



## **Major Leaf Diseases and Pathogenicity of Fungal Flora Associated with *Jatropha curcas* L. Foliar Diseases in Burkina Faso**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.*

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### **ABSTRACT**

The major foliar diseases and pathogenicity of fungal flora associated with leaf diseases of *Jatropha curcas* L were investigated in Burkina Faso. Prospecting and collection were carried out the plantations and hedges of *J. curcas* distributed in different climatic zones of Burkina Faso. The results indicate that foliar diseases are present in all climatic zones of the country. Mainly 4 foliar pathologies were recorded in the 18 sites of the study. These are yellowing of leaves with brown spots, yellowing of leaves with brown spots and black mycelium, drying of leaves from the border and virus diseases. The frequencies of observation of the various diseases show that the yellowing of the leaves with brown spots is the most disease predominant manifestation with a frequency of observation of 72%. Yellowing of the leaves with a presence of black spots and mycelium occupies the second rank with a prevalence of 33% followed by drying of leaves from borders and viroses with frequencies of 22 and 11% respectively. ANOVA analysis has shown that the climatic zone has a significant effect on the distribution of leaf viruses and leaf burns and a non-significant effect on

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yellowing of the leaves with or without brown spots and mycelium. The characterisation of fungal pathogens associated with these diseases identified *Fusarium oxysporum*, *Fusarium solani*, *Fusarium subglutinans*, *Phoma sorghina*, *Botrytis cinerea*, *Curvularia lunata*, *Botryodiplodia hreobromae*, *Cercospora cesami* and *Curvularia eragrostidis*. *Curvularia lunata* is the most widespread with a frequency of 44% followed by *Fusarium solani* with a frequency of 33%. The most seldom observed are *Cercospora cesami* and *Fusarium subglutinans* with a frequency of 10%. Among these characterised species, pathogenicity tests identified *Botryodiplodia threobromae*, *Curvularia lunata*, *Fusarium solani* and *Curvularia eragrostidis* as the pathogenic species of observed leaf diseases of *J. curcas*. These results confirm that *Jatropha* is infested by many fungal species. There is yet an urgency to develop a plant health program adapted to the local context to fight these fungal pathogens.

**Keywords:** *Jatropha*; diseases; fungal; leaves; Burkina.

## 1. INTRODUCTION

*Jatropha curcas* L. (Euphorbiaceae) is a multipurpose crop widely distributed in tropical and subtropical areas of Africa and Asia [1,2]. It has been noted for its environmental and economic purposes, and has evoked interest all over the tropics as a potential bio-fuel crop. It is a multipurpose crop with valuable attributes and considerable economic potential. *Jatropha* provides various products and benefits that contribute to poverty reduction, including the promotion of income-generating activities primarily for women (sale of seeds and soap) and for control of erosion by planting in hedgerows [4,5,6]. Also, the oil cakes obtained after oil extraction can be transformed into compost and used as green manure. The seed shell can also be used to make briquettes for cooking or for the production of timber [7].

Introduced into Africa during the colonial era, it is the richness of its seeds in oil that can be transformed into biodiesel that has created its new notoriety in many countries of West Africa including Burkina Faso [8]. This oil, considered as a potential fuel for biodiesel substitution, can be used directly in internal combustion engines or after trans-esterification [9,10]. Thus, since the 2000s, in a context of multiple food and energy crises in Africa, the culture of *J. curcas* for biofuel production has been intensified [11]. Several ambitious projects of large *J. curcas* plantations have thus emerged, sometimes spontaneous, sometimes supervised by several structures (NGOs, multinationals, associations, local authorities, etc.) [5,12].

Despite its reputation as a toxic plant for many microorganisms, insects and animals, one of the major constraints of *Jatropha* production today is

pest organisms [13,14,15]. *Jatropha* is infested by many insect pests and often shows symptoms of fungal attacks [16,17,18]. Significant losses have been reported, following damage caused by insects, fungi or viruses [6,16]. The fungal pathogens attack all organs (roots, neck, stem, leaf, fruits ...) of the plant at all stages of development including seeds and cause enormous damage [17,18]. This damage can cause significant yield losses [7], hence the need to develop an adapted method for control of pathogens [1,19]. Such approaches require knowledge of the pathogens involved and their damage in order to develop adequate control methods. Unfortunately, this approach is limited in Burkina Faso because of very little information on the fungal species associated with leaf diseases of the specie and their degree of pathogenicity. Indeed, the knowledge of fungal pathogens of *J. curcas* and their severity have not been established. This study was initiated in the aim to contribute to the knowledge of the main fungal leaf diseases of *J. curcas*, associated pathogens and their pathogenicity. It consisted in listing the main foliar diseases of the species in Burkina Faso, characterising the associated fungal flora and studying their pathogenicity.

