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Research Article

GEOGRAPHICAL DISTRIBUTION AND IDENTIFICATION OF FUNGI ASSOCIATED WITH SOYBEAN GROWING IN BURKINA FASO

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ABSTRACT

Soybeans are a major crop in developing countries, mainly as seeds in human and animal nutrition and food processing. However, production is affected by various infectious diseases, particularly fungal diseases. This study aimed to analyse geographical distribution and identify fungal pathogens associated to soybean cultivation to help improve soybean health. A total of three soybean production regions were studied and 278 samples were collected in the provinces of Comoé, Houet, Kénédougou and Sissili. Samples were collected using the yield square method. Fungi associated with leaves, stems, roots and pods were cultured on blotting paper and PDA medium. The isolates were identified using the key of Mathur and Kongsdal (2003) and Champion (1997). Seventeen (17) genera were identified, including five dominant genera on leaves: *Phoma* (17.56%), *Colletotrichum* (12.88%), *Fusarium* (11.08%), *Macrophomina* (10.13%), *Cercospora* (8.21%). Ten (10) species of common parasitic fungi, namely *Alternaria, Cercospora, Cladosporium, Colletotrichum, Curvularia, Fusarium, Macrophomina, Myrothecium, Phoma, Fusarium, Rhizopus* were identified on leaves, stems, roots and pods. Fifteen (15) genera were identified, of which three dominated stems and roots: *Phoma* (20.23%), *Fusarium* (18.06%) and *Colletotrichum* (11.27%). Ten (10) genera were identified, including three dominant genera: *Fusarium* (28.80%), *Colletotrichum* (22.22%), *Phoma* (18.89%) and *Cercospora* (16.67%) in pods only.

Keywords: *Glycine max* L., Pathogenic fungi, Identification, Burkina Faso.

INTRODUCTION

Glycine max [L.] Merr. occupies a critical position on the global economic stage as an essential crop for ensuring global food security (Hartman *et al*., 2011). The world's three largest soybean producers, Brazil, the United States, and Argentina, together accounted for 81% of total production in the 2019/2020 growing season (USDA, 2020). In Burkina Faso, soybean is the fourth most important cash crop after cotton, peanuts, and sesame (MAAH, 2016). Soybean has become a strategic crop due to its importance from both an economic and a nutritional perspective. The crop is produced in seven regions of the country, mainly in the central-eastern, central-western and eastern regions (MAAH, 2016). According to Bila *et al*. (2009), soybean has a number of different uses in the food, industrial, and pharmaceutical sectors. Despite its importance, soybean cultivation is subject to several phytosanitary constraints, particularly fungal diseases caused by a number of pathogenic species. Pimentel *et al*. (2022) reported several fungi of the genera *Fusarium*, *Rhizoctonia*, *Mucor*, *Phoma*, *Macrophomina*, *Alternaria*,

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and *Phomosis* that are known soybean pathogens in the USA. Soybean plants can be colonized by various fungi including pathogenic and non pathogenic(Fernandes *et al*., 2015; Impullitti & Malvick, 2013; Pimentel *et al*., 2006). According to Bandara *et al*. (2020) and Wrather *et al*. (2010), soybean fungal diseases rank among the diseases that have consistently decreased soybean yields in the United States over the past two decades. The multiple pathogens present in the same field require different management approaches, making disease management difficult. Therefore, accurate pathogen identification is important (Hartman *et al*., 2016). In Burkina Faso, preliminary studies conducted by Kafando (2009) and Bado (2012) identified specific fungi on soybean seeds. Although there are numerous published reports on soybean parasitic fungi in different soybean producing countries, the correlation between these parasitic fungi and yield reduction seems to be the first of its kind in Burkina Faso. For this reason, this study focused on characterizing the parasitic fungi of soybean in the main producing regions of Burkina Faso in order to provide valuable resources for research on effective and sustainable management of these diseases. The aim of this work is to contribute to the improvement of the health status of soybean by analyzing the geographical distribution and identifying the pathogenic fungi associated with its cultivation.

MATERIALS AND METHODS

Site presentation and sample collection

A survey was carried out in four (4) provinces: Comoé, Houet, Kénédougou and Sissili, which are the main soybean a production areas in Burkina Faso. A total of 278 samples of diseased organs (leaves, stems, roots, pods) were collected (Figure 1). In each site, farmers' fields were selected at random. Five sampling squares with a surface area of 1 m² were measured, within which two diseased plants were taken and placed in envelopes, all of which were placed in a cooler. In each field, diseased organs were taken from a total of ten seedlings. The growth stages of the seedlings ranged from emergence to flowering.

