Original Research Article

Phenotypic characterization of fungal pathogens associated with the main mycoses of cashew(*Anacardium occidentale* L.)

ABSTRACT

Aims: To study the diversity of fungal pathogens associated with cashew mycoses in Togo.
Study design: This research project was initiated by the Mycology Research and Applications Unit of the Botany and Plant Ecology Laboratory (LBEV) in order to have adequate information on cashew mycoses in Togo.
Place and Duration of Study: Laboratory of Botany and Plant Ecology (LBEV) of the University of Lomé (UL) and Laboratory of Crop Protection and Biosafety Laboratory of Togolese Institute of Agronomic Research (ITRA), February to August 2020.
Methodology:A total of 148 symptomatic samples (leaves, buds, inflorescences, nuts, and apples) were collected from cashew trees in the East Mono prefecture of Togo. Malt-agar medium supplemented with chloramphenicol at 0.5g/l was used for the isolation of fungal pathogens. The characterization of these fungal pathogens was carried out from the 7th day based on their macroscopic(texture, color, diameter of growth) and microscopic(hypha, spore, fruiting body) characters.
Results: This study revealed the presence of five mycoses in cashew orchards in the East Mono prefecture. These are leaf anthracnose, bud's dieback, black rust, leaf yellowing, and powdery mildew. In total, 12 fungal genera were encountered and 14 species of fungal pathogens were identified on all the samples collected : *Rhizopus sp., Penicillium sp., Mucor sp.,Sporotrichum sp., Fusarium nivale, Fusarium moliniforme, Fusarium moliniformevar. subglutinans, Curvularialunata, Curvulariageneculata,Alternaria tenuissima, Alternaria brassicisola, Beltraniarhombica*Penz., *Thielaviacoactilis*Nicot, *Helminthosporiumavenae, Helminthosporiumsiccans, Phomaeupyrena, Aspergillus flavus, Aspergillus niger.*
Conclusion: It would be of great interest to train small cashew and cashew producers in the East Mono prefecture on the recognition of the symptoms of these mycoses and their management.

Keywords: Anacardium occidentale L., mycoses, fungal pathogens, phenotypic characterization, Togo.

1. INTRODUCTION

Cashew (*Anacardium occidentale* L.) is a tropical perennial plant native to the Brazilian coast [1]. From Brazil, this plant has been introduced to other tropical regions of the world including Africa where interest in its cultivation is becoming increasingly important with a share in global production of 37% in 2018 [2]. It is cultivated for the production of cashew nuts and its pseudo fruit (apple) due to their high nutritional value [3]. The demand for cashew nuts and other cashew products is constantly increasing. However, the sustainability and stability of cashew production in these tropical areas, particularly in the West African sub-region, is increasingly threatened in the current context of global change. The change in agro-pedoclimatic conditions, especially the irregularity of rainfall, the attacks of bio-aggressors in the cultivation areas,

sometimes associated with the lack of good agricultural practices, are at the origin of the low productivity of cashew trees. Among the pests of cashew, many fungal pathogens are reported to be associated with various mycoses symptoms observed on different plant organs. Production losses due to pests can reach 72% for anthracnose [4], 50 to 70% for powdery mildew [5, 6] and 48.8% for black rust [7].

Various epidemiological studies on cashew worldwide have shown that cashew is subject to attack by fungal pathogens, some of which are directly responsible for mycoses and others which are considered to be superinfection agents.

Reference [8] shows that the fungi *Colletotrichum gloesporoiodes*, *Oidium anacardii* and *Phomopsis anacardii*are respectively responsible for anthracnose (leaves, nuts, and pseudofruits), powdery mildew (leaves, inflorescences, and pseudofruits) and buds dieback. Other fungal pathogens such as *Aspergillus*spp, *Fusarium*spp, *Penicillium*sp, *Curvularia*sp, have been associated with various organs (leaves, flowers, buds, nuts, apples, and bark) showing symptoms of mycoses [9].

