Journal Homepage: - www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/17243
DOI URL: http://dx.doi.org/10.21474/IJAR01/17243

RESEARCH ARTICLE

ASSESSMENT OF THE PREVALENCE OF MAJOR FUNGAL DISEASES IN CASHEW ORCHARDS IN PÉNI, BURKINA FASO

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Manuscript Info

Manuscript History
Received: 15 May 2023
Final Accepted: 19 June 2023
Published: July 2023

Key words:-
Anacardium Occidentale, Cashew Nut, Fungal Diseases, Disease Frequency, Burkina Faso

Abstract

Cashew tree (*Anacardium occidentale* L.) occupies an important place in the fruit growing of Burkina Faso. Its nuts are the object of an increasingly strong internal and external demand. However, fungal diseases are the cause of considerable losses and reducing the quality of cashew nuts in plantations. The present study was therefore aimed at identifying the fungal pathogen profile associated with cashew orchards in Péni. For this experiment, symptomatic samples of leaves, flowers and fruits were harvested in 60 orchards from 12 villages of Péni, and the fungi associated with the symptoms were isolated on potato dextrose agar (PDA) plates. Then, an identification of fungi was carried based on the morphological characters of colonies, hyphae and conidia, using identification keys. The results revealed that six fungal species were associated to diseases symptoms. Among these fungi, *Pestalotia heterocormis*, *Colletotrichum gloeosporioides*, and *Aspergillus niger* were the most frequently isolated from symptoms, 61.67%, 38.33% and 25.00%, respectively. Our findings indicate that cashew orchards in Péni are attacked by a complex of phytopathogenic fungi that may affect fruit quantity and quality. Therefore, there is a need to seek effective fungal disease management strategies to ensure sustainable cashew production in Burkina Faso.

Introduction:-

Cashew tree (*Anacardium occidentale* L.) is cultivated mainly for the cashew apple and the cashew nut whose kernel is the main object of world trade (Soniyo Yomichan, 2020 ; Diallo and Coly, 2022 ; Israrullah et al., 2022). It is nowadays one of the most important cash crops in Africa, which accounted for more than 55% of the world's cashew nut production (N'Djolosse et al., 2020). These nuts offer considerable potential for the socio-economic development of producing countries through export, processing and local marketing, but also as a bio-pesticide from the liquid extract of the nuts (Audouin and Gonin, 2014 ; Garcia et al., 2017). Africa has especially been the world's leading producer of unshelled mahogany nuts for the past decade (FAOSTAT, 2022 ; Angamuthu, 2022). For instance, more than 50% of India's raw nut requirements in 2019 were covered by imports from African countries

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(Babli and Nayak, 2019). Côte d'Ivoire (West Africa) was the world's largest producer and exporter of raw cashew nuts in 2022, with a production of 848,700 tons on an area of 2,033,886 hectares, or 21% of world production (Angamuthu, 2022 ; FAOSTAT, 2022). Burkina Faso was far behind its neighbor Côte d'Ivoire, with 162,105 tons in 2022. It achieved roughly the same production of unshelled mahogany nuts as its neighbor Benin (190,000 tons), but produced more than Brazil, Ghana, and Thailand, with 13,921 tons, 8,420 tons, and 2,420 tons, respectively (FAOSTAT, 2022).

The development of cashew plantations in Burkina Faso is very spectacular. From 1,000 hectares planted by the "Cashew" project in the 1980s, cashew orchard areas have grown to nearly 80,000 hectares more than 40 years later (Audouin and Gonin, 2014). Four regions of the country (Cascades, Hauts-Bassins, South-West, Centre-West) stand out as the largest cashew producers and account for nearly 99% of the total number of cashew trees in the country (MAHRH, 2008). Cashew nut production in Burkina Faso is estimated at about 100,000 tons per year. These nuts currently rank 3rd among agricultural exports in Burkina Faso after cotton and sesame (Gecit, 2020). However, they are processed and marketed locally. There are 21 processing units in the country, with a high concentration in the Hauts-Bassins (80%) and Cascades (14%) regions. The volume of nuts processed in 2021 was 17,087 tonnes, representing a processing rate of 16% (Commodafrica, 2023). They therefore represent a product with high economic potential, having generated FCFA 117 billion (€ 178 million) in 2018 compared to FCFA 99 billion (€ 151 million) in 2017 (Gecit, 2020). However, the area devoted to this crop remains low in the country. It is therefore necessary to promote this cash crop while considering the biotic constraints that handicap the crop in the field.

