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Foliar fungal diseases of banana in the central region of Burkina Faso: Characterisation of pathogens

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Banana plantations in Burkina Faso are confronted with climatic hazards and parasitic attacks resulting in a considerable yield drop. Unfortunately, few studies have been devoted to banana leaf diseases. This study was initiated to characterize the major foliar fungal pathogens that hinder the development of this crop in the Centre region. Four communes were involved which are Koubri, Loumbila, Pabré, and Tanghin-Dassouri, where a total of eighty samples of symptomatic leaves only were taken at random. From the different analyses, seven genera which are *Alternaria*, *Bipolaris*, *Mycosphaerella*, *Colletotrichum*, *Curvularia*, *Exserohilum,* and *Fusarium,* and fifteen species were identified. In terms of diversity, the commune of Koubri contains fourteen of the species identified, but the commune of Tanghin-Dassouri has the highest infestation rate (42.52%). The high prevalence of the disease in the commune of Tanghin-Dassouri could be explained by environmental conditions because in this site banana production is done in the shallows. To improve banana production in the central region of the country, it is imperative that studies be carried out to gain an exhaustive knowledge of the harmful fungi in this sector and that prophylactic measures be taught to all producers in order to promote the sector.

Key words: Pathogenic fungi, leaves, banana, Kadiogo province, Burkina Faso.

INTRODUCTION

In Burkina Faso, banana is the third most important fruit crop after mango and citrus (Coulibaly, 1999). Bananas were first grown traditionally in the lowlands before becoming a more or less intensified crop following the intervention of the 'Fruit Project' in the mid-1970s (Festas, 1996). Indeed, the Fruit Project, which later became Flex Faso, was the first structure responsible for popularising banana varieties and modern banana growing techniques in Burkina Faso (Coulibaly, 1999). From then on, a number of producers started growing bananas, giving rise to numerous small farms grouped around water points such as rivers and dams (Festas, 1996). Since then, the primary objective of banana production in Burkina Faso has been to satisfy growing

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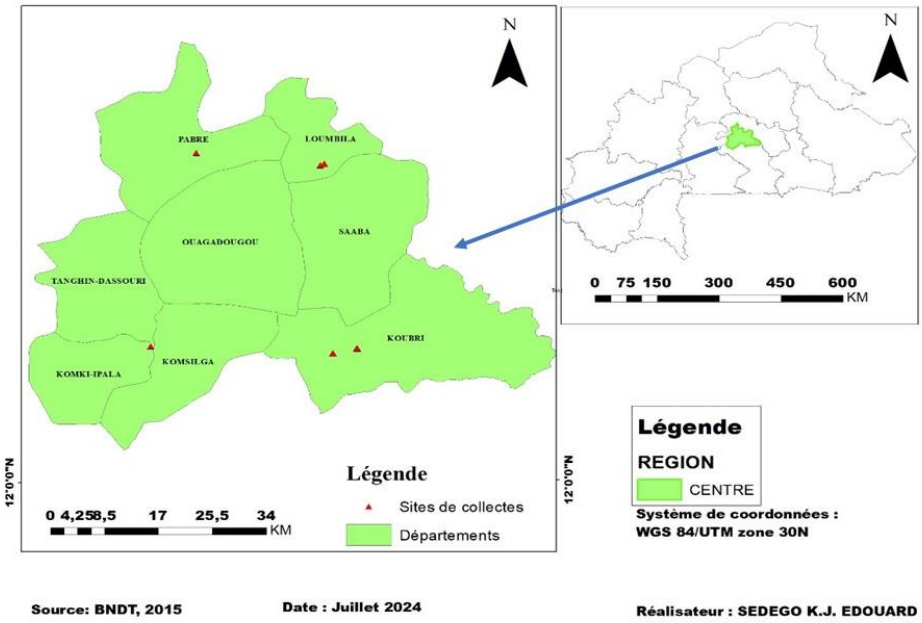