## 2. MATERIALS AND METHODS

### 2.1 Sampling

The prospecting-collection was carried out between February and April 2017. During the survey, plants of *J. curcas* on plantations and in hedges (about ten per climatic zone) were observed and the plantations whose plants presented symptoms of foliar diseases were selected for further study. Eighteen (18) *J. curcas* plantations and hedges in the three climatic zones of the country namely Sahelian zone,

Sudano-sahelian zone and Sudanian zone were selected. The sampling thus constituted a collection of leaves showing symptoms. These leaves were put in labelled made with kraft paper and sent to the laboratory where they were kept in a refrigerator at 4°C.

## 2.2 Description of Sampling Zones

**The Sudanian zone:** It occupies the entire southern region of the country with a rainy season that lasts 6 months and a rainfall of 1200 mm and more. The number of rainy days is generally greater than 60. It is a zone of low thermal amplitude both daily and annually and high atmospheric humidity, especially in the rainy season.

**The Sudano-Sahelian zone:** It has between 900 and 600 mm annual rainfall and spreads all over the center of Burkina. It is the largest climatic zone in the country. The rainy season lasts 4 to 5 months. All climate parameters have medium values. It is a climate transition zone.

**The Sahelian zone:** It represents 25% of the territory of Burkina Faso and is bounded on the south by the 600 isohyet. It is the least rain-fed region whose levels sometimes go down to 150 mm with an average number of days of lower rainfall. The rainy seasons hardly exceed 3 months. The extremes and thermal amplitudes, both daily and annual, are very high, leading to a high loss of atmospheric humidity. The vegetation is mostly steppe adapted which includes several types; the shrub steppe with trees, the shrub steppe (tiger bush), the shrub steppe, the grassy steppe.

## 2.3 Identification of Foliar Diseases

It consisted of a description of the symptoms presented by diseased leaves [19].

## 2.4 Isolation and Characterisation of Fungi from Diseased Tissue

### 2.4.1 Isolation of pathogens

The isolation of pathogens was made on Potato Dextrose Agar (PDA) following the protocol described by Sharma et al. [23]. Leaves with fungal disease symptoms were used for pathogen isolation. Parts of leaves with disease symptoms were cut into small 5mm × 5mm fragments using a scalpel. The fragments obtained were disinfected respectively with 70 °

alcohol for thirty seconds and 1% bleach for one minute and then a series of three (03) rinses with sterile water. The fragments were then placed on Petri dishes at the rate of five (5) fragments per Petri dish containing PDA medium. The Petri dishes were then closed and sealed with parafilm then labeled and incubated under 12h of alternating UV light with 12h of darkness at 22°C for 96 hours.

### 2.4.2 Characterisation of pathogens

Pathogens purification and characterisation were performed according the method described by [20]. After 96 hours, each fungus growing from a sample was transferred individually to PDA-medium and cultures were incubated at 28°C under alternating cycles of 12 hours of light and 12 hours darkness for 7 days. After purification, the identification key of Mathur and Kongsdal [22] was used to identify the different fungal species according to the method described by [23]. Viruses' diseases were not taken into account later in the study.

## 2.5 Pathogenicity Tests

The pathogenicity tests were carried out according to Koch's postulate, which aims to prove that a fungus associated with a diseased tissue is the cause of the observed disease. Tests were made in the greenhouse. To carry out the test, seedlings were produced and artificial inoculations were carried out on the young leaves with inocula of the characterised fungal strains.

**Seedling production:** The seeds were sown to a depth of 2 cm using pots with a 2-liters capacity. Each pot contains a mixture of sand, potting soil and organic manure in the proportions 3/1/1. This mixture, previously sterilised at 120°C for four (04) hours, allows good aeration of the roots and contains enough nutrients for the development of the plant. Pots were maintained in the greenhouse and were then watered every day.

