

SPATIAL DISTRIBUTION, PREVALENCE, AND SEVERITY OF FUNGAL DISEASES AFFECTING BLACK PEPPER CULTIVATION IN CÔTE D'IVOIRE

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ABSTRACT

Black pepper (*Piper nigrum* L.) is a globally important spice crop. In recent years, black pepper production has faced challenges due to various factors, including diseases. However, the black pepper cultivation is significant in Côte d'Ivoire, the specific diseases affecting the crop have not been comprehensively characterized. This study aimed to identify and characterize fungal diseases of black pepper in Côte d'Ivoire. Surveys were conducted in major black pepper-growing regions from November 2019 to July 2021. Samples of symptomatic leaves, stems, berries, and soil were collected from black pepper plants in three regions: South, Central, and West Côte d'Ivoire. Black pepper disease prevalence and severity were assessed to identify areas with higher disease incidence. Five primary disease symptoms were observed: white spot, necrotic spot with yellow halo, apical necrosis, large concentric brownish spot, and apical desiccation. White spot had the highest average prevalence (67%), followed by necrotic spot with yellow halo (56.89%). Average black pepper disease severity was most pronounced for white spot (25.34%) and decreased for the other symptoms. *Colletotrichum* and *Fusarium* were the predominant fungal genera isolated from diseased black pepper plant tissues. Pathogenicity tests confirmed the causal role of these fungi in black pepper disease development. Principal component analysis and hierarchical cluster analysis of pathogenicity data grouped 200 *Colletotrichum* and 93 *Fusarium* isolates into three distinct clusters, respectively. These findings provide a foundation for developing effective disease management strategies for black pepper in Côte d'Ivoire.

Keywords: Côte d'Ivoire, black pepper fungal diseases, disease prevalence, disease severity.

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INTRODUCTION

Spices have been a vital ingredient of food all over the world since time immemorial. Among the spices, black pepper (*Piper nigrum*, 2n=52) is the most widely used and commercially the most important one, aptly known as 'the king' of spices (Vijesh *et al.*, 2024). Black pepper is a perennial, woody climbing vine belonging to the family *Piperaceae*. *Piper nigrum* L. is one of the oldest and the most popular spices in the world. It is indigenous to the Malabar Coast of India, and its cultivation has spread to several other tropical countries like Indonesia, Malaysia, Sri Lanka, Thailand, China, Vietnam, and Cambodia (Addharu *et al.*, 2022). As an

important cash crop in many tropical and subtropical developing countries, black pepper cultivation area comprises over 0.4 million hectares, given 510,045 metric tons of production and 2 billion US dollar trade value in 2017 (Xue *et al.*, 2023).

Black pepper, often referred to as the "king of spices," is a globally prized commodity with applications extending far beyond culinary enhancement. Its incorporation into a wide range of dishes, from fried foods to pastries and baked goods, has solidified its status as a culinary cornerstone. Beyond its gastronomic appeal, black pepper possesses notable medicinal properties. Studies have demonstrated its potential in combating cancer, diabetes, and microbial infections, underscoring

its significance for human health (Kumar *et al.*, 2017). The cultivation of black pepper not only satisfies the global demand for this spice but also provides a substantial income source for farmers (Addharu *et al.*, 2022).

Côte d'Ivoire, a West African nation, has embraced black pepper cultivation, with production concentrated in the southern regions of Aboisso, Agboville, Azaguié, Dabou, N'Douci, and Sikensi, as well as Saïoua in the west and Toumodi in the central part of the country. Despite its cultivation, the nation's production falls short of domestic consumption, necessitating imports to meet local demand. Annual black pepper production is estimated at 180 tonnes, while consumption exceeds 280 tonnes (FIRCA, 2020).

Like many other crops, black pepper is susceptible to a range of diseases, particularly those caused by pathogenic fungi. These diseases pose a significant threat to black pepper production, leading to yield losses and economic hardship for farmers. In regions such as Malaysia and Vietnam, fungal diseases have been identified as major constraints to black pepper cultivation. Downy mildew (*Phytophthora capsici*), wilt (*Fusarium solani*), and anthracnose caused by *Colletotrichum* species have been implicated in substantial pre-harvest losses in Malaysia (Zakaria and Noor, 2020). Similarly, anthracnose has been reported to cause up to 40% yield reductions in Vietnamese pepper fields (Santoshreddy *et al.*, 2021). The pathogens can infect several parts on crops, while the infection during postharvest and ripening stages causes major economic losses (Zakaria, 2021).

Despite the economic importance of black pepper in Côte d'Ivoire, a comprehensive understanding of natural threat such as fungal diseases affecting this crop remains limited. In a general context of One Health approach, it's crucial to address this knowledge gap and support sustainable black pepper production in order to improve people live and fight poverty. In this study, the aim is to (i) assess the prevalence and severity of fungal diseases on pepper; (ii) isolate and identify the most

abundant fungi; and (iii) assess their pathogenicity. The results of this study will enable the development of effective strategies for the management of fungal diseases on pepper in Côte d'Ivoire.

MATERIALS AND METHODS

Surveyed Areas: To comprehensively assess the disease landscape of black pepper in Côte d'Ivoire, a structured survey campaign was implemented from November 2019 to July 2021. The study targeted key black pepper-producing regions, encompassing three primary geographic zones: South, Central, and West.

Within the southern region, sampling was conducted in Aboisso, situated in the Sud-Comoé region, as well as Agboville, Azaguié, N'Douci, Sikensi, and Dabou, located within the Agneby-Tiassa and Grands Ponts regions. The western region's focus was on Saïoua, situated in the Haut-Sassandra region. In the central region, Toumodi, within the Béliér region, served as the sampling site. Collectively, these locations represented 65 black pepper farms that were intensively surveyed (Fig. 1).

A systematic approach was employed for sample collection. The primary focus was on symptomatic leaf tissue, with a total of 259 samples gathered. To expand the disease investigation beyond foliar symptoms, 14 necrotic stem samples and 16 moldy grain samples were also collected. Recognizing the potential involvement of soilborne pathogens, 41 soil samples were included in the study. This comprehensive sampling strategy aimed to capture the full spectrum of diseases affecting black pepper in Côte d'Ivoire.

Fungal Disease Prevalence and Severity Assessment: To assess disease prevalence, a standardized methodology was employed. In each plantation, 30 randomly selected black pepper plants were examined using the square method, as described by Afouda *et al.* (2013). Disease prevalence for a specific symptom was calculated using the following formula:

$$\text{Prevalence} = \frac{\text{Number of plants exhibiting the symptom}}{\text{Total number of plants}} \times 100 \quad (\text{Iwuagwu } et al., 2022)$$

To further characterize disease intensity, the average percentage of leaves with a given symptom per locality (PMFS) was determined. This metric was calculated based on the method outlined by Lakshmi *et al.* (2014). Within each farm, five black pepper plants were randomly chosen from the established observation squares. Leaf symptom assessment was conducted on two opposing sides (east-west or south-north) of each plant, with each side representing one square meter. The PMFS for a given locality was computed as follows:

$$\text{PMFS} = \frac{\text{Number of leaves with the symptom}}{\text{Total number of leaves on two sides}} \times 100 \quad (\text{Banito } et al., 2022)$$

Disease severity was evaluated using a visual rating scale adapted from Belaghihalli *et al.* (2022), ranging from 1 (no symptoms) to 5 (severe symptoms) (Fig. 2).