Figure 1. Location of collection sites.

domestic demand, with the development and expansion of major urban centers such as Ouagadougou and Bobo-Dioulasso (Coulibaly, 1999). According to the same authors, demand rose from 12,000 t in 1996 to 15,000 t between 1998 and 1999. The actual supply is estimated at 6,000 t for an area of 400 ha of banana plantations. Between 2007 and 2019, production rose from 13,000 to 140,000 t, with sales of between CFAF 1.68 billion and over CFAF 40 billion, according to Table Filière Banane (TFB) (2020) for the Hauts-Bassins, Boucle du Mouhoun, South-West and East regions of the country. Unfortunately, this crop is subject to a variety of bio-aggressors, including nematodes, fungi, bacteria, viruses, and insect pests, which cause damage ranging from 20% to total destruction of the banana plantation (Coulibaly, 2002). To ensure sustainable and effective pest management for banana production in Burkina Faso, it is essential to carry out an inventory followed by a characterization of these pests. For this reason, this study was initiated to inventory and identify foliar fungal pathogens of bananas in four localities in the Centre region of Burkina Faso.

MATERIALS AND METHODS

Experimental site

Four rural communes in the province of Kadiogo, which are major banana producers in the Centre region, were surveyed and samples of banana leaves showing symptoms of fungal diseases were collected (Figure 1). These are Koubri, Loumbila, Pabré, and Tanghin-Dassouri, all of which belong to the Sudano-Sahelian zone, where rainfall is very inadequate and irregular from one year to the next. Rainfall rarely exceeds 700 mm per year (Ouédraogo and Piché, 2007).

Collecting samples and obtaining pure isolates

Samples were collected only from diseased leaves of Cavendish Group bananas. These symptomatic leaves were collected in four communes which are Koubri, Loumbila, Tanghin-Dassouri, and Pabré. Diseased leaves were taken from banana trees at random using the Madden (2009) technique, labeled, then packed in sterile mini-grip plastic bags and kept in a cooler containing ice before being transported to the Phytopathology and Tropical Mycology laboratory for analysis. The samples were incubated using the technique proposed by Bouakaz and Oussaid (2013). Conidia were observed using a binocular magnifying glass following the procedure of Mathur and Kongsdal (2003). Finally, the conidia were transferred to the Potato dextrose agar (PDA) culture medium under the same magnifying glass using a flame-sterilized drawn glass capillary (INRA, 2015). Pure isolates were obtained after three to four days of incubation at room temperature (22-25°C) alternating 12 h of near-UV light and 12 h of darkness.

Morphological identification of fungi

The various isolates were characterized morphologically, first with the naked eye and then with a binocular magnifying glass. The technique of Botton et al. (1990) was used to determine the cultural characteristics and color of the colony during its development. Microscopic examination of the isolates was carried out using a ZEISS Primo star binocular optical microscope. The method proposed by Badillet et al. (1987), was used to identify septate or siphonal thallus, the shape of the spores, and the presence or absence of the protective forms of the chlamydospores. All photos were taken at the highest magnification (10×100). Identification was possible using the identification keys proposed by Mathur and Kongsdal (2003).

Table 1. Distribution of pathogenic fungi by communes.

Fungus	Communes					
	Koubri	Loumbila	Pabré	Tanghin-Dassouri	Total isolates	Percentage
Fusarium incarnatum	x	x	xxxx	-	6	11.11
Fusarium chlamydosporium	x	x	xxxx	-	6	11.11
Fusarium longifundum	x	x	xxxx	-	6	11.11
Fusarium spp.	-	x	xxxx	-	5	9.26
Curvularia lunata	x	-	-	x	1	1.85
Curvularia spp.1	x	x	-	x	3	5.55
Curvularia spp.2	x	x	-	xxxx	3	5.55
Colletotrichum spp.	x	-	xx	-	7	13.00
Exserohilum rostratum	x	-	-	-	1	1.85
Exserohilum spp.	x	-	-	-	1	1.85
Alternaria spp.1	x	-	xx	-	3	5.55
Alternaria spp.2	x	-	xx	-	3	5.55
Bipolaris spp.1	x	-	-	x	2	3.70
Bipolaris spp.2	x	-	-	x	2	3.70
Mycosphaerella spp.	x	-	xx	xx	5	9.26
Total species by site	14	6	8	6	54	100

x = 1 isolate, xx = 2 isolates, xxxx = 4 isolates.