Among the biotic constraints, pests and diseases are responsible for the significant decline in cashew yield and quality in the orchards (Malekela and Kitali, 2021 ; Monteiro et al., 2022). Considered minor in the past, some of the cashew diseases have turned out to be serious today, causing considerable losses in cashew plantations (Khatoon et al., 2017). More than twelve (12) diseases have been identified as infecting cashew worldwide and most of them are caused by fungal (Afouda et al., 2013; MADR, 2017). Indeed, Khatoon et al. (2017**)** identified seven fungal species (*Pestalotiopsis palmarum, Phyllosticta sp*., *Colletotrichum gloeosporioides*, *Botryodiplodia theobromae*, *Fusarium oxysporum*, *Rhizoctonia solani* and *Chaetomium brassiliense*) responsible for a variety of diseases on various organs of cashew in Odisha, India. In addition, fungal species like *Alternaria alternata, Aspergillus sp, Cephalosporium sp*, *Colletotrichum gloeosporioides*, *Curvularia lunata*, *Lasiodiplodia theobromae*, *Penicillium sp.*, *Pestalotia heterocornis*, *Trichoderma viridae* and *Cryptosporiopsis sp*., have been isolated from diseased cashew leaves and nuts in Benin, Brazil, Ghana, Cameroon, Tanzania and India (Freire et al., 2002; Majune et al., 2018; Hammed and Adedeji, 2008; Suleiman, 2010; Afouda et al., 2013; Nyaka et al., 2021; Muntala et al., 2021).

In Burkina Faso, in a preliminary study in four major areas, Wonni et al. (2017) shown that anthracnose (*Colletotrichum gloeosporioides*), leaf pestalotiosis (*Pestalotia heterocornis*), bacterial canker on leaves and cashew nuts (*Xanthomonas citri* pv. anacardii), and gum disease (*Lasiodiplodia theobromae*) were major diseases of the cashew trees in the field. According to their findings the incidence of each disease varied according to the locality of production. Unfortunately, data on diseases and causative pathogens at the scale of cashew producing areas are scarce in Burkina Faso (Wonni et al., 2017). It seems urgent to reverse this trend by identifying the different pathogens and their frequency, incidence, and economic impact, in order to have specific data for each cashew producing area in Burkina Faso. Thus, the present study aimed at identifying cashew fungal diseases and their frequency in Péni in the Hauts-Bassins region. This work could contribute to the design of effective control measures against the fungal diseases that will eventually be identified, and limit production losses.

Materials and Methods

Description of the study area

The present study was conducted in a rural commune of Burkina Faso named Péni and located 35 km from Bobo-Dioulasso, on the National Road N° 7 (RN7) linking Bobo-Dioulasso to Banfora. With an area of approximately 1,246 km², Péni is part of the Houet province and has 24 villages. The soils are gravelly, clayey, sandy, sandy-loamy, sandy-clayey and silty-clayey. The climate is of the tropical Sudanese type with two seasons. Average annual rainfall in the commune during the year 2021 ranged from 39.1 to 112 mm, with average annual temperatures ranging from 27.3 °C to 30.9 °C.

Assessment of fungal symptoms on cashew trees

Based on a summary catalog of the symptoms of cashew fungal diseases developed (Afouda et al., 2013; MADR, 2017; Woni et al., 2017 ; Ngoh Dooh et al., 2021), we observed and recorded fungal diseases symptoms using

numbers and letter codes. The data related to the symptomatic characteristics of the fungal diseases were collected with the help of inventory forms elaborated for this purpose.

Collection of diseased samples

Villages were selected based on a purposive sampling approach with a minimum distance of 8 km between sampled villages (Soro et al., 2020). The survey and collection of samples were conducted in twelve (12) villages in the commune of Péni (Figure 1). These villages are : Dissini, Dodougou, Finlandé, Koumandara, Marabagasso, Nyafongon, Péni, Samaradougou, Taga, Tien, Tiemeredji and Tiougouana. A total of sixty (60) orchards were visited for 2 months, with 5 orchards randomly selected per village.

Considering that each orchard represents a quadrilateral (7m x 7m), at each corner and in the center of each orchard, 6 cashew trees were selected for health observations (Ouédraogo, 2002). Thus, in each village, 30 trees were studied, or a total of 360 trees observed. 130 symptomatic samples consisting of leaves, flowers, apples, and nuts were collected from the orchards on the basis of visible disease symptoms. These samples were kept in cold storage boxes and transported to the National Forest Seed Center (CNSF) laboratory for identification and isolation of pathogens.