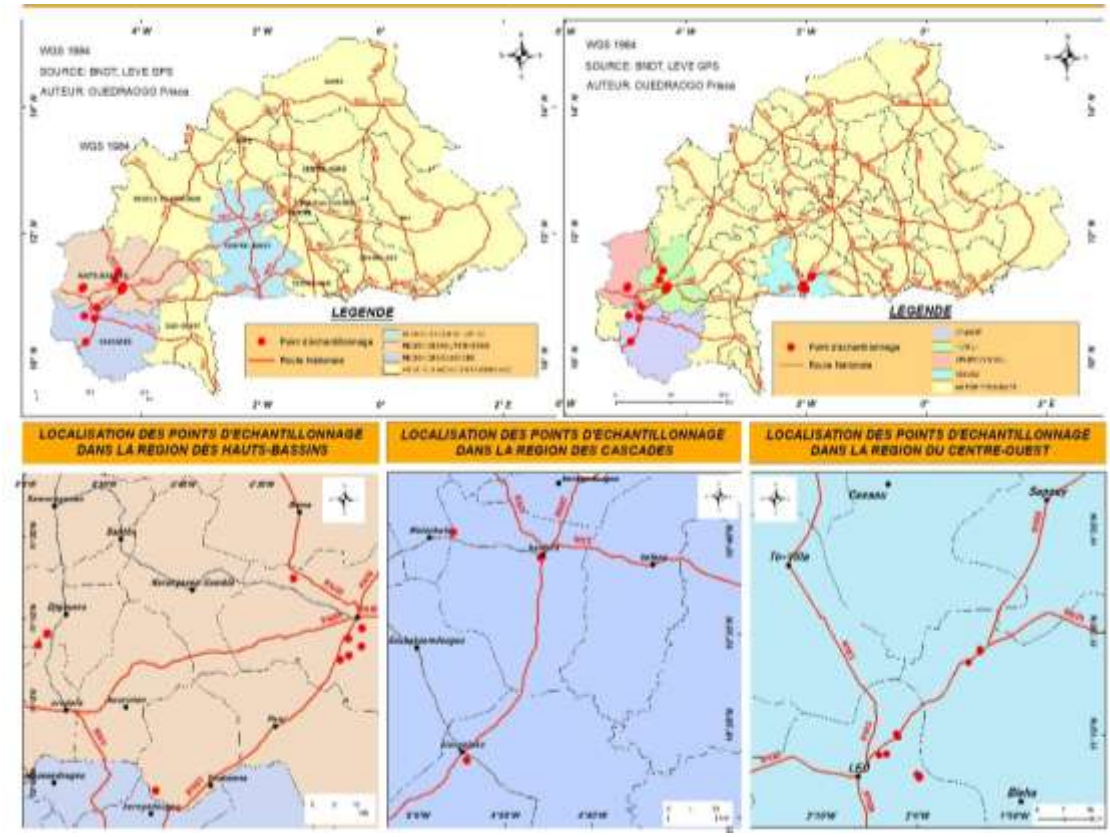


Figure 1. Location of sample collection sites on the map of Burkina Faso.

Techniques for isolating and identifying fungi

Once in the laboratory, the samples were washed in tap water, disinfected by soaking in a 1% NaOCl solution for 30 seconds and then rinsed thoroughly with distilled water for one minute. They were then dried on sterile paper. Finally, a fragment of the diseased parts was cut out and placed in a Petri dish lined with blotting paper lightly soaked in distilled water. The whole dish is incubated in a chamber for 2 - 3 days at 22°C, with alternating light for 12 hours. The fungi that grew on the blotting paper (Mathur and Kongsdal, 2003) were transferred to potato dextrose

agar (PDA) amended with septromycin (1g/L). Incubation was carried out under the same conditions as above.

The fungi were identified on the basis of their cultural characteristics (macroscopic and microscopic). Slide and slide preparations of fungal mycelium from pure isolates were observed using a Euromex Microscopes-Holland DC.5000F CMEX 5 Camera light microscope. Fungi were identified using the micromycete identification key from Mathur and Kongsdal (2003) and Champion (1997). The wet chamber method (described above) was used to re-isolate fungi that had induced symptoms on leaves, stems, roots and pods. These fungi were identified using the method described in the methodology.