Prevalence of foliar phytopathogenic fungi at collection sites

Prevalence was assessed by dividing the number of symptomatic foliar samples by the total number of samples collected from the site. The formula used is as follows:

$$\text{Prevalence} = \frac{\text{number of diseased samples}}{\text{total number of samples on the site}} \times 100$$

Analysis and expression of results

The software Microsoft Excel 2019 was used for data entry, data organization, and graphics.

RESULTS

Diversity of foliar parasitic fungi of banana identified according to collection sites. A total of 80 samples of diseased banana leaves were collected in the communes of Koubri, Loumbila, Pabré, and Tanghin-Dassouri. The analysis identified seven (7) genera of fungi containing phytopathogenic species which are *Alternaria*, *Bipolaris*, *Mycosphaerella*, *Colletotrichum*, *Curvularia*, *Exserohilum*, and *Fusarium*; and fifteen (15) species, namely *Fusarium incarnatum*, *Fusarium chlamydosporium*, *Fusarium longifundum*, *Fusarium* species., *Curvularia lunata*, *Curvularia* species 1, *Curvularia* species 2, *Colletotrichum* species, *Exserohilum rostratum*, *Exserohilum* species, *Alternaria* species 1, *Alternaria* species 2, *Bipolaris* species 1, *Bipolaris* species 2 and *Mycosphaerella* species. In the commune of Koubri, all species were represented except *Fusarium* spp. In the commune of Pabré, however, eight (8) species were counted. Finally, in the communes of Loumbila and Tanghin-Dassouri, six (6) pathogenic fungal species were identified in each of these sites (Table 1). In terms of species distribution, no fungal species were common to the four sampling sites. However, three species (*F. incarnatum*, *F. chlamydosporium*, *F. longifundum*) were common to Koubri, Loumbila, and Pabré, and four species (*Curvularia* spp.1, *Curvularia* spp.2, *Colletotrichum* spp., and *Mycosphaerella* spp.) to Koubri, Loumbila and Tanghin-Dassouri. A summary of the distribution of fungal species by site is shown in Figure 2.

Infestation rate at collection sites

Characterization of the fungi enabled the isolation of 54 isolates from the 70 samples collected. The diversity results show that the commune of Koubri has the greatest fungal diversity with fourteen (14) species out of fifteen (15) identified. But calculating prevalence shows that the commune of Tanghin-Dassouri (42.52%) has the highest infestation rate, followed by Pabré (32.29%), Koubri (26.34%) and Loumbila (5.4%) (Figure 3).

Description of symptoms on banana leaves

Several symptoms were observed on the leaves. These consisted of small dots and dashes on the underside of the leaves. Other rounds (Figure 4A) to elliptical (Figure 4B) leaf spots with regular and irregular outlines (Figure 4C),