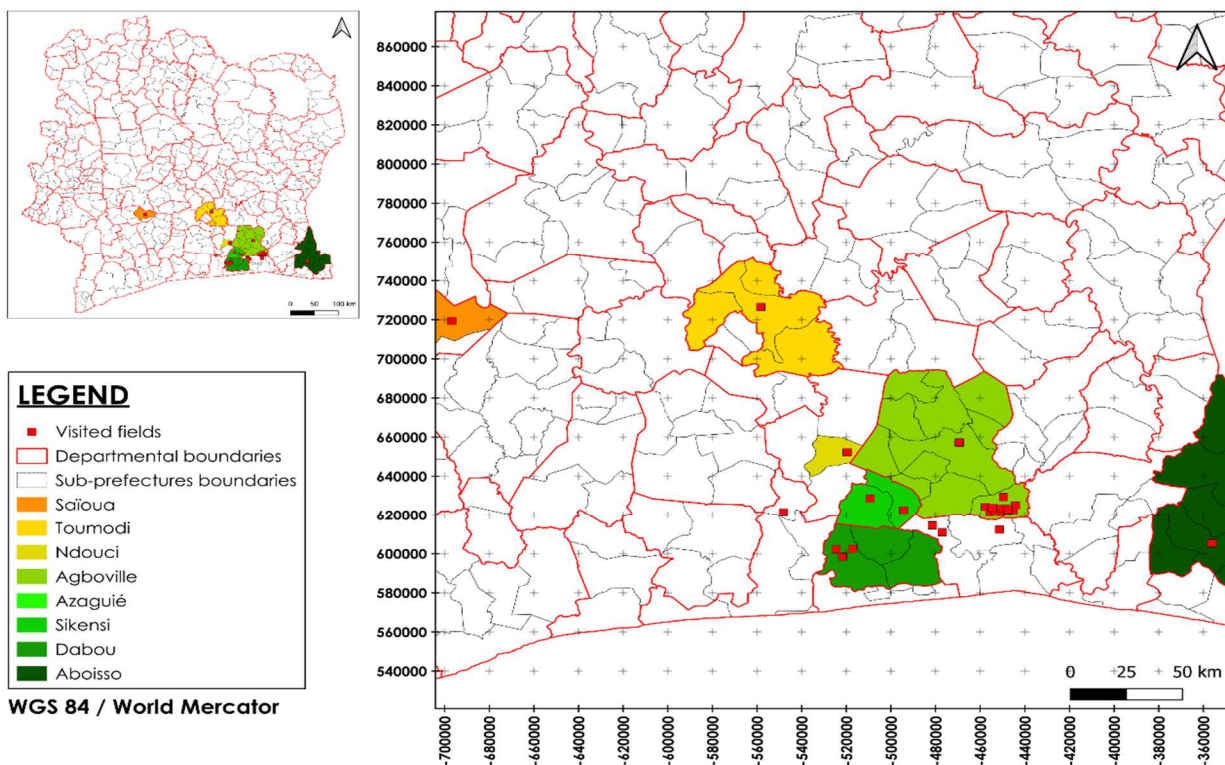


Fig. 1: Farms visited in black pepper-growing areas of Côte d'Ivoire



- 1: apparently healthy organ or no symptoms
- 2 : Very few small lesions
- 3: Many small lesions or one large spot
- 4: Necrotic beach or several large spots

Fig. 2: Visual rating scale for pepper disease severity (Belaghihalli *et al.*, 2022).

A symptom-based disease severity index (I_s) for each farm was calculated using the formula:

$$I_s = \frac{\sum(X_i \times n_i)}{N \times Z} \times 100 \text{ (Pethybridge and Nelson, 2012)}$$

Where I_s = Disease severity index on the farm, X_i = Sum of disease severities i on the plant, n_i = Number of plants of severity i , N = Total number of plants observed, and Z = Highest severity scale, 5 in this case.

Isolation of Fungal Agents Associated with Black Pepper Plant Diseases: Fungal pathogens were isolated from infected black pepper leaf, stem, and fruit tissues. Surface sterilization was conducted by initially washing samples with tap water to remove extraneous debris. Subsequently, four 1-cm² sections were excised from the transition zone between diseased and healthy tissue on leaves and stems. Fruit pericarp sections were obtained

directly from infected areas. Samples were immersed in a 3.6% sodium hypochlorite solution diluted 1:1 with sterile distilled water for 2 minutes, followed by three sequential rinses in sterile distilled water for 2 minutes each. Sterile blotting paper was used to dry the samples. Treated leaf, stem, and fruit sections were placed on potato dextrose agar (PDA) in 90-mm Petri dishes.

Soil-borne fungal isolates were obtained using the suspension-dilution method. Briefly, 10 g of soil was suspended in 90 ml of sterile distilled water and mixed for 30 minutes to create a stock solution. Serial decimal dilutions were prepared by transferring 1 ml of the previous dilution into 9 ml of sterile distilled water, resulting in dilutions of 10⁻¹, 10⁻², and so on up to 10⁻⁶. One milliliter of the final dilution was added to PDA medium, which was then cooled to 40°C. Petri dishes

were manually agitated in a horizontal figure-eight pattern to ensure uniform distribution of the inoculum. All plates were incubated at 28°C until fungal colonies were visible (Ehui *et al.*, 2019).

Identification of Isolated Fungal Agents: Fungal isolates were characterized based on macroscopic and microscopic morphological features. Macroscopic observations included colony color, texture, and the presence of diffusible pigments. Microscopic analysis was performed using a TECLAST Motic model BA310LED optical microscope. Fungal structures were mounted on slides for examination. Identification was based on thallus morphology, reproductive organ types, and their corresponding characteristics, following the identification key of Mathur and Kongsdal (2003).

Pathogenicity test

Inoculum production : Pathogenicity tests were conducted in vitro following the protocol of Than *et al.* (2008). Inoculum was prepared from 200 *Colletotrichum* and 93 *Fusarium* isolates cultured for one week as monospores. Conidia were harvested by scraping the fungal growth with a sterile spatula and suspending the scrapings in 5 ml sterile distilled water. The suspension was vortexed for one minute at 1500 rpm and then filtered through Whatman paper to remove mycelial fragments. Conidial concentration was adjusted to 4×10^6 spores/ml using a hemocytometer and sterile distilled

water, with a drop of Tween 20 added to the final suspension.

Inoculation of Detached Black Pepper Leaves : Healthy, three-month-old leaves from three black pepper (*Piper nigrum* L.) accessions (Lampong-Djibo, Muntok-Djibo, and Muntok-CNRA) were used. Leaf surfaces were sterilized with 70% ethanol for 30 seconds, rinsed thrice with sterile distilled water, and dried with sterile blotting paper. Three leaves (three replicates) were placed on three layers of sterile blotting paper moistened with sterile distilled water in 120 mm Petri dishes. A 10 µl droplet of spore suspension from each isolate was applied to the underside of each leaf. Control leaves received 10 µl of distilled water containing Tween 20 (Koffi, 2014). Petri dishes were incubated in the dark at 28°C for 48 hours.

Observations : Leaves were observed daily for symptom development beginning 48 hours post-inoculation. The incubation period for each isolate was recorded. Infection rate was calculated as the percentage of leaves showing symptoms ten days post-inoculation. Symptom severity was assessed using a 0-5 rating scale (Amrani *et al.*, 2014) based on infected leaf area: 0 = no symptoms, 1 = <20% infected, 2 = 21-40% infected, 3 = 41-60% infected, 4 = 61-80% infected, 5 = >80% infected (Table 1).

Table 1: Rating scale from Amrani *et al.* (2014)

Scoree	1	2	3	4	5
Infected leaf area (%)	1 - 20	21 - 40	41 - 60	61 - 80	>80

The disease severity index (IS) was calculated using the formula:

$$IS = (\sum Xi \times ni / 5 \times Nt) \times 100 \quad (\text{Kranz, 1988})$$

IS = Disease severity index; Xi = infected leaf area score; Ni = Number of leaves with severity i; Nt: Total number of leaves observed; 5: Highest score on the scale.

Isolates causing symptoms were reisolated on PDA and compared to the original isolates.

Statistical analyses: Statistical analyses were performed using STATISTICA version 7.0 software. Data normality was assessed using the Shapiro-Wilk test to determine if the data followed a normal distribution. For normally distributed data, analysis of variance (ANOVA) was conducted at a significance level of $\alpha = 0.05$ to compare means between groups. Following a significant ANOVA result, Fisher's Least Significant Difference (LSD) test ($\alpha = 0.05$) was employed for multiple comparisons and to separate means of prevalence and disease severity into statistically homogeneous groups. For non-normal data,

Kruskal-Wallis test would be employed to compare medians between groups.

Hierarchical ascending classification (HAC) was performed using XLSTAT version 2016 software to group fungal isolates based on shared morphological and pathogenicity characteristics. This analysis helps to identify distinct morphotypes among the isolated fungi. Additionally, principal component analysis (PCA) was conducted to visualize the relationships between incubation periods, prevalence, and severity of *Colletotrichum* and *Fusarium* isolates. PCA is a dimensionality reduction technique that identifies underlying patterns in the data and highlights potential correlations between these variables.

RESULTS

Principal Symptoms of Fungal Diseases in Côte d'Ivoire Black Pepper Orchards: A variety of symptoms were observed on different black pepper

(*Piper nigrum* L.) plant organs (Fig. 3). Abnormalities were noted on leaves, stems, and fruits.

Leaves exhibited the most diverse range of symptoms. The most common symptoms included circular necrotic spots surrounded by a yellow halo, large brownish spots arranged in concentric layers, apical necrosis forming an inverted “V” shape with a black

border and occasionally a yellow halo, apical desiccation resembling an inverted “V” shape, and white spots or powdery patches on the leaf surface.

About the peppercorns, early fruit drop and necrosis of clusters were observed.

Stem symptoms included rot, desiccation leading to plant death, and black pepper stem cankers.