**Pathogenicity test:** Four weeks after sowing, five plants of each accession were inoculated with all of the isolates species that were recovered that were previously produced. All sepecies were cultivated on Potato Dextrose Agar (PDA) medium during two weeks to produce inoculums. 20 ml of sterile water were poured into each Petri dish (containing a culture of pure strain) to obtain the maximum conidia, and the surface of the colony is minutely brushed using a fine brush. The obtained spore

suspension was filtered with muslin to separate the conidia from the mycelia fragments. The conidial suspension collected is added with two drops of 10% Tween 80. Counts of conidia are then done under a Malassez counting cell microscope, and the concentration is adjusted to  $2.10^6$  conidia.ml<sup>-1</sup>. Pathogenicity tests were performed in the greenhouse according to the method described by Hernandez-Cubero et al. [21] after rubbing on the leaves of the carborandum, an abrasive powder which creates micro wounds on the leaves.

## 2.6 Statistical Analysis

Statistical analysis was performed using XLSTAT Version Pro-2017 and the graphs were drawn using Graph Pad Prism software version 5.0. The parameters were subjected to one-way analysis of variance at the 5% level and the effect of climatic zones in the diseases distribution was evaluated.

## 3. RESULTS

### 3.1 Main Foliar Fungal Diseases in Burkina Faso

The symptoms observed (Fig. 1) on the leaf samples collected were described. The description of symptoms in Table 1 shows a

prevalence of leaf diseases in all climatic zones of the country. Mainly 4 foliar pathologies were recorded in the 18 sites. These are yellowing with leaf blight, yellowing with leaf blight and black mycelium, drying of leaves from the border and virus diseases. The frequencies of the observation of the various diseases (Fig. 2) show that yellowing with leaf blight is the predominant manifestation with an observation frequency of 72 % in the plantations surveyed. Yellowing with leaf blight and presence of black mycelium rank second with 33 % followed by drying and viral diseases (which are essentially in the form of mosaics) with frequencies of 22 and 11% respectively.

### 3.2 Diseases Distribution in Relation to the Climatic Zone

The ANOVA analysis showed that the climatic zone has a significant effect on the distribution of virus diseases and leaf drying and a non-significant effect on the distribution of yellowing with leaf blight and presence or not of black mycelium. Thus, viral diseases are mainly found in the Sudano-sahelian and Sahelian zones, whereas they are almost non-existent in the southern Sudanian zone. Also, leaf burns do not exist in the Sahelian zone as they are found in other climatic zones.

**Table 1. List of observed symptoms by provenance**

Climate zone	Provenance	Symptoms observed
Northern Sudan	Kari 1	Yellowing with leaf blight
		Leaf drying by borders
	Kari 2	Yellowing with leaf blight
		Yellowing with leaf blight and presence of black mycelium
	Saria	Virus diseases Leaf drying by borders
	Koudougou	Yellowing with leaf blight
Sahelian	Imkouka	Yellowing with leaf blight
	Biba	Yellowing with leaf blight
	Kielbo	Virus diseases
		Yellowing with leaf blight and presence of black mycelium
	Koulpéllé	Yellowing with leaf blight
	Fada	Yellowing with leaf blight
Southern Sudan		Yellowing with leaf blight and presence of black mycelium
	Bana1	Yellowing with leaf blight
	Omléassan	Yellowing with leaf blight
	Yoro	Yellowing with leaf blight and presence of black mycelium
	Konkodjan	Yellowing with leaf blight and presence of black mycelium
		Yellowing with leaf blight
	Béréba	Yellowing with leaf blight
	Boulo	Yellowing with leaf blight
Nébbou	Yellowing with leaf blight	
	Kouakoualé	Leaf drying by borders
		Yellowing with leaf blight and presence of black mycelium



Fig. 1. Some symptoms observed on collection samples

### 3.3 Purification and Characterisation of Strains

The fungal species associated with foliar pathologies of the species were purification and characterised. Based on the morphology (colors, appearance, etc.) (Fig. 3A, 3B) and spore structure, nine species distributed into 6 genres of fungi were be characterised. These are *Fusarium oxysporum*, *Fusarium solani*, *Fusarium subglutinans*, *Phoma sorghina*, *Botrytis cinerea*, *Curvularia lunata*, *Lasiodiplodia threobeomae*