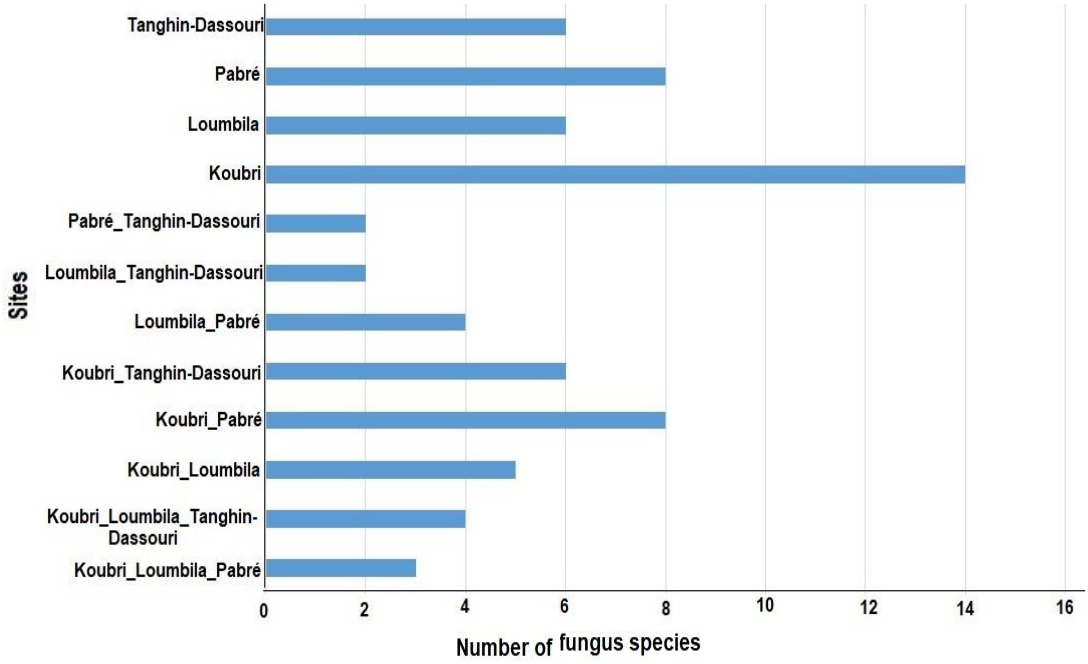

Figure 2. Distribution of the number of pathogenic fungal species by site.

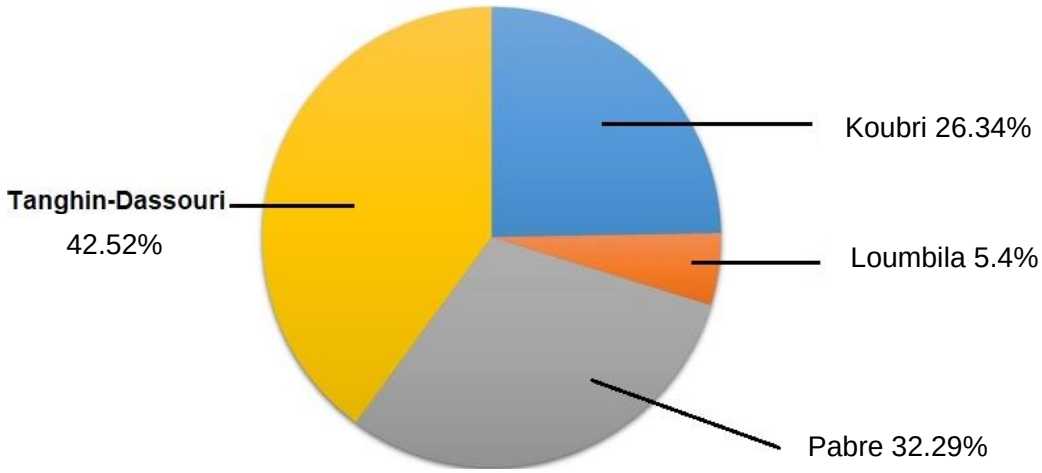

Figure 3. Disease prevalence by collection site.

dark brown to blackish up to 1 to 2 cm long or even 3 cm long were observed on almost all farms. These spots were often greyish in the center, limited by a dark border, and sometimes surrounded by a yellowish halo (Figure 4D). Also, the leaves of some banana trees were dotted with greenish to blackish flecks, then turned yellow and brown with age, especially on older leaves (Figure 4E). When the leaves were severely affected, it was as if they had been sunburnt, which could reach the main veins (Figure 4F), causing them to dry out. In most cases, these symptoms were observed mainly on the periphery of the leaves.