Parameters assessed

The following parameters were assessed:
The frequency (F) of fungi identified: this was determined by dividing the number of samples contaminated by the fungus by the total number of samples. The following formula was

$$F_{(\%)} = \left(\frac{Ei}{Et}\right) x100 \qquad (1)$$

Ei = number of contaminated samples per fungus and Et = total number of samples collected.
The rate of infection of soybean seeds (TI) by a fungus was determined by the number of grains infected by fungus out of the total number of grains examined per seed sample:

$$F_{(\%)} = \left(\frac{Ngi}{N}\right) x100 \qquad (2)$$

Ngi = number of grains infected per fungus; N = total number of grains examined per seed sample.

Data analysis

The data was entered and the graphs produced using Microsoft Excel 2018 and then analysed using XLSTAT 2016. Statistical analysis was performed using the Fisher test at the 5% probability threshold.

RESULTS AND DISCUSSION

Figure 2 shows the incidence of fungal diseases recorded in the different regions: 68% in the Haut-Bassins region (Houet and Kénédougou provinces), 17% in the Cascades region (Comoé province) and 15% in the Centre-Ouest region (Sissili province). The Hauts-Bassins region has the highest incidence of the disease, followed by the Cascades. Observation of the symptoms revealed the presence of brown and greyish spots, soft rot, chlorosis, wilting and dry rot at different stages of plant development (Figure 3). Analysis of leaf organs enabled us to identify seventeen (17) genera of fungi in all the study sites. Of these, *Phoma* was the most common (17.56%), followed by *Colletotrichum* (12.88%), *Fusarium* (11.08%), *Macrophomina* and *Curvularia* (10.37%) and *Macrophomina* (10.13%) (Figure 4).

The distribution of fungi identified on leaf organs is shown in Table 1. A total of seventeen (17) genera of fungi were identified. The dominant genera in the province of Houet were *Colletotrichum* with a rate of 23.32%, followed by the genus *Fusarium* (15.87%) and *Curvularia* (7.93%). In Kénédougou province, *Phoma is* the most widespread genus (17.39%), followed by *Curvularia* (13.04%), *Fusarium* (10.87%) and *Botritys* (10.87%). In Comoé, the *Phoma* genus predominates *(*21.28%), followed by the *Macrophomina* genus (12.53%) and the *Curvularia* genus (11.70%). Finally, in the province of Sissili, the leading genus is *Phoma* (1.78%), followed by *Cercospora* (12.70%), *Curvularia* (9.21%) and *Colletotrichum* (9.21%). Observation of the symptoms revealed the presence of brown spots on the stems and charcoal rot on the roots and pods at various stages of the plant's development (Figure 5). With regard to the distribution of these fungi according to collection sites, in the province of Houet, for the three most frequent genera, we note an incidence rate of 18.79% for the genus *Colletotrichum*, *Fusarium* (16.97%) and *Cercospora* (13.33%). Similarly, in the province of Kénédougou, the genera *Curvularia*, *Fusarium* and *Phoma* each had a rate of 21.05%, followed by *Colletotricum* and *Cercospora with* a rate of 10.53% each. In the province of Comoé, the results show that the genus *Phoma* is the most frequent with a rate of 24.59%, followed by the genus *Fusarium* (20.66%) and finally the genus *Macrophomina* (13.77%). In the province of Sissili, fungi of the *Phoma* genus are present at a rate of 18.23%, followed by the *Fusarium* genus (14.78%) and finally the *Macrophomina* and *Curvularia* genera with a rate of 10.84% each (Table 2). The identification of fungi on these organs made it possible to determine fifteen (15) genera on stems and roots in the 4 collection provinces. The *Phoma* genus was the most frequent with 20.23%, followed by the *Fusarium* genus (18.06%) and the *Colletotrichum* genus (11.27%) (Figure 6).