The attack of an organ by a fungal pathogen and its subsequent infection by other superinfectants can slow down the growth of that organ and the development of the plant in general. Consequently, the various mycoses symptoms reported on cashew would have impacts on plant productivity but also on the growth and organoleptic quality of apples and cashews[9, 10]. These biotic constraints lead to significant yield losses.

In Togo, very little scientific work has been done on the study of cashew tree diseases and the various associated pathogens. In the context of sustainable management of resources, particularly agricultural resources, it is essential to obtain this scientific data in order to lay the foundations for the agroecological management of our cashew orchards.

The objective was to study the diversity of fungal pathogens of cashew trees. In order to understand this fungal diversity, different symptoms of mycoses on cashew trees were inventoried and a phenotypic characterization of fungal strains isolated from symptomatic organs was carried out.

2. MATERIAL AND METHODS

2.1 Study Areas

A phytosanitary survey was carried out in February 2020 during the long dry season in seven localities (Elavagnon, Gbadjahe, Kamina, Badin-cope, Moretan, Kpessi, and Nyamassila) in the prefecture of Est-mono. It is located between 6°31 and 8°22 North latitude and between 0°32 and 1°38 East longitude and is in the lowland zone of the plateau region of Togo. In each locality, a cashew orchard of an average size of two hectares was randomly selected and surveyed.

2.2 Plant Material

The cashew tree (*Anacardium occidentale* L.) was the subject of this study. Almost all the cashew orchards surveyed are farmers' orchards, and in most cases, they are a mixture of varieties. These are mainly red, light red, golden yellow and yellow apple varieties.

2.3 Inventory and sampling

Within each orchard, visible mycoses symptoms on cashew plants were identified using a cashew mycoses board and also confirmed by a plant pathologist. Samples of leaves, buds, inflorescences, nuts, and apples showing a symptom associated with a target mycosis were taken from five randomly selected plants at least 60 meters apart. For each sample, a collection number, the name of the site, the date of the survey, the geographical coordinates, and the address of the orchard operator were filled in. The samples were packed in transparent sterile envelopes and transported to the Mycology Research and Applications Unit of the Botany and Plant Ecology Laboratory (LBEV), University of Lomé (UL) where they were stored at 4°C [9].

2.3 Preparation of the culture medium

Malt extract agar was prepared and poured into 9 cm diameter Petri dishes according to the manufacturer's instructions (OXOID CM0059 Malt Extract Agar).

2.3 Disinfection and plating of samples

3 mm explants, taken with a sterile scalpel from the edge of the stains or necroses of the different samples were washed thoroughly with tap water. Then, under a sterile horizontal laminar flow hood, the explants were immersed for 5 minutes in a 1% (v/v) aqueous solution of sodium hypochlorite (20°Chl bleach), then in 96° ethanol for 1 minute and finally rinsed three times with sterile distilled water.

Depending on the site and the symptom of the mycoses, four explants were grown in duplicate on malt agar medium in Petri dishes. These seeded dishes were incubated in the dark at room temperature (25±2°C).

2.4 Spawning and isolation of fungal strains

The inoculated Petri dishes were observed daily from the third day. Any colonies that appeared were individually subcultured onto new Petri dishes containing sterile malt extract agar.

2.5 Characterization of fungal strains

The characterization of fungal strains was carried out following the approach described byDufresne and Guy[11]. This approach is based on macroscopic (texture, color, diameter of growth) and microscopic (hypha, spore, fruiting body)descriptions of the colonies. From the macroscopic and microscopic characteristics of the isolated fungal strains, identification was made using the identification keys [11-15].

2.6 Conservation of fungal strains

The identified fungal strains were kept on Malt-agar medium tilted in the tubes at laboratory room temperature (25±2°C).