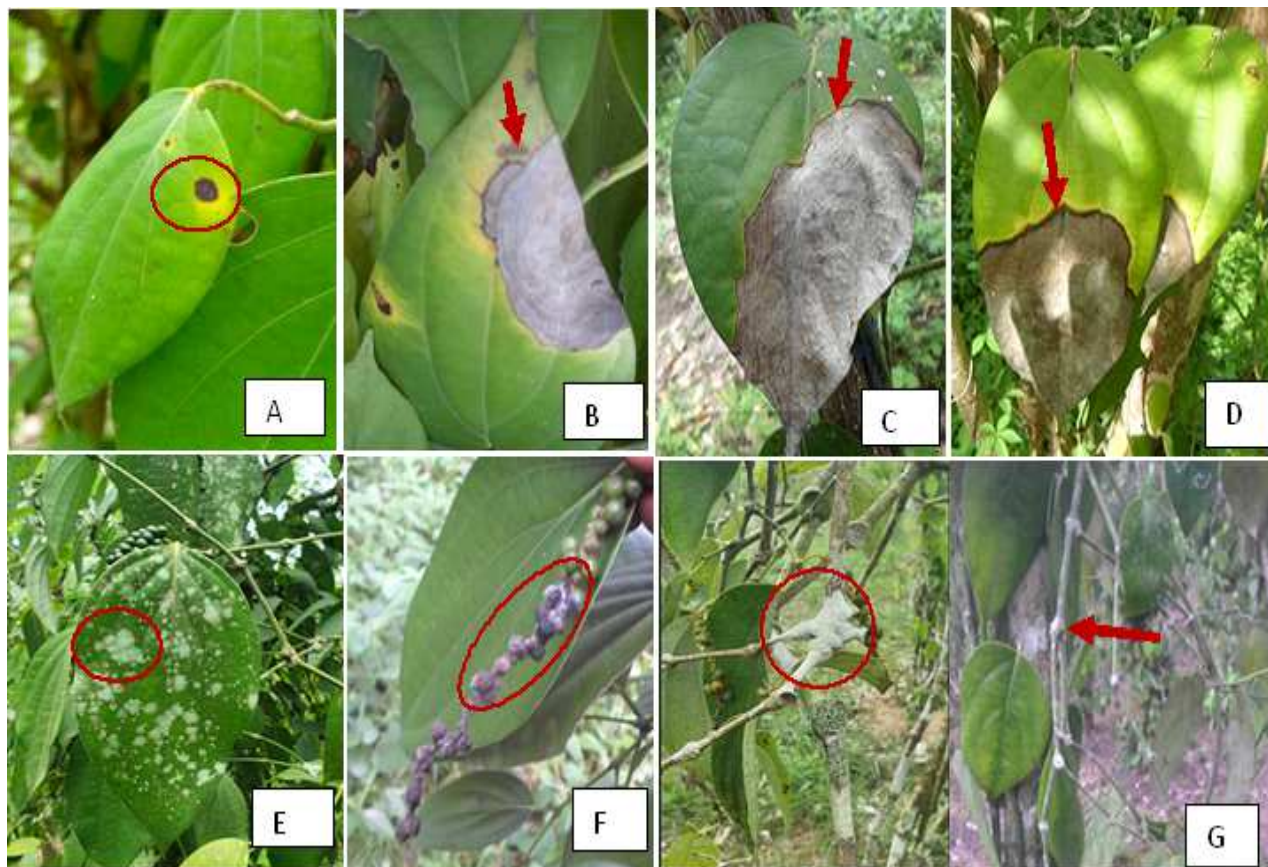


Fig. 3: Symptoms observed in black pepper orchards in Côte d'Ivoire

3A illustrates circular necrotic spots surrounded by a yellow halo. 3B depicts large brownish spots arranged in concentric layers. 3C shows apical necrosis in the shape of an inverted “V” with a blackish border and occasionally a yellow halo. 3D presents apical desiccation in the shape of an inverted “V”. 3E displays white spots or powdery growth on leaves. 3F demonstrates necrosis of fruit clusters. 3G shows stem necrosis.

Prevalence of Fungal Disease Symptoms in Black Pepper Production Areas: Circular necrotic spots with a yellow halo, large brownish spots in concentric layers, apical necrosis in the shape of an inverted “V” with a blackish border and sometimes a yellow halo, apical desiccation in the shape of an inverted “V”, and white spots or powdery growth on leaves were the primary fungal disease symptoms observed in black pepper

production areas (Table 2). White spots or powdery mildew were the most prevalent symptom in all surveyed locations, with a mean incidence of $67.30 \pm 4.68\%$. This was followed by necrotic spots with a yellow halo, apical necrosis, and large concentric spots, which had mean incidences of $56.89 \pm 3.27\%$, $45.32 \pm 4.31\%$, and $34.93 \pm 4.23\%$, respectively. Apical desiccation had the lowest incidence, with a mean of $29.56 \pm 4.49\%$.

The calculated symptom severity index revealed significant differences among the sampled localities (Fig. 4). The highest values were recorded in Saïoua ($57.10 \pm 2.62\%$), Azaguié ($46.28 \pm 7.62\%$), Dabou ($39.86 \pm 8.25\%$), N'douci ($37.96 \pm 6.35\%$), Toumodi ($35.08 \pm 2.24\%$), and Sikensi ($35.06 \pm 6.78\%$). The lowest values were obtained in Aboisso ($26.94 \pm 3.25\%$) and Agboville ($20.44 \pm 12.05\%$).

Table 2: Average prevalence of the main symptoms of Ivorian pepper orchards in 2019-2021

Localities	White powdery	Necrotic spots with yellow halo	Apical necrosis	Large brownish spots	Apical desiccation	Average of all symptoms	P-value
Aboisso	100.00 ± 3.03cA	55.55 ± 7.73aB	28.88 ± 9.68aC	0 ± 0Ad	26.66 ± 0.21bC	52.77 ± 5.16	0.040
Agboville	46.66 ± 3.13aA	76.66 ± 11.70aA	65.00 ± 2.01aA	6.66 ± 5.2aA	73.33 ± 0.9aA	53.66 ± 4.6	0.544
Azagué	61.82 ± 7.83aA	63.62 ± 5.08aA	56.41 ± 8.35aA	45.77 ± 12.65aA	38.88 ± 7.23cA	53.3 ± 8.22	0.057
Dabou	93.33 ± 3.16cA	70.55 ± 8.49aB	56.66 ± 12.51aC	36.66 ± 8.92aAB	45.55 ± 2.01abC	60.55 ± 7.01	0.019
N'douci	64.17 ± 3.40bA	8.33 ± 1.66cA	25.83 ± 5.83aA	31.07 ± 13.92aA	25.00 ± 8.33bA	30.88 ± 6.62	0.338
Saïoua	0 ± 0dAB	60.00 ± 0.10aA	26.66 ± 2.77aB	40.00 ± 5.00aB	0 ± 0dAB	42.22 ± 2.62	0.0040
Sikensi	57.09 ± 9.95aA	48.37 ± 6.96bB	40.55 ± 9.09aB	25.29 ± 10.17aC	10.95 ± 2.38dAB	36.45 ± 7.71	0.0003
Toumodi	0 ± 0dC	18.33 ± 1.66cB	50.00 ± 2.57aA	46.66 ± 4.20aA	33.33 ± 0.10bB	37.08 ± 2.13	0.0010
Average for all zones	67.30 ± 4.68	56.89 ± 3.27	45.32 ± 4.31	34.93 ± 4.23	29.56 ± 4.49		
P-value	0.007	0.007	0.17	0.45	0.002		

Mean ± standard error values followed by the same lowercase letters for comparisons within rows, and those followed by the same uppercase letters for comparisons within columns, were not significantly different at the 5% significance level, according to Fisher's Least Significant Difference test.