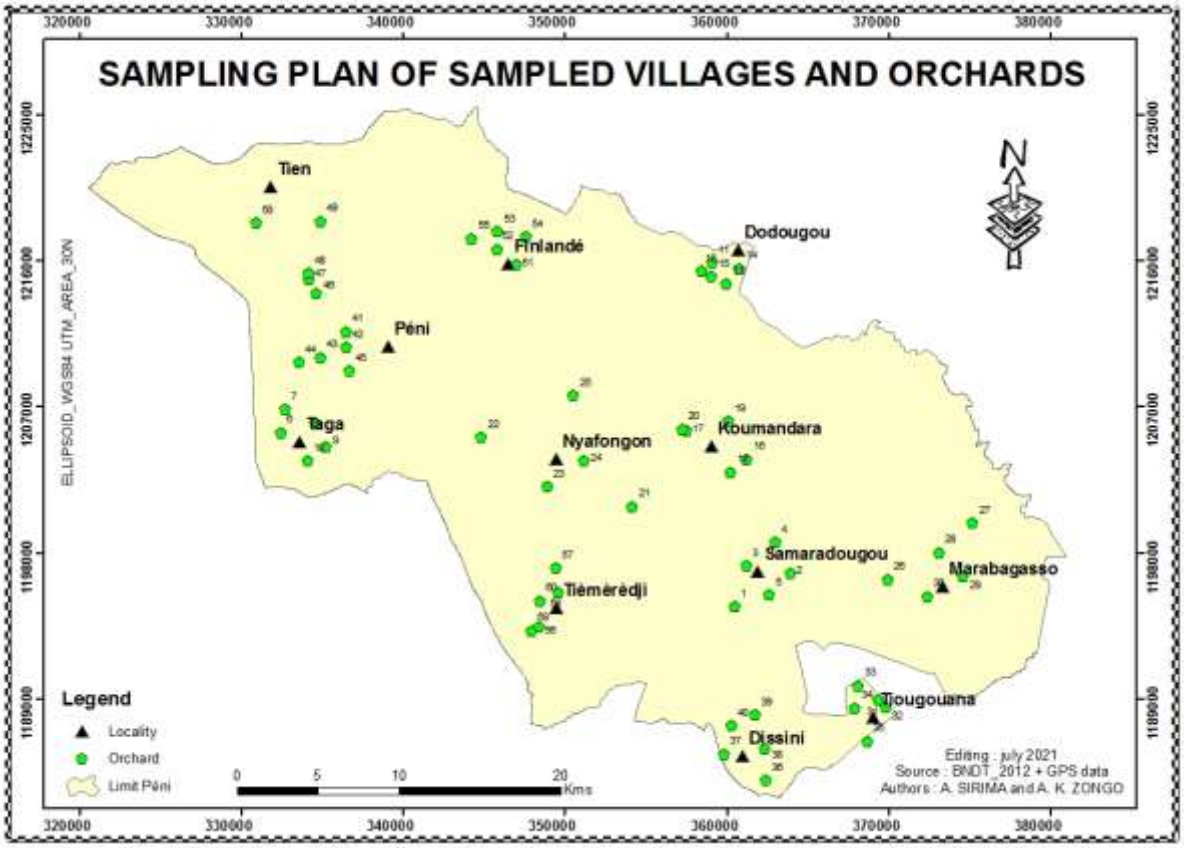

Figure 1:- Survey plan of the study villages and orchards.

Identification and Isolation of associated Fungi

The symptomatic samples were incubated in Petri dishes on moist blotting paper, and kept in the laboratory at 25 °C ± 1 °C, under a 12 :12 UV light/dark photoperiod during six days. Then, samples were examined under a magnifying glass in order to describe of hyphae and reproductive organs of the fungi. The identification of fungi was carried out using identification keys (Mathur and Kongsdal, 2003 ; Navi et al., 1999), based to the appearance and colors of colonies, the morphological characters of the hyphae and conidia. The fungi were then isolated on PDA plates and incubated at 25 °C ± 1 °C with 12 hours of light and 12 hours of darkness during five (5) days for sporulation. The hyphae and conidia of the fungi were observed again under a light microscope to confirm the identity of the fungi. Photographs were taken to illustrate the observations.

Frequency of pathogen isolation

The frequency of isolation (FI) of each fungus per village was determined following to the formula 1, adapted from of Iqbal and Saeed (2012)**:**

$$FI = \frac{NF}{NT} \times 100$$

NF = the total number of samples from which a given fungus was isolated,
NT = the number of samples from which isolations were made.

Statistical analysis of the data

The different data collected were entered, organized, and processed using Microsoft EXCEL 2016 software and subjected to analysis of variance (ANOVA). The means were separated by the DUNCAN test at the 5% threshold using XLSTAT 2016 software. The elaboration of the different maps and survey design was done using ArcGIS 10.5 software.

Results

Types of fungal disease symptoms

During this study, ten (10) categories of fungal diseases symptoms were observed on differents organs of cashew trees in the commune of Péni (Figure 2 and Figure 3).

On the one hand, small black necrotic spots on the upper surface of the leaves (Figure 2 A). On the other hand, rough brownish necrotic spots (Figure 2 B; C) or non-rough spots (Figure 2 D), initially isolated on the leaves, but tending to join with the progression of the infection. Discoloration of plant leaves has also been observed (Figure 2 E ; F). Some appear as transparent halos at first, then turn reddish-brown and necrotic.

In addition, desiccation of apples (Figure 3 G) and flowers (Figure 3 H) was observed. Brownish spots on nuts (Figure 3 I ; J) and apple rot (Figure 3 K) were also observed. These various problems observed on the plants would cause early leaf and fruit drop and branch desiccation.