3. RESULTS

3.1 Symptoms of fungal diseases inventoried

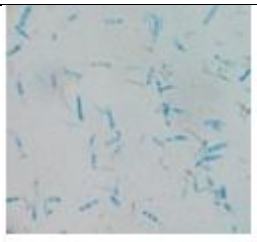
This study identified a total of 5 mycoses on cashew trees in the Est-Mono prefecture through their respective symptoms on infected organs. These are leaf anthracnose (Figure 1A), bud's dieback (Figure 1B), black rust (Figure 1C), leaf yellowing (Figure 1D), and powdery mildew (Figure 1E).

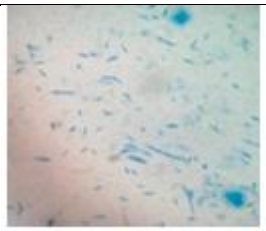
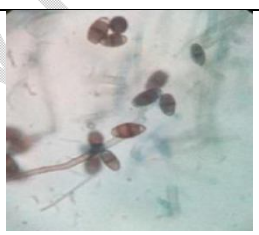
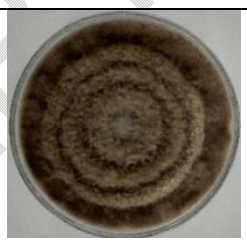
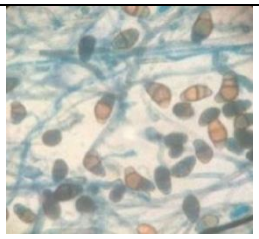
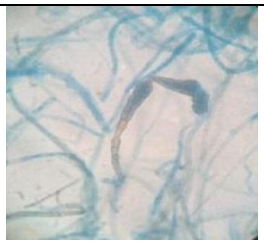
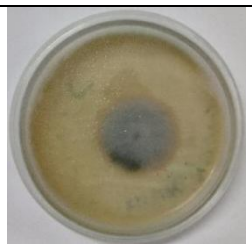
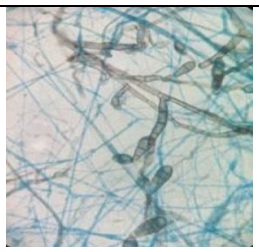
Figure 1: Symptoms associated with identified fungal diseases in cashew
Leaf anthracnose (A), bud's dieback (B), black rust (C), leaf yellowing (D), and powdery mildew (E) [16].

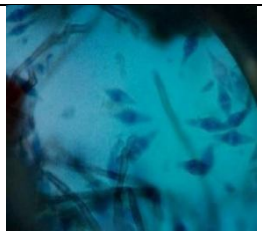
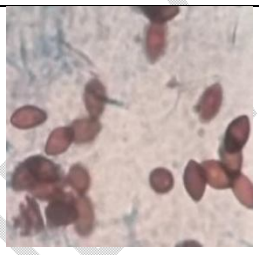
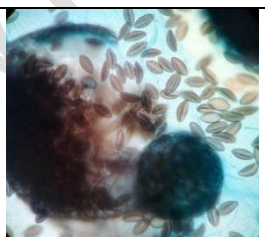
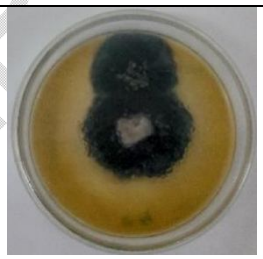
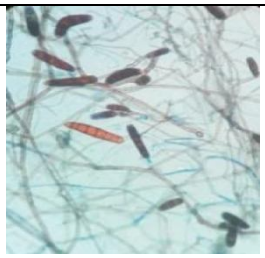
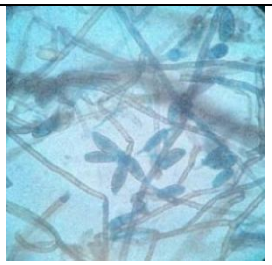
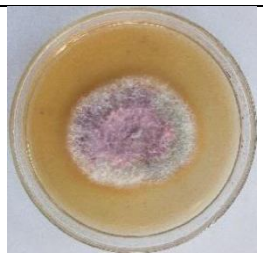
3.2 Isolation and characterization of fungal pathogens

From the organ samples collected during our survey, a total of 102 fungal strains were isolated. The identification allowed us to count twelve (12) fungal genera divided into 14 characterized fungal species and 4 undetermined fungal species (Table 1). The latter were classified according to the mycosis's symptoms identified (Table 2).