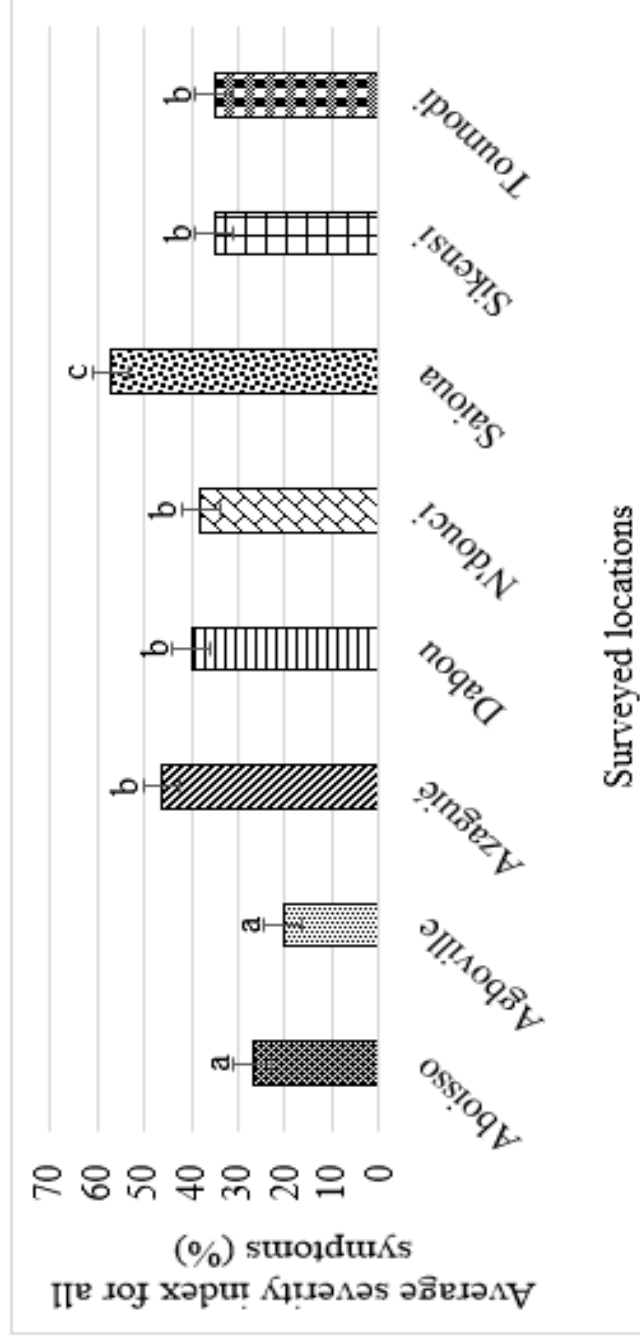


Fig. 4: Average severity index of fungal diseases at the surveyed sites

Bars with the same letters are not significantly different at the 5% significance level, according to Fisher's Least Significant Difference test

Diversity of identified fungi: Overall, six fungal genera were identified from infected black pepper organs: *Colletotrichum*, *Fusarium*, *Curvularia*, *Alternaria*, *Rhizoctonia*, and *Aspergillus* (Fig. 5). A total of 424 isolates were identified. Of these, 328 were isolated from symptomatic leaves, with the highest frequency of isolation from the *Colletotrichum* species (87.20%), followed by *Fusarium* (11.28%). For the other genera—*Curvularia*, *Alternaria* and *Rhizoctonia*—the isolation frequencies were 0.91%, 0.3% and 0.3% respectively. From necrotic grains, 36 isolates were obtained. Isolation percentages were zero for the genera *Colletotrichum*, *Curvularia*, *Alternaria*, and *Rhizoctonia*, and 100% for the genus *Fusarium*. Seven isolates were identified from necrotic stems, with isolation rates for *Colletotrichum*

and *Fusarium* at 57.14% and 42.86%, respectively. Fifty-three isolates were identified in the soil, with 39.62% belonging to the genus *Fusarium* and 60.38% to *Aspergillus*.

There was no clear relationship between fungal genera and the type of symptoms described. In other words, a specific symptom was not consistently associated with fungi from a single genus. The same fungal genus could cause multiple symptoms, and several fungal agents could also produce identical symptoms.

The processing of the samples in the laboratory resulted in isolates of *Colletotrichum* that varied in thallus colouration on PDA medium ranging from dark grey to white. Furthermore, the cylindrical conidia observed. The colonies of *Fusarium* isolates showed yellow-orange to pink pigmentation on PDA medium and variable conidial morphology.

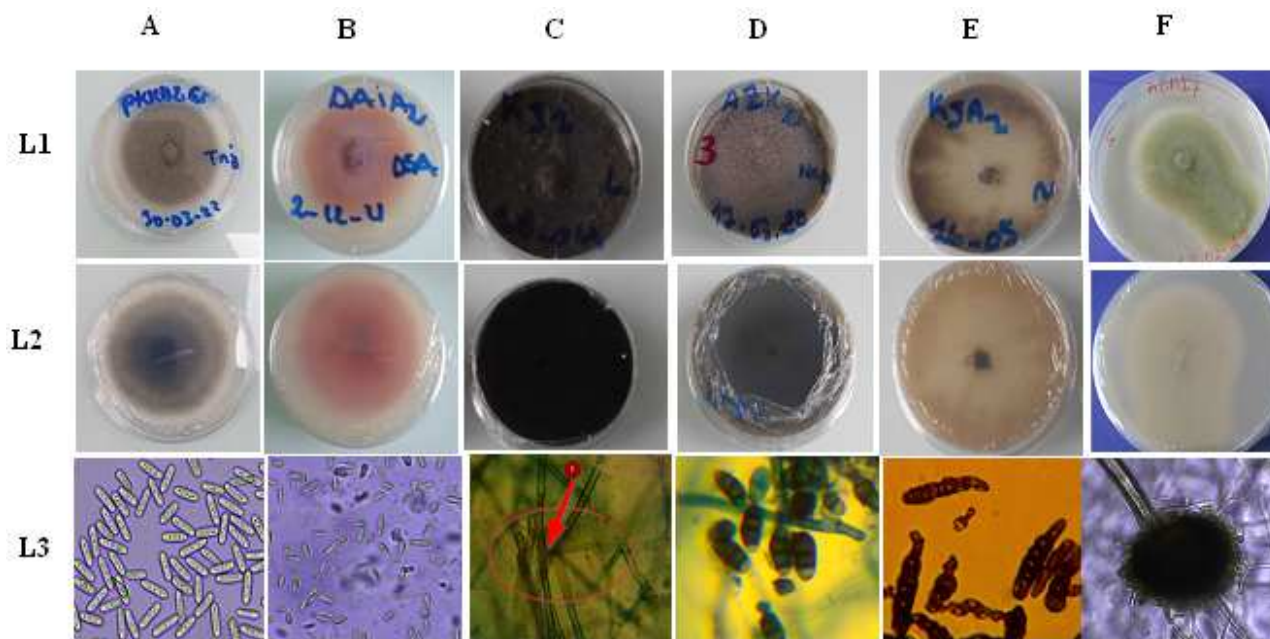


Fig. 5: Macroscopic and microscopic aspects of a 7-day culture on potato dextrose agar medium

L1: plates top view, L2: plates bottom view, L3: Spores. A: *Colletotrichum* sp, B: *Fusarium* sp, C: *Rhizoctonia* sp, D: *Curvularia* sp, E: *Alternaria* sp, F: *Aspergillus* sp

Pathogenicity of fungal isolates:

Symptom Description: Leaves from the Muntok and Lampong types of black pepper were inoculated using the "soft inoculation of detached leaves" method with 200 isolates of *Colletotrichum* and 93 isolates of *Fusarium*. All isolates produced symptoms except for one *Fusarium* isolate. Ten days post-inoculation, the size of the spots varied between the different types of black pepper. The observed symptoms included circular necrotic spots with a yellow halo and large brownish spots arranged in concentric layers (Fig. 6). There was no specific

relationship between the fungal genera and the type of symptom observed. In other words, the symptoms were not associated with fungi from a single genus.

Incubation Period: The incubation period following inoculation varied for all isolates across the three black pepper accessions. Tables 3 and 4 indicate that the incubation period ranged from 2.33 ± 0.70 to 5 ± 0.00 days for the Lampong-Djibo accession, from 2.28 ± 0.57 to 5.42 ± 0.37 days for the Muntok-CNRA accession, and from 3.16 ± 0.33 to 4.44 ± 0.40 days for the Muntok-Djibo accession. No significant differences were

observed between the Lampong-Djibo and Muntok-Djibo accessions for isolates belonging to the *Colletotrichum* genus across the eight locations. However, isolates inoculated on the Muntok-CNRA accession showed a significant difference. For isolates of the *Fusarium* genus, the average incubation period ranged from 2.00 ± 0.01 to 5.66 ± 1.20 days for the Lampong-Djibo accession, from 2.00 ± 0.00 to 5.00 ± 1.00 days for the Muntok-CNRA accession, and from 1.75 ± 1.18 to 5.00 ± 1.00 days for the Muntok-Djibo accession. Statistical analysis revealed that only isolates inoculated on the Lampong-Djibo accession showed a significant difference.

Occurrence of symptoms on detached leaves : The incidence of symptoms on detached leaves of the three

black pepper accessions was recorded. Tables 5 and 6 present the average incidence of symptoms induced by *Colletotrichum* and *Fusarium* isolates, respectively. The Lampong-Djibo accession exhibited a symptom incidence ranging from 50% to 70%, followed by Muntok-CNRA and Muntok-Djibo, which showed incidences ranging from 47% to 77% and 55% to 78%, respectively. For *Fusarium* isolates tested on Lampong-Djibo, the incidence ranged from 44% to 88%, followed by Muntok-CNRA and Muntok-Djibo, with incidences ranging from 46% to 69% and 25% to 100%, respectively. No significant differences in mean symptom incidence were observed between the isolates, regardless of the accession tested.