(*Botryodiplodia threobroma*), *Cercospora cesami*, *Curvularia eragrostidis*. The frequencies of fungal pathogens observation are presented in Fig. 4. The results show that *Curvularia lunata* is the most widespread with an observation rate of 44 % followed by *Fusarium solani* with a frequency of observation of 33 % then of *Phoma sorghina* and *Lasiodiplodia threobeomae* with a frequency of 28%. The most weakly observed were *Cercospora cesami* and *Fusarium subglutinans* with a frequency of 10%.

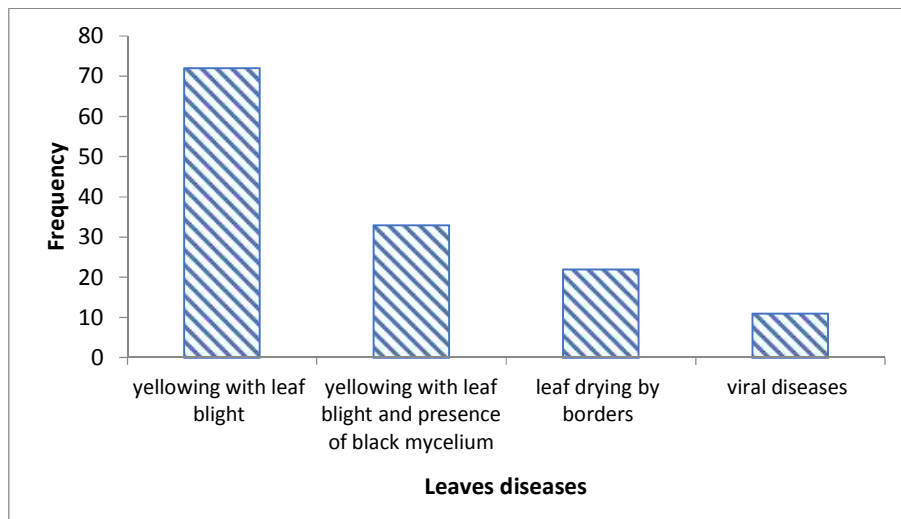


Fig. 2. Frequency of observed leaf diseases

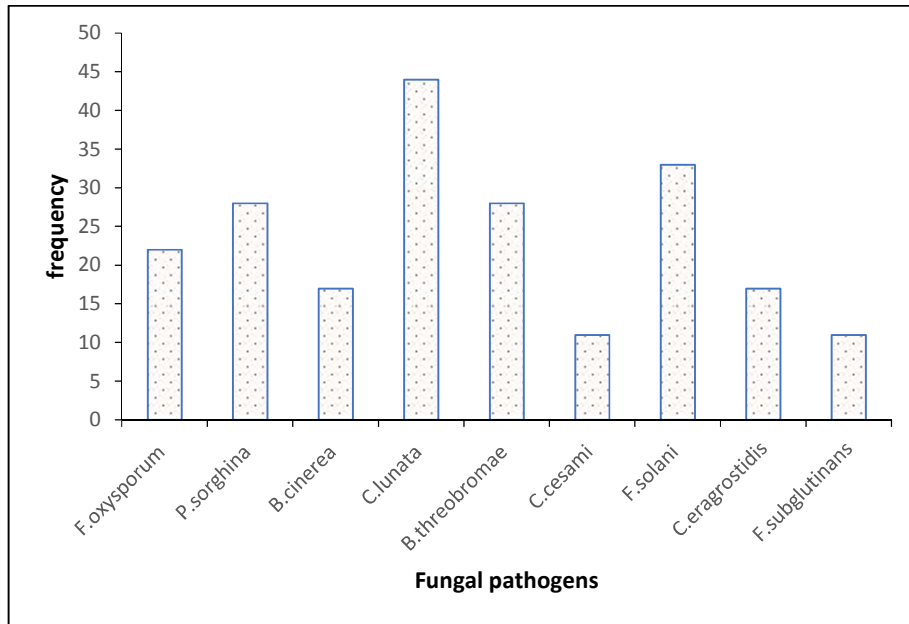
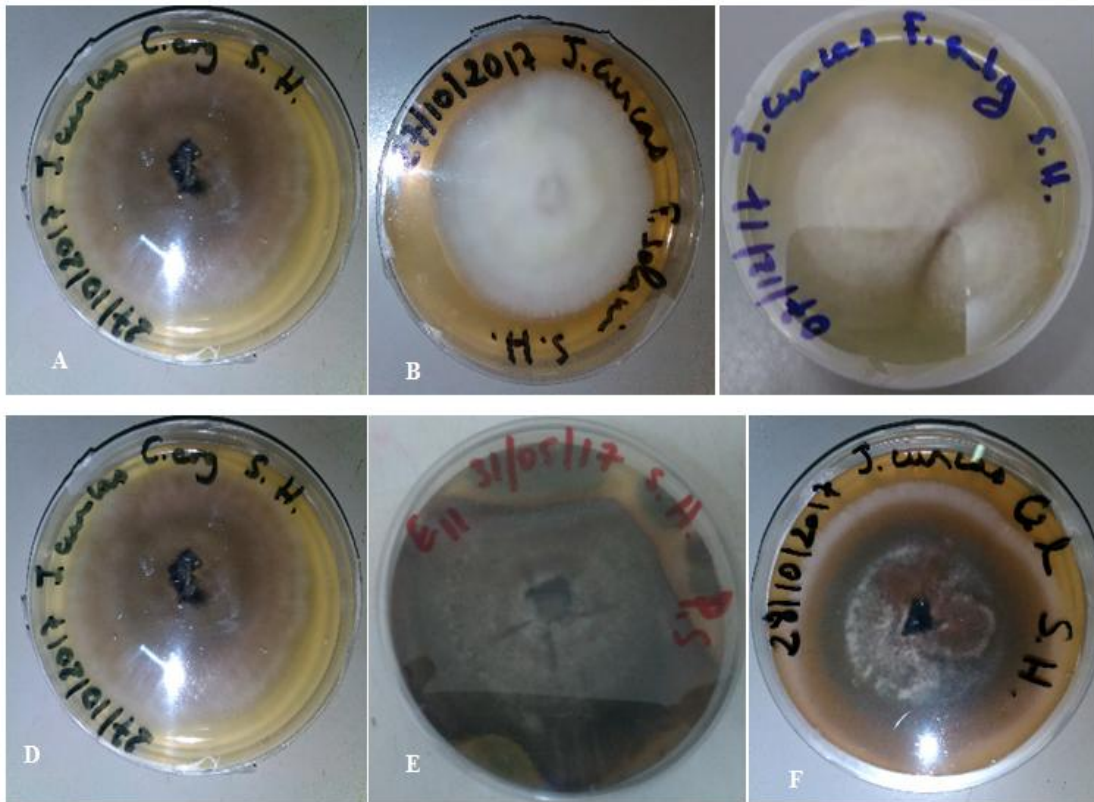
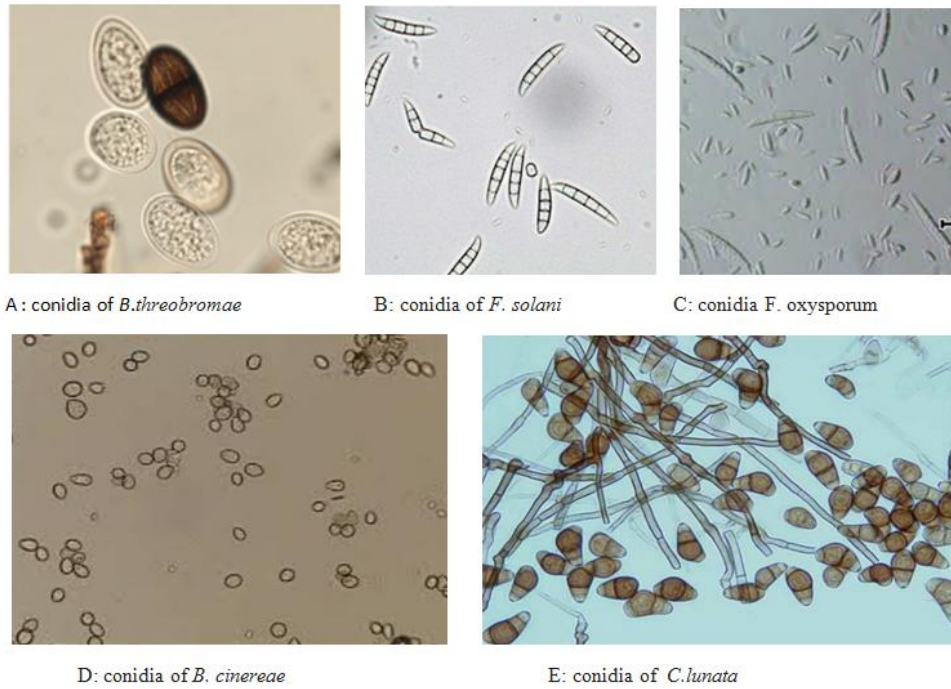