Identification of some of the pathogenic fungi collected

The collection, isolation, and purification work enabled us to identify 15 species which were *F. incarnatum*, *F. chlamydosporium*, *F. longifundum*, *Fusarium* spp., *C. lunata*, *Curvularia* spp.1, *Curvularia* spp.2, *Colletotrichum* spp., *E. rostratum*, *Exserohilum* spp., *Alternaria* spp.1,

Figure 4. Different fungal symptoms were observed on banana leaves. A) brown streaks, B) ellipsoid shapes of symptoms, C) irregular contours, D) grey center with a yellow halo, E) browning of older leaves, and F) drying stage.

Alternaria spp.2, *Bipolaris* spp.1, *Bipolaris* spp.2 and *Mycosphaerella* spp. The isolates, hyphae, and conidia of some of the fungal species are shown in Figure 5.

DISCUSSION

Like all other plants, bananas are subject to numerous fungal diseases, as shown by the results of the characterization of fungi isolated from symptomatic leaves sampled at our sites. The results show that all banana crops in our four communes are subject to parasitic attacks of a fungal nature, as evidenced by the seven (7) genera identified such as *Alternaria*, *Bipolaris*, *Mycosphaerella*, *Colletotrichum*, *Curvularia*, *Exserohilum* and *Fusarium*. Of these, the genera *Mycosphaerella*, *Colletotrichum*, *Exserohilum,* and *Fusarium* are thought to contain pathogenic species that are highly damaging to banana production (Jones, 2000a). These so-called fungal diseases are most often caused by spores or conidia which, in favorable conditions, germinate and give rise to mycelial filaments that develop in the tissues of the host plant, extracting nutrients and exuding substances that are toxic to the plant (Naika et al., 2005). These diseases are mostly induced by phytopathogenic fungi or even a complex (Meddah et al., 2014). This could justify the presence of other genera such as *Curvularia*, *Bipolaris,* and *Alternaria*, which would therefore contain associated parasitic fungal species called “parasites of weakness”.

According to Péfoura and Mourichon (1990), cercosporiosis is one of the main foliage diseases most damaging to bananas. They cause leaves to dry out and it has been shown that the reduction in this resource has a marked effect on fruit filling (Ramsey et al., 1990). However, banana leaf removal experiments show that fruit filling is little affected as long as 5 to 7 leaves are present from flowering to harvest (Daniells et al., 2001). In addition, Robinson et al. (1992) reassure that photosynthetic compensation mechanisms have been demonstrated in young banana leaves when low leaves

Figure 5. Illustration of some isolates, hyphae, and/or conidia observed under the light microscope.
(A) isolate of *Exserohilum rostratum*, (B) hyphae and conidia of *Exserohilum rostratum*, (C) isolate of *Fusarium longifundum*, (D) conidia of *Fusarium longifundum*, (E) isolate of *Curvularia lunata*, (F) hyphae and conidia of *Curvularia lunata*, (G) isolate of *Curvularia* spp.1, (H) hyphae and conidia of Curvularia sp.1, (I) isolate of *Mycosphaerella* sp., (J) conidia of *Mycosphaerella* sp., (K) isolate of *Alternaria* spp.1, (L) hyphae and conidia of *Alternaria* spp.1, (M) isolate of *Bipolaris* spp.1, (N) hyphae and conidia of *Bipolaris* spp.1, (O) isolate of *Bipolaris* spp.2, (P) hyphae and conidia of *Bipolaris* spp.2.

are removed mechanically by growers following cercosporiosis. Bingham et al. (2009) add that a high phyllochron maximizes photosynthetic activity in the upper part of the foliage, which is also the part that contributes most to the production of assimilates because it is the part that intercepts the most solar radiation.