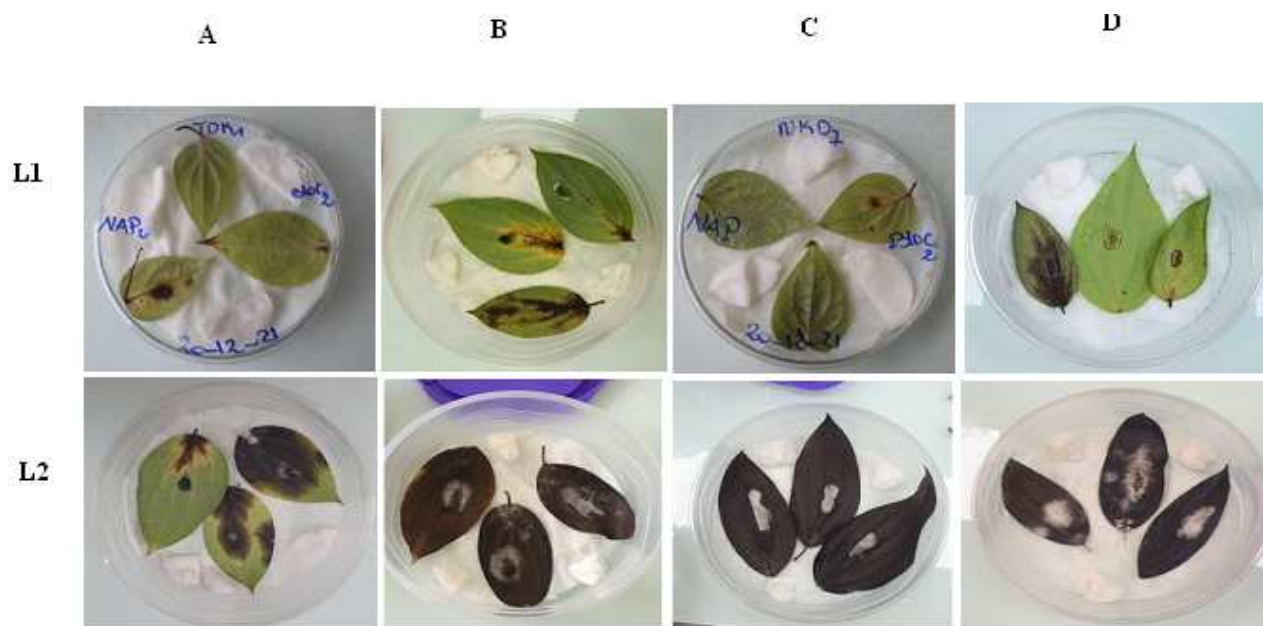


Fig. 6: Post-inoculation symptoms on detached black pepper leaves

L1: 2 days post-inoculation, **L2:** 10 days post-inoculation, **A and B:** *Colletotrichum* on Muntok and Lampong types respectively, **C and D:** *Fusarium* sp. Muntok and Lampong types respectively

Table 3: Incubation period of *Colletotrichum* isolates inoculated on detached leaves

		Incubation period (days) on black pepper accessions		
		Lampong-Djibo	Muntok-CNRA	Muntok-Djibo
Localities of <i>Colletotrichum</i> isolates	Aboisso	2.33± 0.70a	5.42 ± 0.37c	4.14± 0.55a
	Agboville	3.00 ± 0.63a	2.28± 0.57a	4.44 ± 0.40a
	Azaguié	2.90 ± 0.32a	3.63 ± 0.24ab	3.16 ± 0.33a
	Dabou	3.54 ± 0.45a	3.60± 0.52ab	4.42 ± 0.42a
	N'douci	3.80± 0.71a	3.20 ± 0.35ab	4.61 ± 0.46a
	Saïoua	4.00± 1.00a	5.00± 1.26bc	3.83 ± 1.16a
	Sikensi	2.90 ± 0.3a	3.58± 0.28ab	3.63 ± 0.29a
	Toumodi	5.00 ± 0.00a	3.50 ± 1.08ab	4.20± 0.80a
	P-value	0.478325	0.000321	0.184966

Means ± standard error values followed by the same letters in a column are statistically identical at the 5% significance level according to Fisher's Least Significant Difference test.

Table 4: Incubation period for *Fusarium* isolates inoculated on detached leaves

		Incubation period (days) on black pepper accessions		
		Lamong-Djibo	Muntok-CNRA	Muntok-Djibo
Localities of <i>Fusarium</i> isolates	Aboisso	5.66 ± 1.20c	3.66 ± 0.37a	1.75 ± 1.18a
	Agboville	2.00 ± 0.1a	2.40 ± 0.67a	4.00 ± 1.09a
	Azaguié	2.23 ± 0.47a	2.60 ± 0.58a	3.23 ± 0.52a
	Dabou	2.5 ± 0.5a	4.37 ± 0.42a	4.50 ± 0.80a
	N'douci	3.66 ± 0.21ab	2.00 ± 0.01a	3.50 ± 0.50a
	Saïoua	2.33 ± 0.33a	5.00 ± 1.00a	5.00 ± 1.00a
	Sikensi	3.77 ± 0.55ab	2.33 ± 0.53a	3.85 ± 0.46a
	Toumodi	3.50 ± 1.50ab	3.25 ± 0.53a	2.60 ± 1.28a
	P-value	0.0232	0.1132	0.4232

Mean ± standard error values followed by the same letters in a column are statistically identical at the 5% significance level according to Fisher's Least Significant Difference test.

Table 5: Symptom incidence for *Colletotrichum* isolates inoculated on detached leaves.

		Incidence (%) on black pepper accessions		
		Lamong-Djibo	Muntok-CNRA	Muntok-Djibo
Localities of <i>Colletotrichum</i> isolates	Aboisso	0.55 ± 0.14a	0.54 ± 0.05a	0.57 ± 0.07a
	Agboville	0.50 ± 0.13a	0.47 ± 0.11a	0.76 ± 0.06a
	Azaguié	0.65 ± 0.06a	0.63 ± 0.04a	0.55 ± 0.06a
	Dabou	0.69 ± 0.08a	0.66 ± 0.079a	0.68 ± 0.06a
	N'douci	0.70 ± 0.12a	0.77 ± 0.07a	0.78 ± 0.06a
	Saïoua	0.66 ± 0.19a	0.53 ± 0.17a	0.72 ± 0.16a
	Sikensi	0.66 ± 0.06a	0.66 ± 0.05a	0.66 ± 0.05a
	Toumodi	0.66 ± 0.33a	0.5 ± 0.14a	0.6 ± 0.06a
	P-value	0.942115	0.209193	0.155758

Mean ± standard error values followed by the same letters in a column are statistically identical at the 5% significance level according to Fisher's Least Significant Difference test.

Table 6: Symptom incidence for *Fusarium* isolates inoculated on detached leaves.

		Prevalence (%) on pepper accessions		
		Lamong-Djibo	Muntok-CNRA	Muntok-Djibo
Localities of <i>Fusarium</i> isolates	Aboisso	0.44 ± 0.11a	0.55 ± 0.055a	0.25 ± 0.16a
	Agboville	0.63 ± 0.18a	0.53 ± 0.13a	0.73 ± 0.19a
	Azaguié	0.61 ± 0.12a	0.46 ± 0.10a	0.72 ± 0.09a
	Dabou	0.56 ± 0.04a	0.46 ± 0.06a	0.61 ± 0.05a
	N'douci	0.88 ± 0.07a	0.66 ± 0a	1 ± 0a
	Saïoua	0.51 ± 0.14a	0.66 ± 0.33a	0.5 ± 0.16a
	Sikensi	0.69 ± 0.08a	0.69 ± 0.08a	0.55 ± 0.08a
	Toumodi	0.66 ± 0a	0.66 ± 0a	0.4 ± 0.19a
	P-value	0.177814	0.177814	0.079506

Mean ± standard error values followed by the same letters in a column are statistically identical at the 5% significance level according to Fisher's Least Significant Difference test.

Symptom severity index on detached leaves: The data presented in Tables 7 and 8 illustrate the severity indices. The isolates of the *Colletotrichum* genus tested on the Lampong-Djibo accession exhibited severity indices ranging from 11.11% to 33.33%. This was followed by the Muntok-CNRA and Muntok-Djibo accessions, which recorded severity indices ranging from 10.66% to 32% and 13.68% to 23.33%, respectively. Statistical analyses

indicated no significant difference among the *Colletotrichum* isolates from the eight localities when tested on the Lampong-Djibo and Muntok-Djibo accessions. However, a significant difference was observed among the isolates inoculated on the Muntok-CNRA accession. Similarly, the isolates of the *Fusarium* genus tested on the Lampong-Djibo accession showed severity indices ranging from 8.89% to 21.54%, followed

by the Muntok-CNRA and Muntok-Djibo accessions, with severity indices ranging from 8.44% to 13.33% and 5% to 25.55%, respectively. Statistical analysis revealed no significant differences among the *Fusarium* isolates.

Table 7: Severity index of *Colletotrichum* isolates on detached leaves of *Piper nigrum* accessions.