This study presents the distribution of parasitic fungi on the leaves, stems, roots and pods of soybean (*Glycine max*) produced in Burkina Faso. Samples analysed on blotting paper and PDA medium revealed the presence of pathogenic fungal organisms: parasites in the strict sense or associated parasites or weaknesses. The results show that the Haut-Bassins region has the highest incidence of fungal diseases, followed by the Cascades and Centre-East regions. This could be explained by the environmental conditions and the sampling period. Most of the plots sampled in the Haut-Bassins were at the reproductive stage, while those in the Cascades and Centre-Est regions were mainly at the vegetative stage. Reznikov *et al.*(2018) in their study, showed that the incidence of anthrax was high at the R7 growth stage. During this reproductive phase, the plant has a large surface area to accommodate fungi. Secondly, the incidence of fungal diseases is unevenly distributed across the regions. This is due to a number of factors. Environmental conditions, cultivation practices, the

history of previous diseases and the selection of susceptible cultivars all influence the development of diseases(Bradley *et al*., 2021). Host plant susceptibility and interaction with other soil microbes are key factors shaping the composition of the fungal community. The previous crop used in a cropping system has an impact on the composition of the microbial community in a given location by favouring the reproduction of pathogenic and/or mutualistic organisms that are closely associated with that host plant (Benitez and Lehman, 2017; Edwards *et al*., 2015). In the United States, many regions are already experiencing dramatic changes in weather patterns that may alter the traditional range of some pathogens and therefore affect the disease pressure they exert on crops (Delgado-Baquerizo *et al*.,2020 ;Velásquez *et al*., 2018).

From this study, seventeen (17) genera of fungi were identified on soybean in the 4 study sites. These fungi are essentially members of the Ascomycetes group. Several of our isolates have previously been documented as soybean pathogens (Shovan *et al*., 2008). The genera of parasitic fungi commonly found in the three regions on soybean leaves, stems and roots were *Phoma Fusarium*, *Colletotrichum*, *Macrophomina* and *Cercospora*. The *Phoma* genus had the highest incidence rate on soybean leaves 18% and 20% on stems and roots respectively. As for the genus *Fusarium,* it was identified in soybean plants that suffered from vascular wilting, root rot and stem rot, among others. (Pitt and Hocking, 2009). In fact, species such as *Fusarium solani, F. oxysporum, F. proliferatum, F. graminearum and F. sporotrichioides* according to (Abdelmagid *et al*., 2021; Broders *et al*., 2007; Chang *et al*., 2015; Arias *et al*., 2013; Farias and Griffin, 1989; Killebrew *et al*., 1993; Rizvi and Yang, 1996) are known to be causal agents of soybean root rot and the species *Fusarium fujikuroi* has been reported to be responsible for pre- and postemergence damping-off in soybeans (Pedrozo *et al*., 2015; Chang *et al*., 2020). *Fusarium thapsinum* and *F. equiseti* have also been reported as seed-borne pathogens of soybean (Pedrozo and Little, 2014). The genus *Phoma* on the other hand has been recognised as an opportunistic, cosmopolitan and ubiquitous pest of diseased or dead plants (Kövics *et al*., 2014) This is the case on spoiled mung bean and soybean sprouts (Sato *et al*., 2014). *Macrophomina* is a cosmopolitan soil saprophyte and is well known as a facultative and opportunistic plant pathogen that infects plants exposed to certain stress conditions (Wrather *et al*., 1997;Wrather *et al*., 2001). It is seed-borne (Kunwar, 1986). It causes charcoal rot by infecting roots through the adhesion of microsclerotia to the integuments during germination and emergence. (DeMooy and Burke, 1990). Temperatures close to 30°C and dry conditions make this pathogen prevalent in regions with subtropical and tropical arid climates such as Pakistan (Khan,2007), China (Xiao jian *et al*., 1988) and India (Suriachandraselvan and Aiyyanathan, 2006) where yield losses caused by this fungus can be as high as 90%. A comparison of the various soybean organs attacked showed that leaf organs were more heavily attacked than stems, roots and pods. Miller and Roy (1982) explain this finding by the fact that leaves have a greater surface area exposed to inocula, unlike pods, which are the seeds protective envelopes. In fact, competition between fungi, differences in the nutritional status of plant organs and the differential responses of each fungus to temperature may have influenced the stratification of fungi on the soybean plant (Miller and Roy, 1982).

