and a balance were used; a refrigerator used as a thermoregulator for the conservation of our samples; 70° alcohol and bleach were used to disinfect the leaves and the equipment; dye (cotton blue) was used to highlight the spores and hyphae.

2.4 Methodology

2.4.1 Sampling

The survey was held on 13 September 2021 at the Saria research station and covered seven (07) varieties. The symptoms cowpea (chlorosis, necrosis, leaf distortion) were observed during the surveys (figure 2). This observation was followed by sampling, which consisted of taking three random samples of three diseased leaves per plant and per variety. A sterile slide was used to cut the diseased leaves. Before any sampling, the hands were disinfected with 70° alcohol. The samples were collected in plastic bags, labelled and sealed. The whole set was stored in a cooler containing ice and taken to the laboratory for examination. Once at the laboratory, the samples were kept in the fridge for analysis.



I. Samples labeled as agged

Figure 2. Collection and storage of samples

2. 4. 2 Cowpea seed quality analysis

As the seed quality could not be assessed by visual observation, objective methods were developed. The sanitary test aimed to detect the different pathogenic fungi in a seed lot of each variety. It is therefore an analysis of the physical parameters and physiological qualities of a seed lot on the basis of a representative sample. For this purpose, we used the standard blotter method described by Mathur and Kongsdal (2003) with slight modifications, to detect fungi growth from seeds in the presence of humidity. We proceeded first by sterilizing the Petri dishes with alcohol at 70°; then we placed blotting paper in four (4) layers in the different Petri dishes that we moistened with distilled water. Ten (10) cowpea seeds untreated per variety, chosen randomly, were placed in a circular fashion on the surface of the blotting paper and then sprinkled. The Petri dishes were incubated for seven days at 20-25°C (figure 3). After incubation, the seeds were examined individually with a binocular magnifying glass for the presence or absence of fungi. Indeed, the individual seeds were examined for the presence of fungi under a stereomicroscope. A preliminary identification of each fungus developed on the seeds was made by examining the mycelium and/or conidia under a compound microscope and the different strains present on each seed were recorded.



Figure 3. Incubation of cowpea seed

2. 4. 3 Isolation of fungi from leaves

Isolation is carried out from leaves showing the characteristic symptoms of a fungal disease.

The leaves are first cleaned with simple water to remove sand and other debris on the leaves.

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 leaves are cut separately into small fragments using a sterile pair of scissors. These fragments are inoculated into Petri dishes containing PDA medium, prepared according to the manufacturer's instructions, at a rate of five (5) fragments per Petri dish. Inoculation is also done in a fume hood next to the flame of the benzene burner with all possible precautions to avoid contamination (figure 4). The plates were incubated at a temperature of 25°C. Seventy-two (72) hours after incubation, the colonies that have appeared are purified for microscopic examination.

a. Washed Leaves, **b.** Leaves seeding into Petri dishes containing PDA

2. 4. 4 Purification and identification of fungi

Purification consisted in transplanting the colonies obtained after incubation on seeds and leaves successively until pure strains were obtained. Transplanting was done by taking a fragment of a colony using sterilized forceps while avoiding contact with other neighbouring colonies in the same dish (figure 5). Each visible mycelial growth was isolated by collecting a fragment of the mycelium using a sterilized needle that was placed in the center of a new Petri dish containing PDA medium for growth and labelled. The boxes were observed under a magnifying glass to ensure that the colony was free of any contamination and uniform in staining. If not, or otherwise in case of contamination, the fragments are transferred back to new Petri dishes (figure 5). The pure dishes obtained are retained for microscopic observation to identify the species. The identification was done according to the morphological and microscopic criteria of the different colonies. All the pure colonies obtained were subjected to morphological identification carried out first with a magnifying glass showing the presence of sporangia (colour and shape) and then with a microscope showing the presence of filaments or mycelium (septate or not) and spores (size, arrangement and shape). Microscopic studies were made using a fragment of the pure colony taken with hyaline tape, soaked in cotton blue for one or two minutes then rinsed with distilled water and placed on a slide. Microscopic observation was done at X10 and X40 magnification. The identification key of Mathur and Kongsdal [5] was used to identify the fungi.



Figure 4. Incubation of Cowpea leaves collected.