		Severity index (%) on black pepper accessions		
		Lamong-Djibo	Muntok-CNRA	Muntok-Djibo
Localities of <i>Colletotrichum</i> isolates	Aboisso	11.11 ± 2.72a	11.22 ± 1.02a	16.19 ± 4.45a
	Agboville	15.83 ± 4.35a	13.33 ± 4.30a	21.85 ± 3.99a
	Azaguié	16.50 ± 1.99a	15.94 ± 2.32a	16.84 ± 3.34a
	Dabou	15.38 ± 2.54a	16.44 ± 2.33a	13.68 ± 1.19a
	N'douci	18.00 ± 5.17a	32.00 ± 6.41b	27.69 ± 7.19a
	Saïoua	33.33 ± 23.41a	10.66 ± 3.39a	23.33 ± 8.21a
	Sikensi	16.66 ± 2.22a	15.21 ± 1.81a	19.51 ± 2.55a
	Toumodi	13.33 ± 6.66a	10.66 ± 4.52a	20.00 ± 8.43a
	P	0.533384	0.004757	0.505672

Mean ± standard error values followed by the same letters in a column are statistically identical at the 5% significance level according to Fisher's Least Significant Difference test.

Table 8: Severity index of *Fusarium* isolates on detached leaves of *Piper nigrum* accessions.

		Severity index (%) on pepper accessions		
		Lamong-Djibo	Muntok-CNRA	Muntok-Djibo
Locations of <i>Fusarium</i> isolates	Aboisso	8.89 ± 2.22a	12.59 ± 2.06a	5.00 ± 3.19a
	Agboville	12.62 ± 3.80a	10.66 ± 2.66a	14.66 ± 3.88a
	Azaguié	21.54 ± 5.3a	11.11 ± 2.65a	22.05 ± 7.016a
	Dabou	10.51 ± 3.10a	12.50 ± 3.19a	25.55 ± 8.85a
	N'douci	17.77 ± 1.4a	13.33 ± 0.00a	20.00 ± 0.00a
	Saïoua	09.01 ± 3.0a	13.33 ± 6.66a	10.00 ± 3.33a
	Sikensi	18.97 ± 5.33a	8.44 ± 2.10a	16.00 ± 4.94a
	Toumodi	13.33 ± 0.00a	13.33 ± 0.00a	8.00 ± 3.88a
	P	0.722253	0.907190	0.670427

Mean ± standard error values followed by the same letters in a column are statistically identical at the 5% significance level according to Fisher's Least Significant Difference test.

Pathogenicity of *Colletotrichum* and *Fusarium* isolates

: A principal component analysis (PCA) was conducted on isolates of the *Colletotrichum* and *Fusarium* genera based on pathogenic parameters, which include the incubation period, infected leaf surface, average prevalence of symptoms, and average severity index. This analysis aimed to assess the degree of divergence among these isolates.

For the *Colletotrichum* genus, the PCA revealed that the first two components (F1 and F2), with eigenvalues greater than 1, account for 58.38% of the total observed variability (Fig. 7). Variables that are most eccentric and furthest from the factorial plan demonstrated the strongest correlations. The correlation circle indicates that the average prevalence of symptoms, infected leaf surface, and severity index of the isolates tested on the Muntok-CNRA accession are positively correlated with the F1 axis. Conversely, the incubation period of the isolates tested on the Muntok-CNRA accession is negatively correlated with the F1 axis. The incubation period of the isolates tested on the Muntok-

Djibo accession is negatively correlated with the F2 axis, while the average prevalence of symptoms, infected leaf surface, and severity index of the isolates tested on the Muntok-Djibo accession are positively correlated with the F2 axis. Hierarchical ascending classification (HAC) structured the *Colletotrichum* isolates into three groups: G1, G2, and G3, containing 40.81%, 49.65%, and 9.52% of the isolates, respectively (Fig. 8). Group 1 isolates are characterized by long incubation periods of 6 days and low average prevalences (33%). Group 2 isolates exhibit a rapid incubation period of 2 days and a high average prevalence (66.66%). Similar to group 1, group 3 consists of isolates with slower incubation periods but high average prevalences (66.66%).

For the *Fusarium* isolates, the contribution of the F1 and F2 axes to the total inertia is 38.87% (Fig. 9). The correlation circle shows that the average prevalence of symptoms of the isolates tested on the Muntok-CNRA accession, infected leaf area of the isolates tested on the Muntok-Djibo accession, and average severity index of the isolates tested on the Lampong-Djibo accession are

positively correlated with the F1 axis. In contrast, the average prevalence of symptoms of the isolates tested on the Muntok-Djibo accession is negatively correlated with the F1 axis. Variables strongly associated with the F2 axis include the average prevalence of symptoms of the isolates tested on the Lampong-Djibo accession, infected leaf surface of the isolates tested on the Muntok-CNRA accession, average severity index of the isolates tested on the Lampon-CNRA accession, and infected leaf surface of the isolates tested on the Lampong-Djibo accession, all of which are positively correlated with the F2 axis. The

incubation periods of isolates tested on Muntok-CNRA, Muntok-Djibo, and Lampong-Djibo are negatively correlated with the F2 axis. The HAC grouped the *Fusarium* isolates into three categories (Fig. 10), comprising 40.10%, 40.10%, and 15.8% of the isolates, respectively. Group 1 is distinguished by average incubation periods of 3 days and high prevalences (66.66%). Group 2 is characterized by long incubation periods of 5 days and low prevalences (33%). Group 3 is marked by the shortest incubation periods of 2 days.

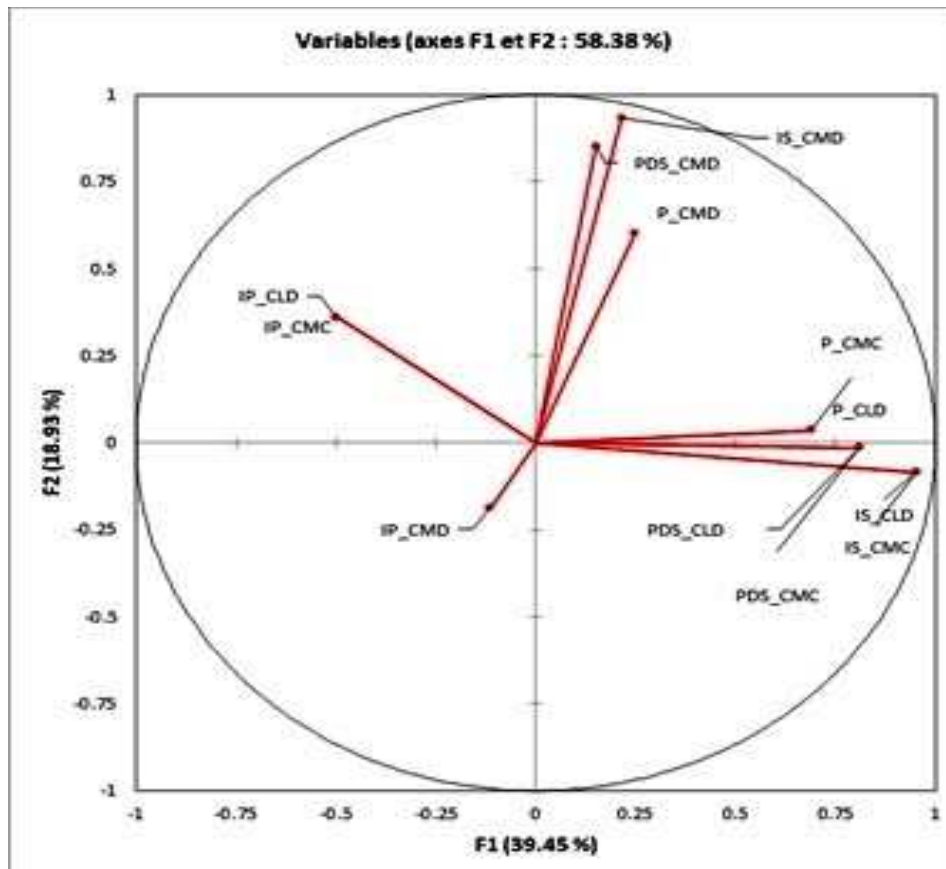


Fig. 7: Correlation circle of pathogenicity data for *Colletotrichum* isolates.

IP-CMC : incubation periods for *Colletotrichum* isolates inoculated on detached leaves of accessions Muntok-CNRA , **IP-CMD** : incubation periods for *Colletotrichum* isolates inoculated on detached leaves of accessions Muntok-Djibo, **IP-CLD**: incubation periods for *Colletotrichum* isolates inoculated on detached leaves of accessions Lampong-Djibo, **P-CMC**: prevalence for *Colletotrichum* isolates inoculated on detached leaves of accessions Muntok-CNRA, **P-CMD**: prevalence for *Colletotrichum* isolates inoculated on detached leaves of accessions Muntok-Djibo, **P-CLD**: prevalence for *Colletotrichum* isolates inoculated on detached leaves of accessions Lampong-Djibo, **PDS-CMC**: percentage of diseased leaf surface for *Colletotrichum* isolates

inoculated on detached leaves of accessions Muntok-CNRA, **PDS-CMD**: percentage of diseased leaf surface for *Colletotrichum* isolates inoculated on detached leaves of accessions Muntok-Djibo, **PDS-CLD**: percentage of diseased leaf surface for *Colletotrichum* isolates inoculated on detached leaves of accessions Lampong-Djibo, **IS-CMC**: severity index for *Colletotrichum* isolates inoculated on detached leaves of accessions Muntok-CNRA, **IS-CMD**: severity index for *Colletotrichum* isolates inoculated on detached leaves of accessions Muntok-Djibo, **IS-CLD**: severity index for *Colletotrichum* isolates inoculated on detached leaves of accessions Lampong-Djibo.