In addition to cercosporiosis, there are leaf diseases caused by *Mycosphaerella* species (Jacome, 2003). These cause moderate to severe damage wherever rainfall is abundant. Black stripe disease, also known as

black cercosporiosis and caused by *Mycosphaerella fijiensis* is very important. It occurs throughout the humid lowlands of the tropics and has a wide host range, including the Cavendish subgroup and plantain bananas. Ploetz et al. (2003) point out that in some tropical regions, foliar diseases caused by *Mycosphaerella eumusae*, Sigatoka disease caused by *Mycosphaerella musicola*, and speckle caused by *Mycosphaerella musae*, are equally if not more important. It can therefore be deduced that the strong presence of *Colletotrichum* fungi (4 isolates) and *Mycosphaerella* (2 isolates) in the Tanghin-Dassouri commune explains its higher infestation rate (42.52%) compared with the other communes. In addition, the environmental conditions certainly favored the development of these strains to the detriment of the other strains, as the Tanghin-Dassouri collection site was sufficiently humid, as in a lowland. The high humidity was probably beneficial for the development of *Colletotrichum* and *Mycosphaerella* fungi and inhibited that of *Fusarium, Curvularia, Exserohilum,* and *Alternaria*. At Koubri, on the other hand, environmental conditions were optimal and favored the development of 15 fungal species.

Fusarium or Panama disease is considered by Kung'u (1995) to be one of the most destructive diseases ever observed in bananas. Its causal agent is a soil-dwelling fungus, *Fusarium oxysporum* Schlecht f. sp. *cubense* (E.F. Smith) Snyd. and Hans. (Foc). It is a lethal and very common disease in bananas (Ploetz and Pegg 2000). It devastated the export trade before the 1960s and has a recently identified variant of the "Tropical Breed 4 (TR4)" which affects Cavendish cultivars, and it threatens production for export, and also the livelihood of small-scale farmers. This fungus is usually spread in the infested waste and plants, as well as in soil, water, tools, and agricultural machinery. It has the ability to survive several years in soils without its host. It can cause serious damage and have a significant economic impact. Early detection allows for appropriate control measures. Quarantine and exclusion measures provide effective control of the disease by restricting movement of bulbs, The results of this study are based on the analysis of the potential for the transfer of releases and soil from infected areas to healthy areas (Ploetz et al., 1995).

Other serious, but less worrying diseases, are the foliar disease caused by *Cladosporium musae* (*Cladosporium* speckle) and the freckle caused by *Guignardia musae*, post-harvest diseases of anthrachnosis and crown rot, caused primarily by *Glomerella musae*, and root rot caused by *Calonectria (Cylindrocladium*) specie. (Jones, 2000a; Jones, 2000b; Jones, 2000c; Muirhead and Jones 2000; Ploetz et al. 2003).

In view of the existence and negative impact of fungal diseases on the banana plant in Burkina Faso, it is imperative to make an exhaustive inventory of the pathogenic fungi that significantly reduce banana production. This should be followed by effective training for growers in the choice of tolerant varieties, early recognition of signs of the disease through regular inspection of all plantations, the creation of a service to monitor the introduction of shoots from infected areas, and the use of appropriate bio-fungicides to control the disease.

Conclusion

Several species of parasitic fungi were isolated from the various banana farms located in the four communes of the central region of Burkina Faso. In all, seven (7) genera and fifteen (15) species were identified. The genera *Mycosphaerella*, *Colletotrichum*, *Exserohilum,* and *Fusarium* are the groups of fungi that contain species potentially damaging to banana production. Identification of symptomatic leaves in the commune of Tanghin-Dassouri contained 2 isolates of *Mycosphaerella* and 4 isolates of *Colletotrichum*. The frequency of the disease explains the high rate of infestation in this commune (42.52%), followed by Pabré (32.29%), Koubri (26.34%), and Loumbila (5.4%). For the sustainable development of the banana sector in Burkina Faso, appropriate prophylactic measures need to be taught to both smallholders and large-scale producers to improve the quality and quantity of bananas, which is a condition of economic survival for producers.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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