Fig. 3A. Frequencies of fungal pathogens observation

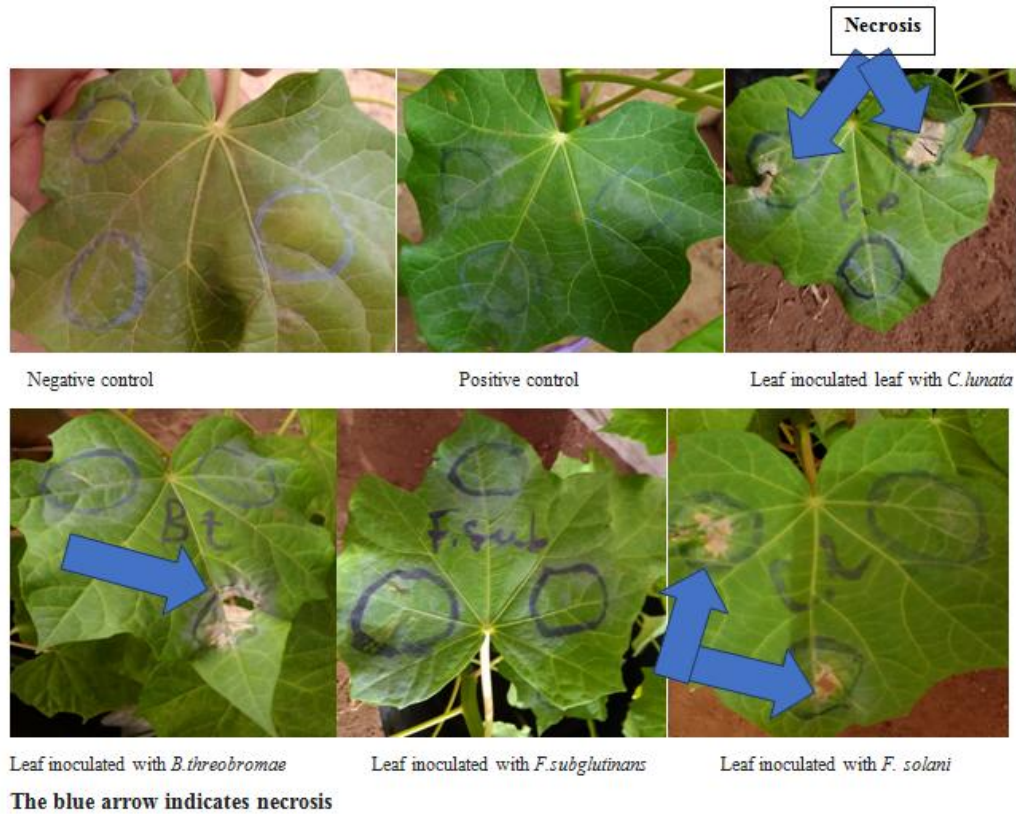


A : *C. eragrostidis* B : *F. solani* C : *F. subglutinas* D : *C. eragrostidis* E : *Phomasorghina* F : *C. lunata*

Fig. 3B. Morphological structure of some fungi characterized



**Fig. 4. Conidia of some purified fungi**



**Fig. 5. Some symptoms observed with pathogenicity tests**

### 3.4 Pathogenicity Tests

The pathogenicity test was performed in order to establish a link between the symptoms observed and the fungi. This test identified *Lasiodiplodia threobeomae*, *Curvularia lunata*, *Fusarium solani* and *Curvularia eragrostidis* as the pathogenic species responsible for observed leaf diseases of *J. curcas*.