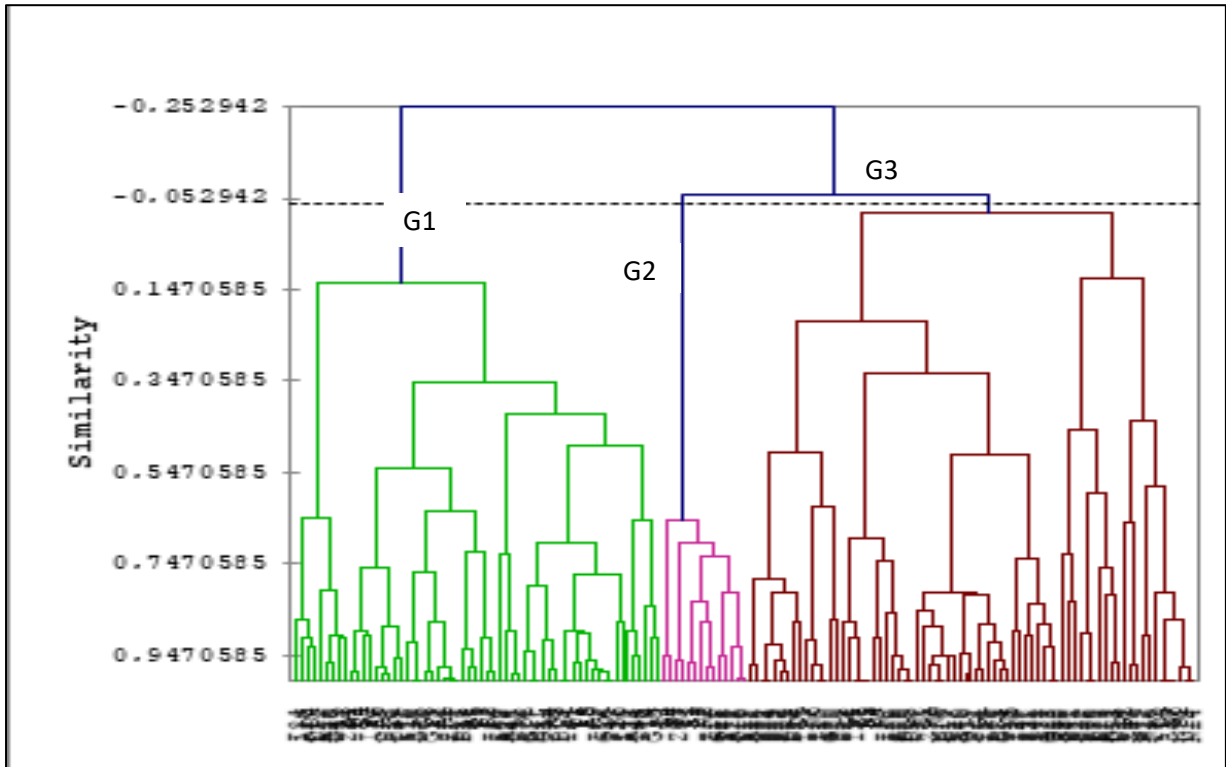


Fig. 8: Bottom-up hierarchical clustering of *Colletotrichum* isolates based on pathogenicity data from detached black pepper leaves.
G1: group 1, G2: group 2 and G3: group 3

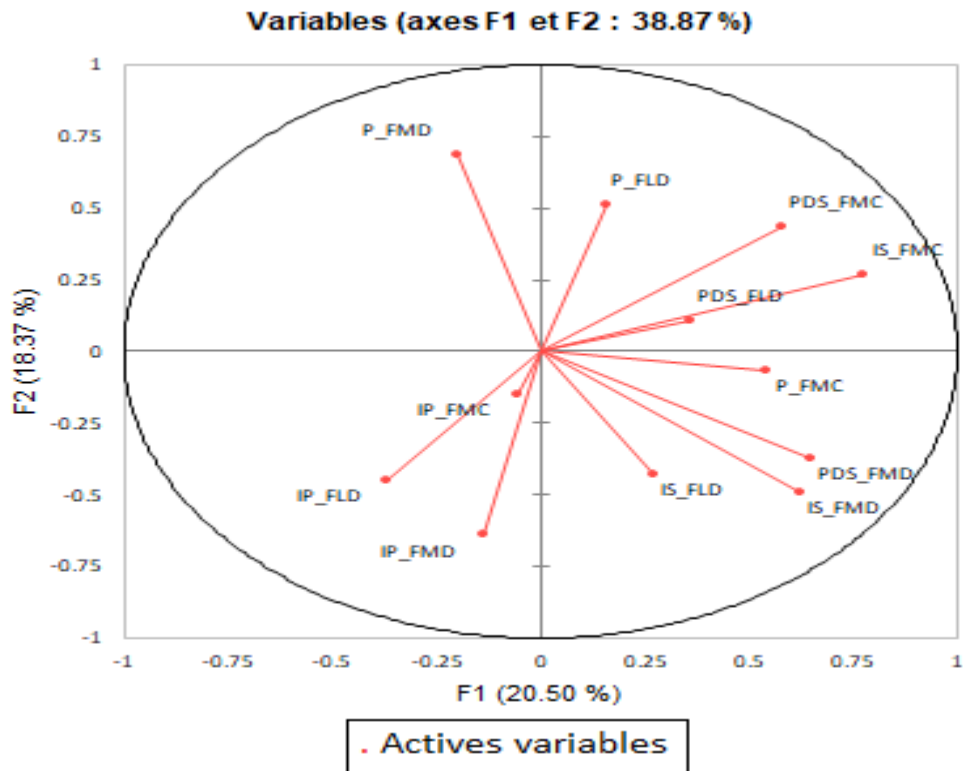


Fig. 9: Correlation circle of pathogenicity data for *Fusarium* isolates.

IP-FMC : incubation periods for *Fusarium* isolates inoculated on detached leaves of accessions Muntok-CNRA , **IP-FMD** : incubation periods for *Fusarium* isolates inoculated on detached leaves of accessions Muntok-Djibo, **IP-FLD**: incubation periods for *Fusarium* isolates inoculated on detached leaves of accessions Lampong-Djibo, **P-FMC**: prevalence for *Fusarium* isolates inoculated on detached leaves of accessions Muntok-CNRA, **P-FMD**: prevalence for *Fusarium* isolates inoculated on detached leaves of accessions Muntok-Djibo, **P-FLD**: prevalence for *Fusarium* isolates inoculated on detached leaves of accessions Lampong-Djibo, **PDS-FMC**: percentage of diseased leaf surface for

Fusarium isolates inoculated on detached leaves of accessions Muntok-CNRA, **PDS-FMD**: percentage of diseased leaf surface for *Fusarium* isolates inoculated on detached leaves of accessions Muntok-Djibo, **PDS-FLD**: percentage of diseased leaf surface for *Fusarium* isolates inoculated on detached leaves of accessions Lampong-Djibo, **IS-FMC**: severity index for *Fusarium* isolates inoculated on detached leaves of accessions Muntok-CNRA, **IS-FMD**: severity index for *Fusarium* isolates inoculated on detached leaves of accessions Muntok-Djibo, **IS-FLD**: severity index for *Fusarium* isolates inoculated on detached leaves of accessions Lampong-Djibo.