Figure 5. Transplanting and purification of colonies obtained from the leaves and seeds incubation.

a. Seeds and leaves incubated, **b.** Colonies obtained after transplanting, **c.** Pure colonies obtained after purification.

3. Results

The isolation and purification of the different samples were carried out on PDA medium and resulted in various colony aspects, textures and colours. The identification was limited to the specific level and was mainly based on the identification key for micromycetes fungi established by Mathur and Kongsdal [5].

3.1 Seeds

Indeed, after the sanitary test of the seeds, four (4) genera of fungi were identified.

These are Aspergillus, Macrophomina, Phoma and Fusarium, of which the genus Aspergillus is the most dominant in terms of species. In terms of percentage, Aspergillus flavus and Phoma sp. recorded a percentage of 100% infection, i.e. seven (7) genotypes. They are followed by Aspergillus niger (71.42) and Fusarium sp. (71.42) i.e. five (5) genotypes. Macrophomina sp. was the fungus that infected fewer genotypes (3 genotypes).

3. 2 Symptomatic leaves

Four (4) genera divided into six (6) species of fungi were also identified from symptomatic leaves. These are: *Phoma*, *Macrophomina*, *Fusarium* and *Rhizopus* of which Fusarium is the predominant genus in number of species, i.e. three (3). *Phoma* sp., *Macrophomina* sp. and Fusarium sp. have a 100% infection rate. They are followed by *Fusarium avenaceum* (71.42), *Rhizopus* sp. (28.57) and finally *Fusarium decemcellulare* (14.28%).

3. 3 Leaf and seed

In summary, the fungal species identified on both seeds and leaves are three (3).

These are *Macrophomina* sp., *Phoma* sp. and *Fusarium* sp. These results show a diversity of fungi found on cowpea seeds and leaves, some of which are saprophytes and others pathogens.

3. 3. 4 Distribution of parasitic fungi identified on cowpea seeds and leaves

3. 3. 4. 1 Seeds

Among the fungi identified, three (3) are fungi that can be classified as parasites or saprophytes namely *Phoma* sp., *Macrophomina* sp. and *Fusarium* sp. These three fungi were identified on the Komcallé, Teeksongo and Yipoussi varieties. *Phoma* sp. and *Fusarium* sp. were identified on Gourgou and KVx 745-11P. The other varieties did not record any parasitic fungi.

3. 3. 4. 2 Symptomatic leaves

Among the fungi identified from symptomatic leaves, two (2) fungi were found to be parasitic fungi, namely *Fusarium avenaceum* identified on Gourgou and KVx 745-11P and *Fusarium decemcellulare* identified on a single genotype KVx 745-11P. We also note four (4) fungi that can be classified as saprophytes or parasites (in case they produce mycotoxins). These are *Phoma* sp., *Macrophomina* sp., *Fusarium* sp. and *Rhyzopus* sp.

3. 3. 5 Detailed descriptions of the fungi identified on the leaves

3. 3. 5. 1 Fusarium decemcellulare

Fusarium decemcellulare is a pathogenic ascomycete. It is characterised by a white colony, microconidia which then release globose conidia. The colony and spores are shown in the figure 6.

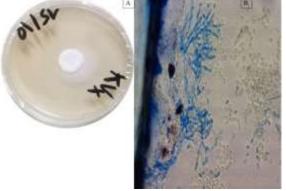


Figure 6. Fusarium decemcellulare A. Colony morphology on PDA medium; B. Microscopic appearance of the spores at $G = \times 40$ and with cotton blue as stain.

3. 3. 5. 2 Fusarium avenaceum

This is a phytopathogenic and cosmopolitan ascomycete found in temperate zones and to some extent in subtropical zones. The mycelia colonies are salmon-yellow. The conidiophores are simple or poorly branched, formed on the aerial mycelium. Spores are curved microconidia with 4-5 septa. The hyphae are septate. The colony, spores and hyphae are illustrated in the figure 7.

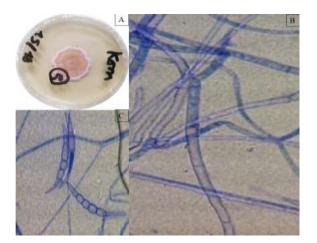


Figure 7. Fusarium avenaceum A: Colony morphology on PDA medium; B: Hyphae appearance under $G = \times 40$ microscope with cotton blue stain; C: Macroconidia appearance under $G = \times 40$ microscope with cotton blue stain.