## 4. DISCUSSION

The study confirmed a prevalence of leaf diseases in all climatic zones of Burkina Faso. These diseases include those caused by fungal pathogens and viruses. In addition, the study found that the climate zone has a significant effect on the distribution of these diseases. Indeed, the prevalence of these diseases is lower in the Sahelian zone. Similar results have been reported by previous work. Indeed, Rouamba [17], Ellison et al. [15] and Nacro et al. [7] have reported similar results. In addition, Rouamba [17] reported the prevalence of these diseases in the southern and northern parts of the country. The study also revealed the prevalence of leaf diseases in all climatic zones of the country. Our results show that yellowing with the presence of brown spots, drying or necrosis of leaves are the most common foliar diseases in Burkina Faso. Similar results have been reported by previous work in Burkina Faso and around the world. Indeed, Rouamba [17], Ellison et al. [15], and Nacro et al. [7] reported necrosis, burns, leaf blight, and yellowing among fungal leaf diseases of *J. curcas* encountered in Burkina. Also, they reported the highlighted prevalence of these diseases in southern and northern zones of country. Machado et al. [16] and Fajri et al. [23] respectively in Brazil and India have reported similar results. Also, Fajri et al. [23] reported the climatic effect on the distribution of diseases. According to their work, the high prevalence of diseases in the southern zone could be explained by the existence of optimal conditions such as humidity and soil temperature, which are necessary for the growth of fungi and infection of plants. The low incidence of leaf diseases in some climatic zones could be explained by unfavorable conditions for the development of pathogens in its localities.

Characterisation and pathogenicity testing identified *Lasiodiplodia threobeomae*, *Curvularia lunata*, *Fusarium solani* and *Curvularia eragrostidis* as *J. curcas* pathogens. Many studies in Burkina Faso and in the world have

reported the pathogenicity of these species on several plant species including *J. curcas*. In Burkina Faso, Nacro et al. [7] identified several fungal species including *Curvularia lunata* and *Fusarium moniliforme* as fungal leaf diseases of *J. curcas* ranging from burns to leaf necrosis. Rouamba [17] and Ellison et al. [15] have characterised many fungal species including *Fusarium*, *Curvularia*, and *Botrytis*, as associated fungal with leaf diseases of *J. curcas*. Sulaiman et al. [24] reported for the first time in Malaysia the pathogenicity of *L. theobromae* (*B. theobromae*) in *J. curcas*. The species is believed to be responsible for leaf blight, canker and dieback. Ji et al. [25] and Santos et al. [26] also reported that *L. theobromae* (Pat.) Griffon and Maubl and *Curvularia lunata* as important pathogens with high potential in *J. curcas*. Fajri et al. [21] reported the pathogenicity of many species of *Fusarium* on *J. curcas*. In Indonesia, Sharma et al. [23] reported that many *Fusarium* species, including *F. solani*, *F. moniliforme*, and *F. oxysporum*, were the leading causes of death for *Jatropha*, with mortality rates of up to 25%. However, some fungi isolated from diseased organs did not show pathogenicity on *J. curcas*. Similar results have been reported by Nacro et al. [7] and Wonni et al. [27] respectively on *J. curcas* and on cashew tree. Also, Wonni et al. reported that some fungi associated with cashew tree as saprophytic parasites but could appear pathogenic under favorable environmental conditions.

## 5. CONCLUSION

In order to generate information on the main fungal leaf diseases of *J. curcas* and the fungal flora associated with these diseases in Burkina Faso, the present study was initiated. Surveys across the different phytogeographic zones have identified four foliar diseases, namely yellowing with brown spots on the leaves, yellowing with black spots with black mycelium, drying and viral diseases. Among them, three were fungal diseases. Laboratory analyses showed the presence of nine species of fungi. The pathogenicity test was positive with four species of fungi namely *Fusarium solani*, *Botrytis cinerea*, *Curvularia lunata* and *Lasiodiplodia threobeomae*. Adequate methods for the control of these pathogens should therefore be sought.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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