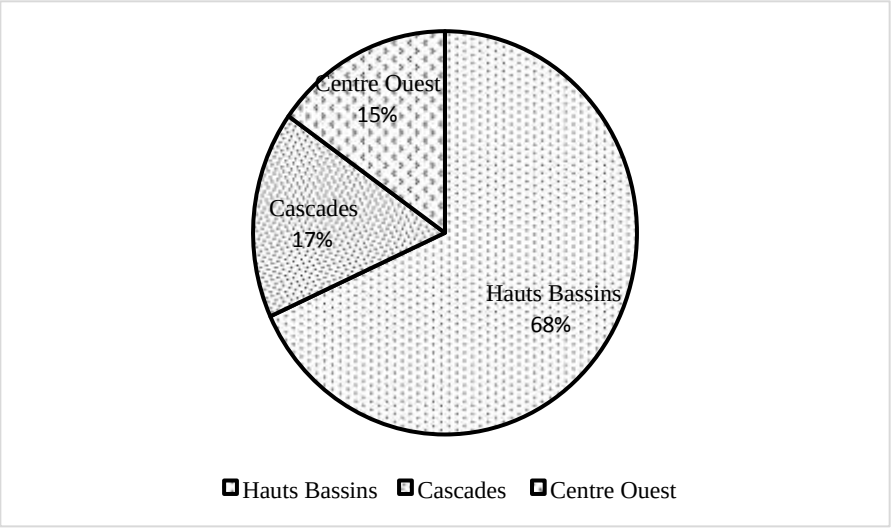


Figure 2. Incidence rates of the disease by collection region.

Figure 3. Symptom status of leaf organs collected. (A) circular brown spots ; (B) necroses; (C) oval brown spots; (D) dotted brown spots; (E) circular grey spots followed by yellowing; (F) brown spot followed by a yellow halo; (G) soft rot; (H) wilting; (I) brown spots along the main and secondary veins; (J) desiccation; (K) leaf chlorosis.

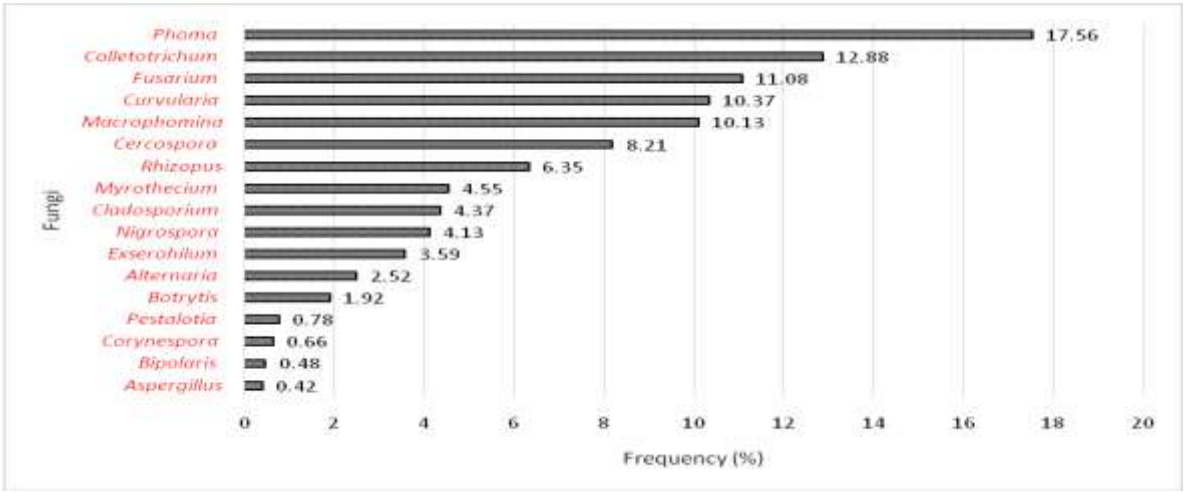


Figure 4. Percentage of fungi identified on the leaf organs collected.

Table 1. Breakdown of fungi by province of collection.

Fungi	Houet	Kénédougou	Comoé	Sissili
Alternaria	2,16	7,61	2,48	1,59
Aspergillus	1,20	0,00	0,24	0,00
Bipolaris	0,96	0,00	0,47	0,00
Botryitis	0,24	10,87	1,06	3,81
Cercospora	6,25	2,17	8,16	12,70
Cladosporium	4,81	5,43	4,14	4,13
Colletotrichum	23,32	6,52	9,81	9,21
Corynespora	0,24	5,43	0,12	1,27
Curvularia	7,93	13,04	11,70	9,21
Exselerohilum	2,40	0,00	4,14	4,76
Fusarium	15,87	10,87	8,75	11,11
Macrophomina	6,73	9,78	12,53	8,25
Myrothecium	6,25	5,43	3,66	4,44
Nigrospora	4,09	1,09	5,08	2,54
Pestalotia	0,72	1,09	0,95	0,32
Phoma	9,86	17,39	21,28	17,78
Rhizopus	6,97	3,26	5,44	8,89

Figure 5. Symptomatological status of stems and roots collected. (L) brown spots on roots ; (M) dry rot with browning ; (M) brown spots on stems ; (O1) dry rot with blackening; (O2) and (O3) dry rot with black mycelium inside the root.