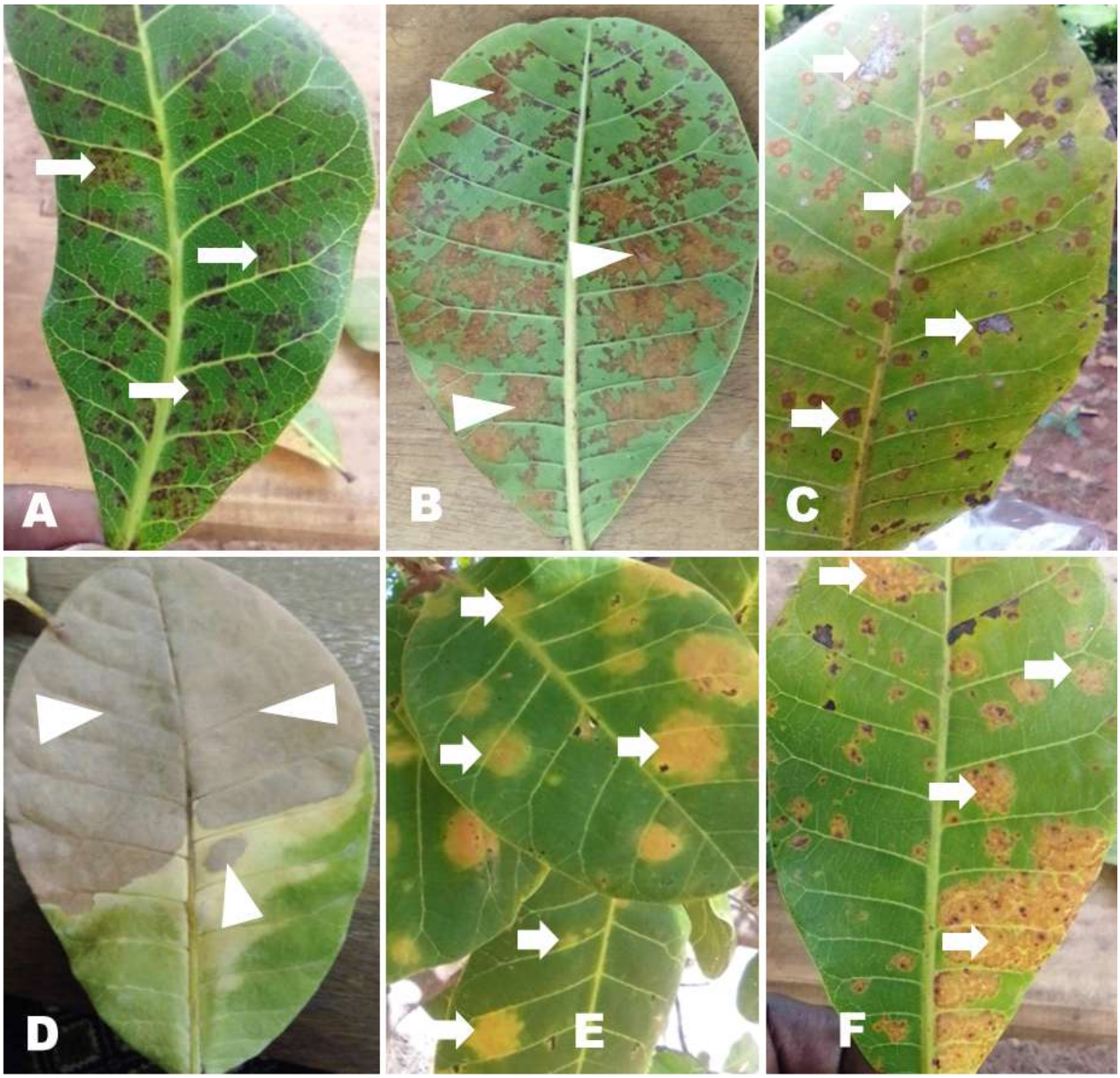

Figure 2:- Fungal disease symptoms on cashew leaves.
(**A**) Black necrotic spots on leaf; (**B** and **C**) Rough brownish necrotic spots on leaf; (**D**) Non-rough brownish necrotic spots on leaf; (**E** and **F**) Leaf discoloration.