3. 3. 5. 3 Rhizopus sp.

Rhizopus is a genus of fungi that grow as filaments in the soil, on fruits and on decaying plants. Some species of *Rhizopus* can cause mucormycosis, an infection that can be lethal to humans and animals. It is a member of the order Mucorales. It is characterised by a white flaky colony with blackish fruiting bodies on the surface, septate hyphae and globose conidia. The colony, spores and hyphae are illustrated in the figure 8.

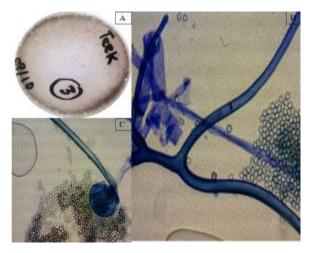


Figure 8. *Rhizopus* sp. A: colony morphology on PDA medium; B: hyphae appearance under $G = \times 40$ microscope with cotton blue stain; C: spore appearance under $G = \times 40$ microscope with cotton blue stain.

3. 3. 6 Detailed descriptions of the fungi identified on the seeds

3. 3. 6. 1 Aspergillus flavus

Aspergillus flavus is a saprophytic ascomycete, very common in tropical areas producing aflatoxins. *A. flavus* is found in cultivated soils, on cereal seeds such as maize or oats.

The mycelia colonies are powdery and green in colour. The hyphae are aseptate and have a double membrane. The spores are globose to ovoid, inamyloid. They are formed in very long chains and spread by air in nature. Viewing with a magnifying glass, the sporangia are white in the immature state and yellow in the mature state. The colony, sporangia and spores are represented in the figure 9.

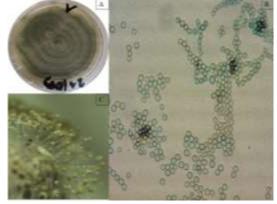


Figure 9. Aspergillus flavus

A: Colony morphology on PDA medium; B: Spores appearance under $G = \times 40$ microscope and with cotton blue as stain; C: Sporangia appearance under magnifying glass.

3. 3. 6. 2 Aspergillus niger

Aspergillus niger is a saprophytic, very common and cosmopolitan aflatoxin-producing ascomycete. It grows on various substrates. The mycelial colonies are powdery and black in colour. The spores are globose to ovoid and inamyloid. They are formed in very long chains and spread by air in nature. Under magnification, the sporangia are black in colour. The colony, spores and hyphae are represented in the figure 10.

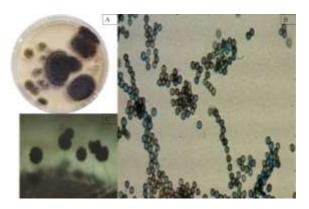


Figure 10. Aspergillus niger A: Colony morphology on PDA medium; B: Spores appearance under $G = \times 40$ microscope and with cotton blue as stain; C: Sporangia appearance under magnifying glass.

3. 3. 7 Description of fungi identified on leaves and seeds

3. 3. 7. 1 Macrophomina sp.

Macrophomina sp. is an ascomycete which could be a parasite or a saprophyte. It is soil- or seedtransmitted. It is characterized by a black, granular colony with septate hyphae. There is an absence of conidia and the presence of chlamydospores (swellings) which allow them to survive from one year to the next on the soil or in decaying plant debris. Under the microscope, chlamydospores are usually spherical and smooth. Observation of the seed with a magnifying glass showed mycelia intercepted from nodes. The colony, chlamydospores and hyphae are shown in the figure 11.

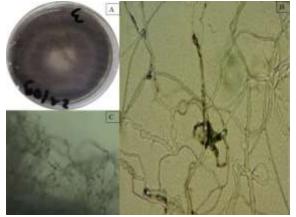
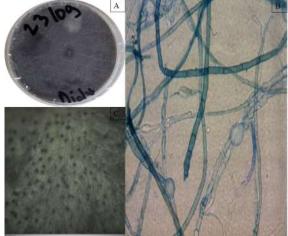
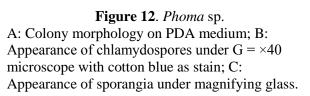


Figure 11. *Macrophomina* sp. A: Colony morphology on PDA medium; B: Appearance of chlamydospores under $G = \times 40$ microscope with cotton blue as stain; C: Appearance of sporangia under magnifying glass.