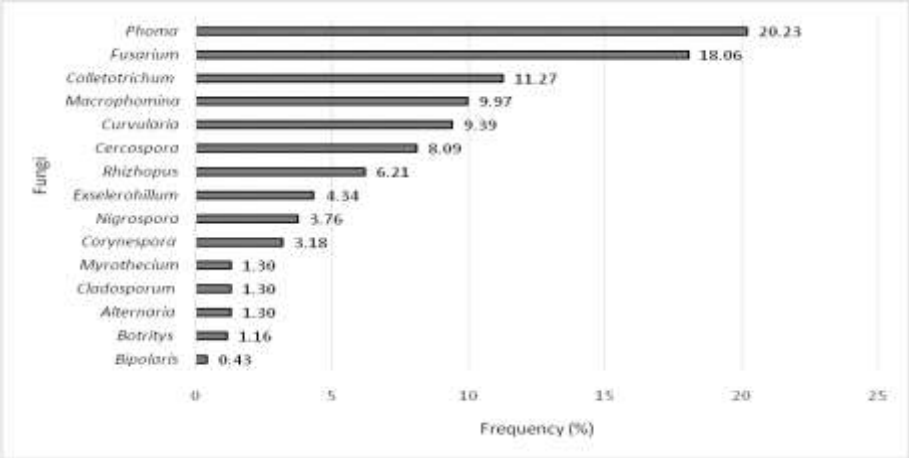


Figure 6. Rate of fungi identified on roots and stems at collection sites.

Table 2. Incidence rates of fungi on stems and roots according to study sites.

Fungi	Houet	Comoé	Sissili	Kénédougou
Alternaria	1,21	1,31	1,48	0,00
Bipolaris	1,21	0,00	0,49	0,00
Botritys	1,21	0,66	1,97	0,00
Cercospora	13,33	5,25	7,88	10,53
Cladosporum	0,00	1,64	1,97	0,00
Colletotrichum	18,79	8,20	9,85	10,53
Corynespora	7,88	0,33	3,45	5,26

Curvularia	7,27	8,85	10,84	21,05
Exselerohilum	3,64	3,28	6,90	0,00
Fusarium	16,97	20,66	14,78	21,05
Macrophomina	2,42	13,77	10,84	5,26
Myrothecium	1,21	0,98	1,97	0,00
Nigrospora	6,06	3,93	1,48	5,26
Phoma	14,55	24,59	18,23	21,05
Rhizopus	4,24	6,56	7,88	0,00

As regards the pods collected, the identification work enabled us to define ten (10) genera of fungi at all the sites (Fig. 7). The results show that the genus *Fusarium* is the most frequent with a rate of 28.80%, followed by the genus *Colletotrichum* (22.22%) and the genera *Phoma* (18.89%) and *Cercospora* (16.67%) (Figure 8).

Figure 7. Symptom status of pods collected. (P1) blackish spot on pods; (P2) and (P3) rot with black mycelium inside the pod; (P4) seed rot with black mycelium; (P5) brown spots on the radical.