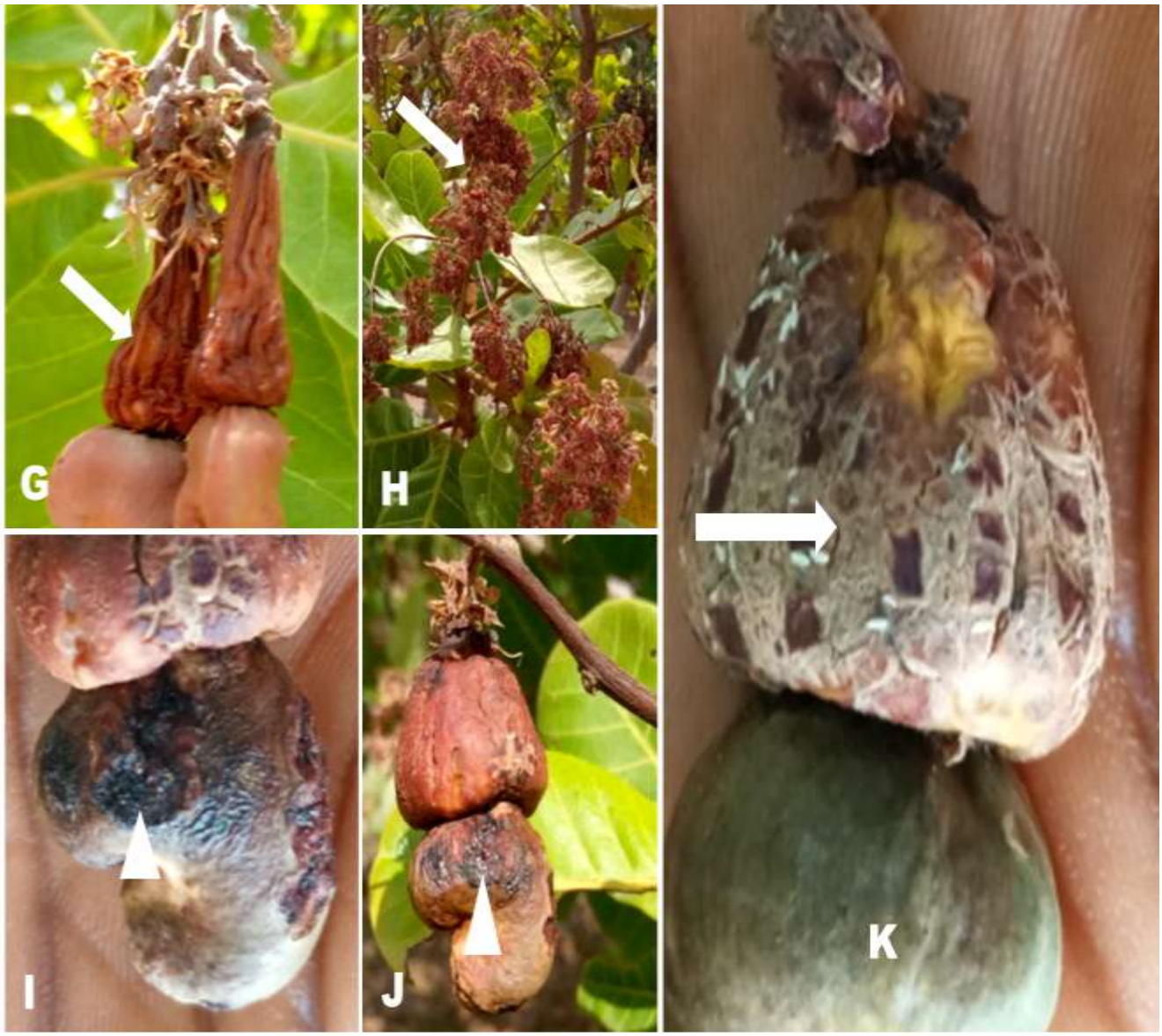

Figure 3:- Fungal disease symptoms on cashew flowers and fruit.
(**G**) desiccation of apples; (**H**) desiccation of flowers; (**I** and **J**) brownish spots on nuts; (**K**) rotting of apples.

Identification of fungi associated with disease symptoms
Figure 4 shows the sporangia and conidia of fungal species associated with different symptoms in cashew orchards. Table 1 presents the fungi isolated per type of symptoms.

Microscopic examination of the isolates identified six fungal species from four fungal genera. These were *Aspergillus niger*, *Aspergillus flavus*, *Colletotrichum gloeosporioides*, *Pestalotia heterocormis*, *Pestalotia guepini*, *Rhizopus microsporus*. Thus, from leaf samples with isolated necrotic spots on the margins and in the center, isolation allowed the identification of *C. gloeosporioides* (Table 1), which causes anthracnose. For leaf samples with reddish-brown necrotic spots, isolation allowed the identification of *Pestalotia heterocormis* and *Pestalotia guepini* (Table 1), responsible for pestaliosis. Pathogens isolated from desiccated samples were *C. gloeosporioides*, *P. heterocormis*, and *A. niger*, while those isolated from black spots were *A. niger*, *A. flavus* and *R. microsporus* (Table 1).