3. 3. 7. 2 Phoma sp.

It is a coelomycete, common in the temperate regions of Eurasia but sometimes also found in other parts of the world and cosmopolitan. The genus *Phoma* has species that adapt easily to the parameters of the indoor environment. They are usually found in the soil. It has a black colony, septate hyphae and chlamydospores that allow them to survive for years on the soil. Observation with a magnifying glass shows black pycnidia included on the seed. The colony, chlamydospores and hyphae are shown in the figure 12.





3. 3. 7. 3 Fusarium sp.

Fusarium is a genus of the most widespread imperfect fungi belonging to the class Ascomycetes. Within this genus, several species (mycotoxin producers) cause plant diseases. The mycelial colonies are white and flaky, septate hyphae with chlamydospores. A magnifying glass shows a cluster of white flaky mycelia. The colony, chlamydospores and hyphae are shown in the figure 13.

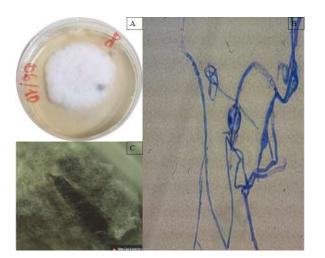


Figure 13. Fusarium sp. A: Colony morphology on PDA medium; B: Appearance of chlamydospores under $G = \times 40$ microscope with cotton blue as stain; C: Appearance of sporangia under magnifying glass.

4 Discussion

This study presents the contamination of *Vigna unguiculata* seeds and leaves produced in Burkina Faso based on the determination of phenotypical characteristics.

4.1 Seeds

The seed samples submitted to the sanitary test were contaminated by several species of fungi. Thus, visibly healthy seeds harbour several fungi within them. The fungi Aspergillus flavus was present in all samples and was found to be at a very high level of infection compared to the other fungi. Aspergillus niger was identified in five (5) samples. This could be explained by the fact that these aflatoxin-producing microorganisms are known to be saprophytic and are able to grow in various climatic conditions. They colonise plants already damaged by wounds, insect bites on the surface of seeds of foodstuffs. They also grow on decaying organic matter in the soil, in compost. These results are in agreement with those of Vasava et al. [6] who had also found these fungi on cowpea seeds. The work of Houssou et al. [7] and Ngombonzokwani et al. [8] also confirmed the presence of fungal strains of Aspergillus flavus and Aspergillus niger species in cowpea seeds respectively. The genus Phoma contains mycotoxin-producing fungi species. The species *Phoma* sp. is also found in all samples. Its presence on seeds can be explained by poor harvesting and storage conditions.

It is often involved in damping off. The early manifestation of the disease is a decrease in the germination capacity of the seeds. Zida *et al.* [9]

mentioned that after germination, seedling damping-off manifests as necrosis of the root system and young stem followed by death of the seedling.

The low contamination of seeds by fungi such as *Fusarium* sp. and *Macrophomina* sp. could be explained by the competitive development of fungi such as *Aspergillus flavus* and *Aspergillus niger* that rapidly invade the seeds and inhibit the development of other fungi.

Indeed, these invading fungi are able to colonise the space in a short time. They are also able to produce (antifungal) substances that inhibit the development of fungi on the seed.

The fumonisin-producing genus Fusarium has been identified in cowpea seeds. This implies that Fusarium sp. found on seeds not only inhibits germination but can also disrupt the later stages of seedling development with consequent yield reduction. Cissé and Hall [10] reported the presence of Fusarium sp. on cowpea seeds. The species Macrophomina sp. is responsible for charcoal rot of seeds. Good seed is an important factor for successful production. In addition to germination capacity, another important aspect of quality seed is the absence of pathogens in the seed. The presence of mycotoxin-producing fungi in stored cowpea seeds could be dangerous for the health of consumers. Some species was identified on seeds of Bambara groundnut (Vigna subterranea (L.) Verdcourt) produced in Burkina Faso by Ouili et al. [15]. It is Aspergillus flavus, Aspergillus niger, Aspergillus parasiticus, Aspergillus fumigatus, sp., Aspergillus Macrophomina phaseolina, Rhizopus sp., Cladosporium sp.