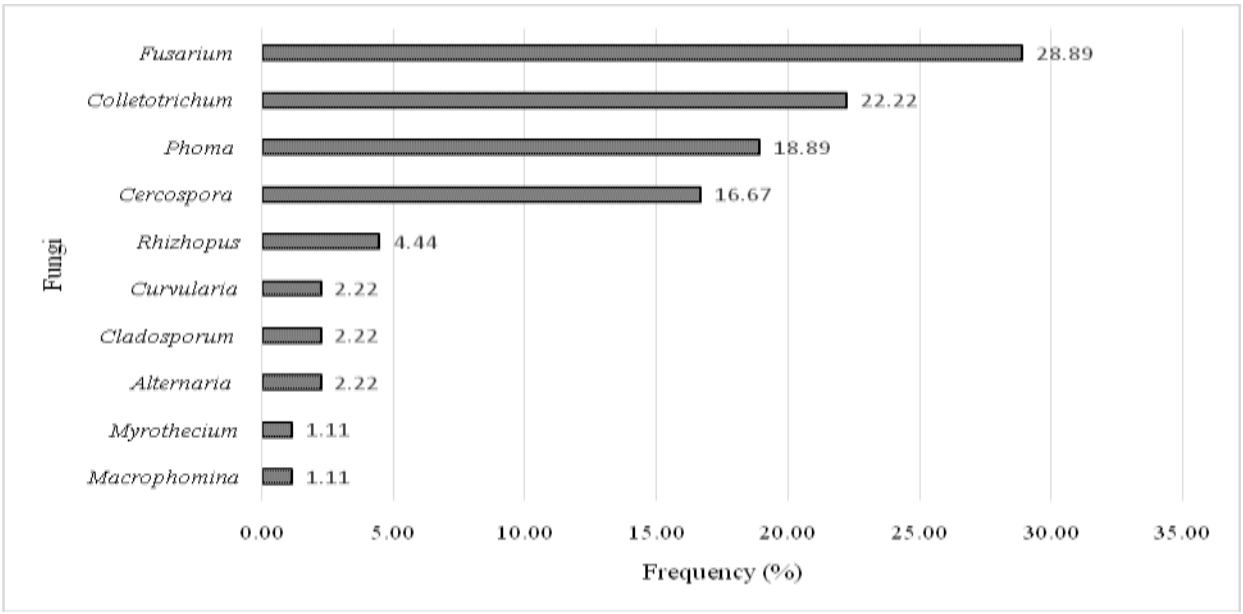
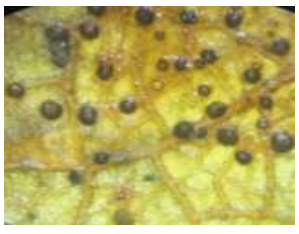


Figure 8. Incidence rate of fungi on collected pods.

Of a total of seventeen fungal genera identified throughout the collection from the 4 collection sites, ten (10) genera were found to be pathogenic to soybean. These are the genera illustrated in the plate below thanks to observation work carried out with the Optika Microscopes magnifying glass (4x) and/or the Euromex Microscopes-Holland DC.5000F CMEX 5 Camera microscope (40x). (Figure 9).

Isolate on culture medium	Observing with a magnifying glass (4x)	Observation au microscope (40x)	Genres
			***Curvularia***
			***Myrothecuim***
			***Fusarium***
			***Macrophomina***
			***Cercospora***
			***Phoma***

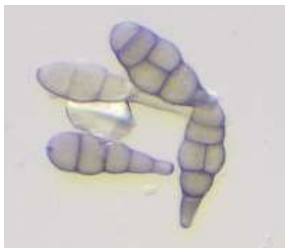
Isolate on culture medium	Observing with a magnifying glass (40x)	Genres
		***Bipolaris***
		***Exserohillum***
		***Alternaria***

Figure 9. Different genera of pathogenic fungi isolated from leaves, stems, roots and pods collected at the study sites.

Legend : (A) surface of a Curvularia colony on a PDA plate, (B) surface of a Myrothecuim colony on a PDA plate, (C) surface of a *Fusarium* colony, (D) surface of a *Macrophomina* colony on a PDA plate, (E) surface of a *Cercospora* colony on a PDA plate, (F) surface of a *Phoma* colony on a PDA plate, (G) surface of a *Colletotrichum* colony on a PDA plate, (H) surface of a *Bipolaris* colony on a PDA plate, (I) surface of an *Exserohilum* colony on a PDA plate, (J) surface of an *Alternaria* colony on a PDA plate.

CONCLUSION

This study enabled us to discover that soyabean is subject to a number of parasitic attacks, particularly fungal attacks. Seventeen genera of fungi were identified, including ten genera recognised as obligatory parasites and seven as optional or weak parasites. The Haut-Bassins region has the highest incidence (17 genera identified), followed by the Cascades and Centre-Ouest regions (14 genera each). The data on the incidence and distribution of fungi generated in this study can be used as a reference in future research to monitor changes in the composition of the profiles of the predominant fungi. Molecular identification work will make it possible to specifically identify the pathogens responsible for soybean cultivation in Burkina Faso. This will enable effective, sustainable and environmentally-friendly control methods to be devised.

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