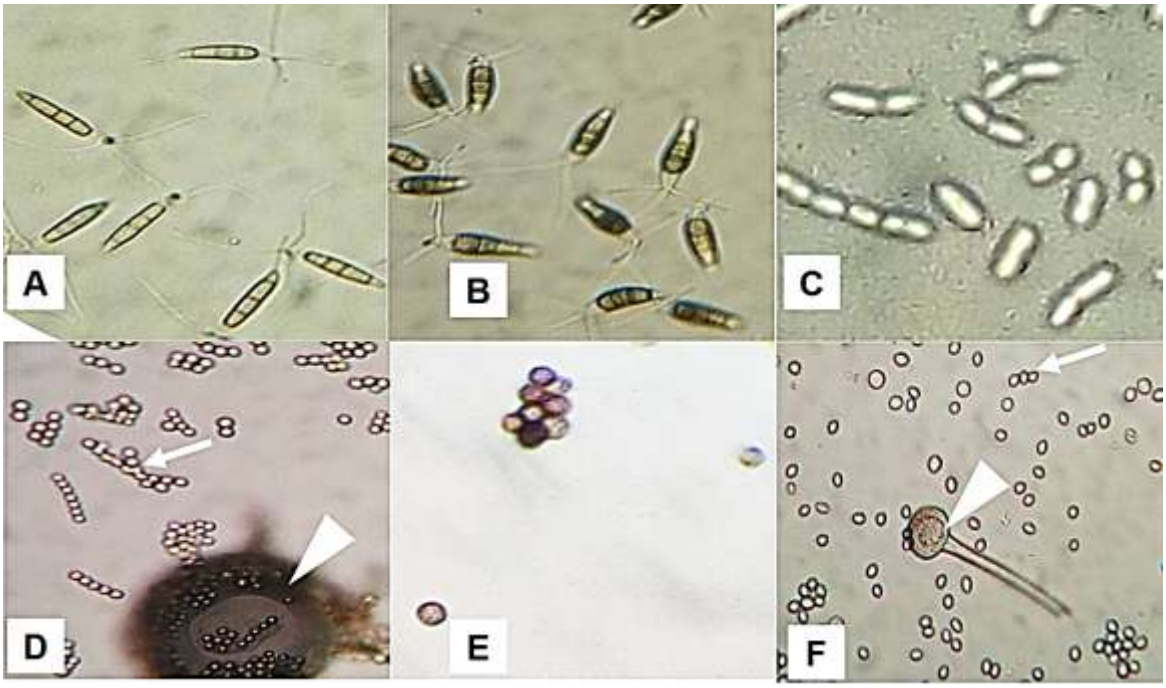

Figure 4:- Conidia of different fungal species identified from symptomatic organs of cashew.
A: Conidia of *Pestalotia heterocormis* (100X) **B**: Conidia of *Pestalotia guepini* (100X)
C: Conidia of *Colletotrichum gloeosporioides* (100X) **D**: Conidia (arrow) and sporangia (arrowhead) of *Aspergillus flavus* (100X),
E: Conidia of *Aspergillus niger* (100X)
F: Conidia (arrow) and sporangia (arrowhead) of *Rhizopus microsporus* (100X).

Table 1: Isolated pathogens associated with observed fungal symptoms.

	Sheets	**Flowers**	**Cashew apples**	**Cashew nuts**
Symptoms	Black, brownish, reddish necrotic spots	Drying out	Rot, Drying out	Black spots, brownish
Fungi isolated	*Colletotrichum gloeosporioïdes*, *Pestalotia heterocormis*, *Pestalotia guepini*, *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus microsporus*	*Colletotrichum gloeosporioïdes*, *Pestalotia heterocormis*, *Aspergillus niger*	*Colletotrichum gloeosporioïdes*, *Pestalotia heterocormis*	*Pestalotia heterocormis*, *Aspergillus niger*, *Aspergillus flavus*,

Frequency of isolation of identified pathogens

Table 2 presents the ANOVA of the mean isolation frequencies of fungi isolated, and Table 3 presents the means of their isolation frequencies. Statistical analysis did not reveal any significant difference between isolation localities ($p > 0.05$). However, she revealed that there is a significant difference between the frequencies isolation of the fungal species isolated ($P < 0.05$). *A. niger*, *A. flavus*, *C. gloeosporioides*, *P. heterocormis* were isolated in the six villages. The most frequently isolated species were *P. heterocormis* (61.67%), *C. gloeosporioides* (38.33%) and *A. niger* (25.00%).

Table 2:- Analysis of variance of isolation frequencies of cashew fungi in Péni.

Source	DDL	Sum of squares	Average of squares	F	Pr > F
Villages	11	1600,000	145,455	0,383	0,957
Fungal agents	5	25533,333	5106,667	13,460	**< 0,0001**
Villages* Fungal agents	50	17500,000	350,000	1,128	0,413

Table 3: Average frequency of isolation of each fungal agent associated with cashew in Péni.