Table 1. Macroscopic and microscopic characteristics of fungal strains

Species	Characterization	Macroscopic aspect	Microscopic aspect
Fusarium nivale	Colony: woolly, pure white, 8.5 cm Hypha: septate Spores: hyaline, only slightly curved macroconidia with 3-7 septa		

Fusarium moliniforme	Colony: woolly, pinkish white, 7.3 cm. Hypha: septate Spores: hyaline, unicellular piriform microconidia mixed with slightly curved macroconidia with 3 to 7 septa		
Fusarium moliniforme Var. subglutinans	Colony: powdery, pinkish white, 5.5 cm Hypha: violet and septate Spores: consisting of claviform microconidia mixed with curved, multi-celled macroconidia.		
Curvularialunata	Colony: woolly, olive-brown, 6 cm. Hypha: brown and septate. Spores: brown, with 3 to 4 septa		
Curvulariageneculata	Colony: woolly, olive brown, 4 cm Hypha: brown and septate, Spores: brown, 3 septate, and curved in the central cell.		
Alternaria tenuissima	Colony: fluffy, greyish white, 2.3 cm Hypha: brown and septate Spores: brown, multi-celled, pyriform and chain-like.		
Alternaria brassicisola	Colony: downy to woolly, grey, 2.7 cm Hypha: brown and septate Spores: brown, multi-celled, short and chain-like		

*Beltraniarhombica*Penz.	Colony: fluffy to powdery, white-black, 2cm Hypha: brown and septate Spores: faintly brown, numerous, unicellular, biconical, hyaline band present.		
Sporotrichum sp.	Colony: velvety to powdery, white, 6.8 cm Hypha: septate Spore: golden yellow, unicellular, oval to ellipsoidal.		
*ThielaviaCoactilis*Nicot	Colony: fluffy to powdery, white, 3.5cm. Hypha: septate Asci: dark brown, subglobose, smooth and thin. Spore: dark brown, unicellular, obovate to ellipsoidal, with subapical pore		
Helminthosporiumavenae	Colony: downy, dark brown, 4.6 cm. Hypha: brown and septate. Spore: dark brown, oblong, multi-celled, rarely curved.		
Helminthosporiumsiccans	Colony: downy, dark green, 5.5 cm. Hypha: brown and septate Spores: brown, almost cylindrical, multi-celled and in an alternating arrangement.		
Phomaeupyrena	Colony: fluffy to powdery, pink-grey, 4.5 cm Hypha: septate Pycnidia: dark brown, with an ostiole, Spores: hyaline, unicellular and cylindrical.		