Thus, the improvement of seed storage conditions for better seed quality requires the use of techniques including adequate storage and preservation equipment.

4. 2 Symptomatic leaves

From the macroscopic and microscopic results and their studied characters, the genus of six isolates including Macrophomina, Phoma. Fusarium and Rhizopus is concluded. These strains are therefore responsible for the infection of cowpea leaves. If we compare these results with the disease symptoms found for each leaf, we find that it is not the same pathogen cited in the literature. the pathogens responsible Indeed. for cercosporiosis and rhizoctonia are Cercospora cruenta and Rhizoctonia solani respectively, while the pathogens responsible for leaf rust and smut are Uromyces appendiculatus and Entyloma vignae respectively.

The genus *Fusarium* is a large cosmopolitan genus of imperfect fungi. It is one of the most important mycotoxigenic fungal genera in food and feed. The well-known Fusarium mycotoxins are fumonisins, deoxvnivalenol zearalenone. and other trichothecenes. The identified Fusarium species are three (3): Fusarium sp., Fusarium avenaceum and Fusarium decemcellulare. Earlier studies by Oluyemisi et al. [11] confirmed the presence of Fusarium sp. Most Fusarium species are able to grow as saprophytes. Burgess *et al.* [1] demonstrated that in the soil, Fusarium can persist for several years through the formation of chlamydiospores or through the development of hyphae on organic residues. This could explain the presence of Fusarium sp. in seeds and leaves. This fungus can be considered as telluric. In addition, many saprophytic species are able to develop as secondary pathogens on senescent plant tissue. Fusarium decemcellulare and Fusarium avenaceum are recognised as pathogens.

The genera *Rhizopus*, *Macrophomina* and *Phoma* contain pathogenic species of cowpea that cause diseases such as black crown rot, seedling blight and leaf spot. They are widespread plant pathogenic fungi. The presence of these fungi on the leaves could be explained by their ability to develop under various climatic conditions. They can also be transported by compost or manure, water or wind to the leaves. The leaf is the essential element for the survival of a plant. The plant feeds itself through the leaves.

Indeed, thanks to the leaves, the plant captures and separates CO_2 to feed itself.

It has the function of photosynthesis and respiration, which are unavailable for the proper growth of the plant.

Thus, the presence of pathogenic and saprophytic fungi can hinder the development process of the plant and even lead to its death. Cowpea leaves used as food vegetables, if infected, can be dangerous for human health.

4. 3 Leaves and seeds

The identification of fungi on cowpea seeds and leaves shows the presence of three fungi identified on both seeds and leaves. This diversity of identified fungi could be explained by certain climatic and soil factors that are favourable to fungal development. Their presence could also be explained by the fact that these fungi are telluric. A telluric fungus is a fungus present in the soil. These organisms are characterised by the fact that they persist and survive for some time in the soil, in infected plant debris and plant residues. Indeed, according to Emechebe and McDonald [13], Emechebe [14] the pathogen is preserved during the dry season by its acervuli in contaminated seeds, soil and plant debris, which constitute the primary sources of inoculum for the disease. The soil is thus a reservoir of inoculum for these pathogens. It is possible that these fungi are also systemic fungi, i.e. they spread through vessels.

5 Conclusion

This study allows the determination of the major fungal strains associated with Bambara groundnut seeds in Burkina Faso. it appears that cowpea seeds and leaves contain a diversity of fungal species.

The inventory and identification revealed two genera of parasitic fungi, one genus of saprophytic fungi and one genus of fungi that can be classified as saprophytic or parasitic in the case that it produces mycotoxins, from the analysis of the seeds. Also, this study revealed that the number of fungi identified varies from one genotype to another.

The analysis of the pathogenic diversity of fungal strains of symptomatic leaves allowed us to identify four genera of parasitic fungi. The results of this study also revealed the existence of several pathogenic fungal strains involved in cowpea leaf and seed disease.

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