Fungal agents	Averages frequencies
Pestalotia heterocornis	61.67 a
Colletotrichum gloeosporioïdes	38.33 b
Aspergillus niger	25.00 bc
Rhizopus microsporus	18.33 cd
Aspergillus flavus	11.67 cd
Pestalotia guepini	5.00 d

The frequency of isolation of each pathogen depended on the villages (Figure 5). *P. heterocormis* had the highest isolation frequencies in Samaradougou, Taga, Dodougou, Koumandara, Péni and Tien. However, he was not isolated in Marabagasso. *C. gloeosporioides* was isolated in all the orchards in the villages surveyed, with the highest frequencies in Nyafongon, Finlandé, Tien and Tiemeredji.

The species *P. guepini* was isolated only in the village of Dissini and Tiongouana with highest frequency in Dissini. *R. microsporus* was only isolated in Nyafongon, Dissini, Tiemeredji. The fungi *A. niger* and *A. flavus* were not isolated in three (03) and six (06) villages, respectively.

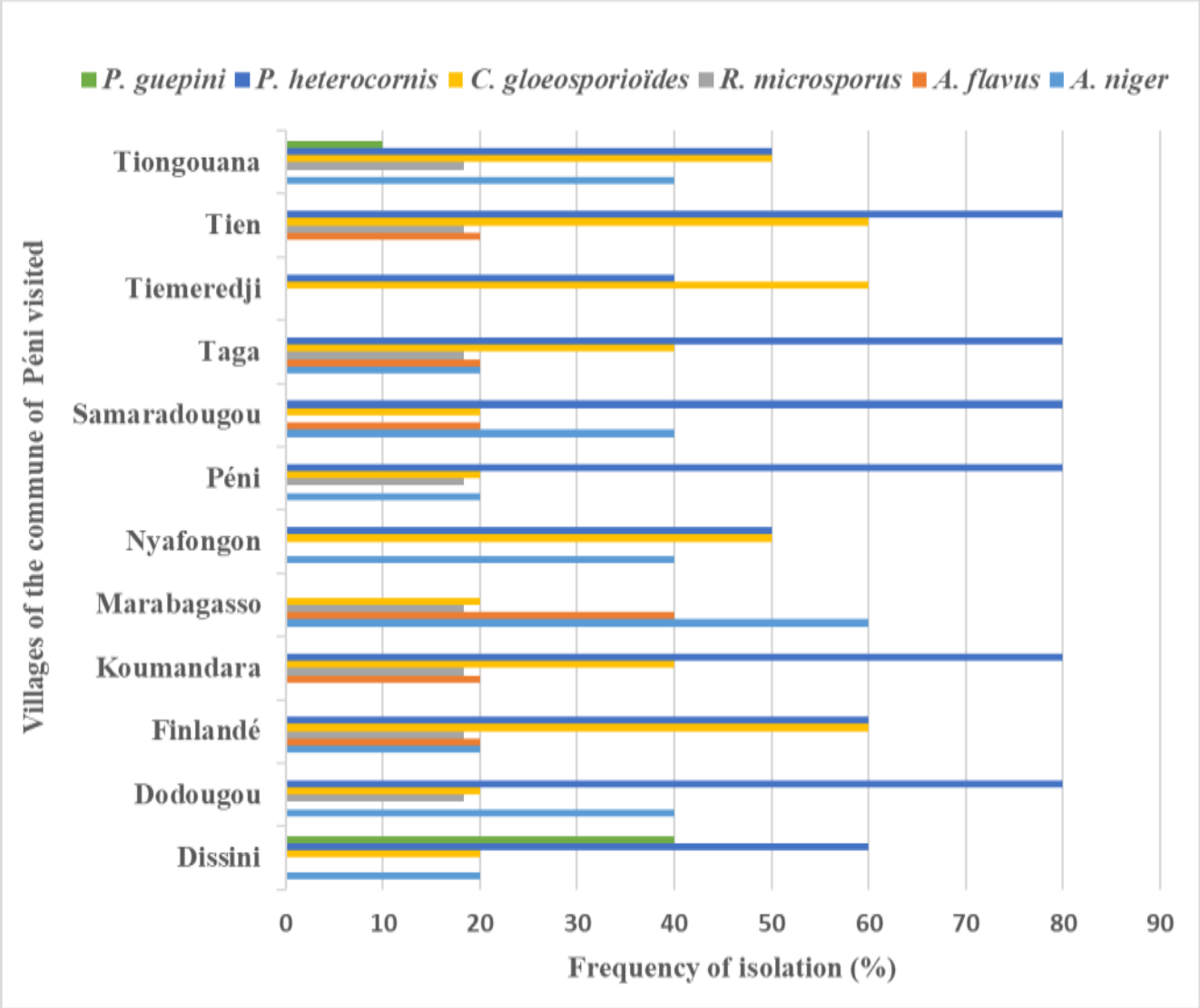

Figure 5:- Frequency of isolation of each pathogen by village.