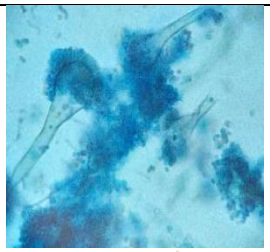
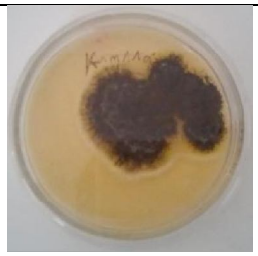
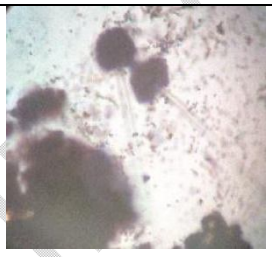
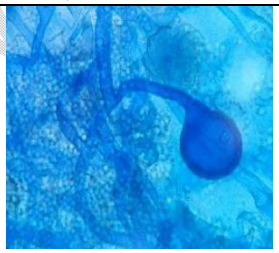
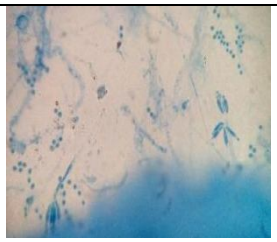
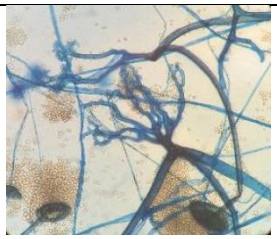
Aspergillus flavus	Colony: powdery, green-yellow, 6 cm. Hypha: brown conidiophores and not septate Spores: more or less dark, globose, borne by an aspergillate head.		
Aspergillus niger	Colony: powdery, dark brown, 4 cm Hypha: conidiophores light brown and not septate Spores: dark, globose, numerous, arranged in a chain like aspergillate head.		
Mucor sp.	Colony: fluffy to powdery, grey-white, 6 cm Hypha: hyaline and rarely septate Spores: hyaline, borne on columella, subglobose.		
Penicillium sp.	Colony: powdery, green-blue, 3.2 cm Hypha: brown and septate Spores: arranged in chains on sterigmata, weakly coloured, subglobose and smooth.		
Rhyzopus sp.	Colony: woolly, white-grey, 1.8 cm Hypha: brown and not septate Spores: brown, subglobose, borne on columella.		

Table 2. Fungal species associated with cashew fungal disease symptoms

Mycoses	Associated fungal species
Leaf anthracnose	*Alternaria tenuissima, Aspergillus flavus, Aspergillus niger, Curvulariageneculata, Curvularialunata, Fusarium nivale, Helminthosporiumsiccans, Mucor* sp., *Penicillium* sp., *Rhizopus* sp.
	Alternaria tenuissima, Aspergillus niger, Aspergillus flavus, Curvulariageneculata,

Buds dieback	*Curvularialunata, Fusarium moliniforme, Fusarium nivale, Helminthosporiumsiccans, Sporotrichum*sp, *ThielaviaCoactilis*Nicot.
Black rust	*Alternaria tenuissima, Aspergilus flavus, Aspergilusniger, Curvulariageneculata, Curvularialunata, Fusarium nivale, Helminthosporiumsiccans, Helminthosporiumavenae, Penicillium* sp, *Sporotrichum*sp
Leaf yellowing	*Alternaria brassicicola, Curvularialunata, Phomaeupyrena*
Powdery mildew	*Aspergillus niger, Beltraniarhombica*penz., *Fusarium moliniforme var subglutinans.*

3.3 Discussion

Phytosanitary surveys in cashew orchards in the Est-Mono prefecture identified the following fungal diseases: leaf Anthracnose, buds dieback, black rust, leaf yellowing, and powdery mildew. Many studies on cashew diseases have reported their characteristic symptoms. On cashew trees, anthracnose has been reported on leaves, branches, inflorescences, young apples, and nuts of cashew. Young infected leaves turn orange-brown to light red with age and sporulation of the fungus [17]. Anthracnose attacks other tropical fruit plants such as avocado, banana, citrus, mango, and papaya [18]. Buds' dieback and twig necrosis are the characteristic symptoms of dry of buds [8,19]. These symptoms are similar to fire blight [16]. The black rust symptom on cashew trees was first reported on cashew nuts in Tanzania [20]. According to the work ofMengee*t al.*[21,22], young infected nuts turn black while older nuts show characteristic dark lesions. Leaf yellowing has been reported in cashew orchards in Benin [23, 24]. These authors observed round yellow spots on the leaf surface of some cashew trees. Finally, powdery mildew attacks the young organs and tender tissues at the upper level of the cashew tree [25]. Its symptom on infected organs is very characteristic. When it attacks the leaves, they show whitish or grey-white sporulating colonies on their upper surface, which are easily visible to the naked eye. In addition, when these fall off, they usually leave purplish-brown spots on the main vein of the leaf [17]. The mycoses symptoms reported in this study are similar to those described in similar studies. Another similar study in the Tchamba prefecture (Togo) revealed the presence of buds' dieback, anthracnose, leaf yellowing, black rust, and gum disease in cashew orchards [26].