Discussion

Surveys conducted in Péni, one of the major cashew nut production areas in Burkina Faso, revealed several cashew fungal disease symptoms. The major symptoms were necrotic spots on the edges and in the center of the leaves (anthracnose), reddish-brown necrotic spots (pestaliose), drying of flowers and apples (anthracnose and pestaliose), browning of nuts (anthracnose), and apple rot. These results are similar to those of several authors in the sub-region. Indeed, symptoms of anthracnose and pestaliose have already been reported on cashew trees in the Hauts-bassins, Cascades, South-West and Centre-West regions of Burkina Faso (Wonni et al., 2017), and in Benin (Afouda et al., 2013). In addition, to these two diseases, symptoms of bud desiccation, downy mildew and Alternaria have been reported on cashew trees in Cameroon (Ngoh Dooh et al., 2021). Banito et al. (2021) similarly observed on cashew trees in the Tchamba prefecture of Togo, bud dryness symptoms with a prevalence of 100% in the field.

The symptoms of fungal diseases identified in the cashew orchards of the commune of Péni, varied from one village to another. Indeed, the symptoms of anthracnose and pestaliose were observed in all the orchards of all the villages except for the village of Marabagasso for the symptoms of pestaliose. These symptoms were observed on flowers, fruits, and leaves over almost the entire study area. Gil Rodrigues dos Santos et al. (2019) observed in their study on the detection of *Colletotrichum gloeosporioides* in local cashew species in Brazil, symptoms of anthracnose on the surfaces of the flower stalk, leaves, and cashew nuts of *Anacardium humile*. Anthracnose and pestaliosis were the most prevalent fungal diseases in all cashews producing villages in the commune of Péni. Their frequency varied depending on the production locality (Wonni et al., 2017; Ngoh Dooh et al., 2021).

The different observations carried out in the laboratory allowed the identification of six fungal species from the symptomatic organs collected. Works in different countries had reported similar results to those obtained in this study for the genera, *Colletotrichum gloeosporioides*, *Pestalotia heterocormis*, *Aspergillus sp*., *Rhizopus sp*. (Afouda et al., 2013; Wonni et al., 2017; Ngoh Dooh et al., 2021). These fungi are known to be involved in leaf deterioration, flower and apple desiccation, and nut deterioration in the field. A species of *P. guepini* was obtained in the sample, and this for the first time in Burkina Faso. However, this result is contrary to many studies who did not identify the species in their studies (Wonni et al., 2017 ; Muntala et al., 2021). It could be explained either by the pathogenic power of the species or by environmental factors or socio-cultural pressures in villages (intensification of annual crops, soil erosion, etc.). Indeed, the villages of Dissini and Tiongouana, located to the south of the commune of Péni, have similar characteristics in terms of land use in the commune.

The most frequently isolated fungal species in the study were *Pestalotia heterocormis*, *Colletotrichum gloeosporioides* and *Aspergillus niger*. The isolation frequencies by village also varied from one zone to another, reflecting differences in the distribution of the diseases observed. Thus, pestalotiosis and anthracnose were the most frequent and the most distributed in the commune of Péni. They are therefore the most important fungal diseases in terms of distribution. This result is similar with that of Silué et al. (2017, 2018). The latter had previously indicated that anthracnose and pestalotiosis, are the most disseminated fungal diseases in cashew orchards in Côte d'Ivoire. Furthermore, in the present study, the villages of Finlandé, Taga and Tien were the localities with the most attacked cashew orchards, in terms of the diversity of fungal agents identified. Similar results were reported by Kra et al. (2017) who observed that in three sub-prefectures (Bouandougou, Tiéningboué, Ouellé) in Côte d'Ivoire, only the one in Bouandougou recorded disease cases on cashew trees with a rate of 6.66%. Symptoms were stunting, leaf curling and drying of part or all of the tree. Beyond climatic factors, the distribution of these different diseases in the study area could be related to the seeds used during the establishment of the orchards, the frequency, and the type of maintenance of the orchards. Soro et al. (2020) showed in their study on the analysis of agronomic factors on the sanitary state of cashew orchards in Côte d'Ivoire, that the origin and type of planting material are strongly linked in the manifestation of cashew pests and diseases.

Conclusion

The present study has shown that a diversity of fungal pathogens is associated with cashew cultivation in the commune of Péni. In addition, it identified for the first time *Pestalotia guepini* associated with cashew trees in Burkina Faso. It also revealed a preponderance of pestalotiosis and anthracnose on flowers, leaves, and fruits. However, further studies are needed to provide information on the real impact of pestalotiosis and anthracnose on the quantitative and qualitative yields of cashew fruits in Péni. In addition, it would be necessary to continue morphological and pathogenic characterization studies of the strains of the different fungi identified in order to anticipate appropriate control methods.

Acknowledgments
The authors would like to thank the managers of the National Forest Seed Center (CNSF/Ouaga) for facilitating the various manipulations.

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