The response of plants to pest attacks is very often expressed externally as symptoms or spots, which are often clearly visible on the infected organs. In cashew, the pathogens responsible for the main mycoses are well known. Afouda*et al*. [27] show that the fungus *Colletotrichum gloeosporioides* (Penz.) is the causal agent of anthracnose. Other studies have shown that *Phomopsis anacardii* species is the causal agent of buds' dieback [8], while black rust is caused by a fungus of the genus *Cryptosporiopsis* sp. [20], obligate parasite of cashew [17]. As for leaf yellowing,Aihounton [23] and Lissava[24]considered it to be a mycosis and its pathogen could be identified by fungal analysis. As for powdery mildew, the species *Oidium anacardii* Noack was identified as the causal agent [5]. It should be noted that these fungal pathogens couldn't be isolated in this study. This would be related to the technique of incubation of the explants and purification of the fungal strains on the one hand, and the choice of the culture medium (MEA) on the other hand. The work ofN'Guettia*et al*. [28] on pathogenic fungi demonstrated that acidified PDA medium (5% citric acid) was able to isolate the fungus of the genus Colletotrichum sp. Furthermore, the efficiency of incubation at 28°C under alternating cycles of 12 hours of light and 12 hours of darkness for 7 days before transferring fungal strains to Malt Extract Agar medium was proven [9]. This may suggest that MEA medium is better suited for the preservation of fungal strains than for isolation. Another plausible reason for the difficulty in isolating *Cryptosporiopsis* sp. and *Oidium anacardii* Noack associated with black rust and powdery mildew respectively is that they are obligate parasites [29].

Fungal analysis from explants collected from symptomatic cashew samples revealed a wide diversity of fungal pathogens. Since the causal agents of the main mycoses of cashew are known, it is clear that the fungi identified in this study should be considered as superinfection pathogens. Our results are similar to those of [9], which reported *Aspergillus flavus*, *Aspergillus niger*, *Mucor* sp., *Penicillium* spp. and *Rhizopus* sp. on cashew organs infected by anthracnose, pestalotiosis and powdery mildew. In addition, *Aspergillus niger*, *Fusarium* sp., *Penicillium* sp. and *Rhizopus* sp. have been implicated in kernel rot of mature and immature cashew nuts [30]. *Alternaria* sp. and *Curvularia*sp. have been reported to be associated with fungal symptoms on infected cashew leaves, nuts or apples [9]. However, *Beltraniarhombica*penz., *Thielaviacoactilis*Nicot. and *Sporotrichum* sp. have so far not been reported in the literature as fungal pathogens associated with cashew trees. Indeed, the species *Beltraniarhombica*penz. [31] and *Thielaviacoactilis*Nicot. [32] have been reported in the literature as simple pathogens and have been reported on soybean seeds in Kenya and on leaves of *Paphiopedilum* sp. in India, respectively. In addition, the species *Sporotrichum* sp. has been reported as a soil-borne fungus [33,34]. These results add to the list of fungal pathogens associated with cashew. Furthermore, these fungi identified as superinfection pathogens can become true pathogens under particularly favorable environmental conditions.

4. CONCLUSION

At the end of this work, it appears that the symptoms of leaf anthracnose, bud's dieback, black rust, leaf yellowing, and powdery mildew are the mycoses identified in cashew orchards in the Est-Mono prefecture in Togo. The characterization of fungal strains associated with these different mycoses' symptoms revealed a great diversity of fungal pathogens in cashew. Based on the results of this study, it is important to sensitize cashew farmers in the study area on good agricultural practices controlling the spread of these fungal diseases.

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