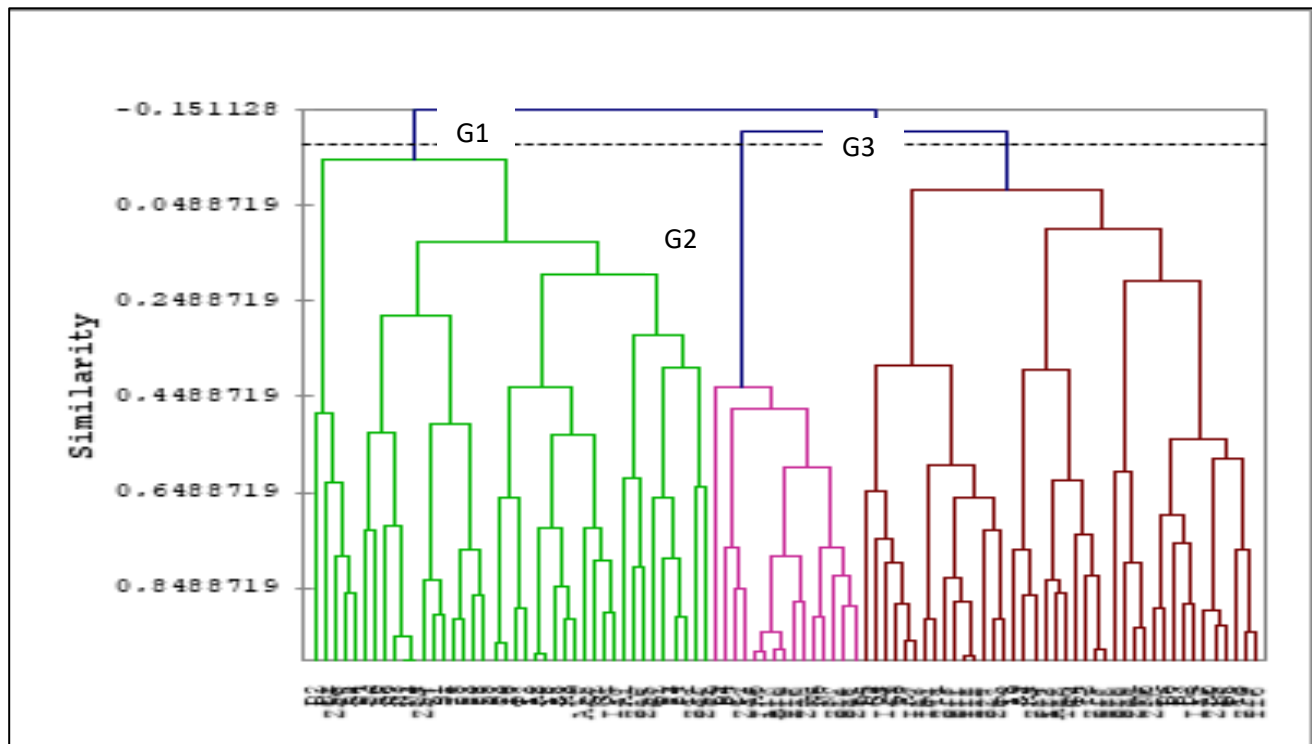


Fig. 10: Bottom-up hierarchical clustering of *Fusarium* isolates based on pathogenicity data from detached black pepper leaves.

G1: group 1, G2: group 2 and G3: group 3.

DISCUSSION

As one of the main economic spice crops of Côte d'Ivoire, pepper has been drawn great hopes in promoting agricultural production and rural incomes. Despite maintaining crop fields to increase yield, monitoring of pepper diseases is important and should be strengthened to reduce crop losses. In this study, several symptoms were observed on black pepper plants, including leaf spots, grape necrosis, and stem necrosis. Among these symptoms, circular necrotic spots with a yellow halo, large brownish spots arranged in concentric layers, apical necrosis, apical drying, and white spots or powdering

were noted across all surveyed localities, except for white spots or powdering, which were not observed in Toumodi and Saïoua. This uneven distribution of symptoms may be attributed to varying climatic conditions—such as temperature and humidity—across different growing zones, which influence the pathogen's development. Consequently, the prevalence and severity of these symptoms suggest they are major issues for black pepper in Côte d'Ivoire.

The symptoms observed in Côte d'Ivoire are consistent with those reported in black pepper plantations in other countries, including Malaysia (Zakaria *et al.*, 2022), India (Chethana *et al.*, 2015), Brazil (Severo *et al.*,

2021), Vietnam (Kumar *et al.*, 2021), and Cameroon (Petchayo, 2022). Specifically, the study by Rajshree *et al.* (2022) highlighted that concentric layered brownish spots and circular necrotic spots with a yellow halo are prevalent symptoms of black pepper in India. The prevalence of symptoms varied by locality; for instance, white spots, necrotic spots, and apical necroses were notable concerns in Aboisso, Azaguié, Dabou, N'douci, Saïoua, and Sikensi, whereas apical drying required particular attention in Agboville. Conversely, large brownish spots arranged in concentric layers were less critical across all localities.

The high prevalence of symptoms in the surveyed areas is likely linked to favorable climatic conditions such as precipitation and humidity. Increased precipitation promotes extended periods of humidity, which facilitates symptom development. This finding aligns with the results of Amrani *et al.* (2014), who demonstrated that infection and symptom development correlate with favorable infection periods. Temperatures in the surveyed localities ranged from 21.8°C to 28.5°C throughout the year, with precipitation reaching up to 2500 mm (Silué, 2019). According to Lourd *et al.* (1979), the optimal temperature for symptom development ranges from 18 to 30°C, regardless of the plant species.

Several fungal genera affecting various organs of the black pepper plant were identified in this study, including *Colletotrichum*, *Fusarium*, *Curvularia*, *Alternaria*, *Rhizoctonia*, and *Aspergillus*. Among these, *Colletotrichum* and *Fusarium* were the most significant. These findings are consistent with Petchayo (2022), who reported a diversity of fungal agents, notably *Colletotrichum*, *Fusarium*, and *Lasiodiplodia*, associated with yellowing of black pepper leaves. Additionally, *Colletotrichum* and *Fusarium* have been reported on black pepper seeds and leaves (Lestari *et al.*, 2021; Lin *et al.*, 2022; Zakaria *et al.*, 2022). N'Guettia (2015) and Silué (2019) reported the presence of certain *Colletotrichum* and *Fusarium* species on mango and cashew trees in Côte d'Ivoire, suggesting a possible cultural and pathogenic similarity between these genera affecting black pepper plants in Côte d'Ivoire and those in other countries with similar climatic conditions.

Colletotrichum was predominantly isolated from black pepper leaves and stems, while *Fusarium* was isolated from leaves, stems, grains, and soil. This indicates that all parts of the black pepper plant are susceptible to various fungal agents. The observed diversity of fungal agents and the symptoms they cause may be attributed to both favorable climatic conditions and the presence of nearby host plants, which facilitate infection. Petchayo (2022) reported three fungal genera (*Colletotrichum*, *Lasiodiplodia*, and *Fusarium*) associated with black pepper plantations in Cameroon.

The processing of the samples in the laboratory resulted in isolates of *Colletotrichum* that varied in

thallus colouration on PDA medium ranging from dark grey to white. This difference in colouration could be related to the environmental conditions of the pepper-growing areas but also to the management and agronomic practices prevailing in pepper tree orchards. Furthermore, the cylindrical conidia observed are characteristic of the species *C. gloeosporioides* (Dembélé *et al.*, 2020; Kouadia *et al.*, 2023). However, although the literature defines the species *C. gloeosporioides* as the only species responsible for anthracnose in pepper shaker (Chethana *et al.*, 2015; Rajshree *et al.* 2022; Zakaria *et al.*, 2022), the identification of *Colletotrichum* species based of these morphological characteristics remains difficult because of the enormous variations within this genus (Kouadia *et al.*, 2023). The morphological characters of each isolate of *Fusarium* were different from one another. Colour of the aerial mycelium varied showed yellow-orange to pink of results was obtained by Pavithra *et al.*, (2023) wherein 22 species of *Fusarium* isolates were isolated from pepper ecosystem and observed orange yellow to pink pigmentation and varying pattern of conidial morphology. According to these authors, the showed yellow-orange to pink pigmentation with micro conidia and macro conidia is typical of *Fusarium oxysporum*.

Therefore, in order to complete this traditional morphological identification, molecular identification are still required to confirm the identity of isolates of this *Colletotrichum* species. Experimental infections under controlled conditions would be needed to confirm the pathogenicity of these fungal genera.

Each isolate of *Colletotrichum* and *Fusarium* proved pathogenic to at least one of the three black pepper accessions. The first symptoms appeared on inoculated leaves between 3 and 3.88 days after inoculation. According to Waller (1992) and summarized by Pinard *et al.* (1998), this variation is influenced by the juvenile or adult nature of the leaves. Younger leaves tend to be infected more rapidly. However, this result may contradict experiments where all leaves were harvested at the same stage, suggesting that genotypic variability of the isolates or accessions may be a factor. Pathogenicity tests revealed that isolates from both genera can cause large lesions on inoculated leaves, indicating that species potentially endophytic in other crops can be pathogenic to black pepper.

Analysis of symptom prevalence and severity by locality for the three black pepper accessions indicated significant differences in virulence, particularly for the Muntok-CNRA accession ($P < 0.05$). Principal component analysis revealed that this difference was due to isolates N1, N3, N6, and N14, which had severity indices of 80%, 60%, 80%, and 33%, respectively. The lesions caused by these isolates from N'douci appeared distinct from those from other localities. Additionally, experiments with *Colletotrichum* species on detached black pepper leaves achieved severity indices of 92.59%

(Azrul *et al.*, 2019). The Muntok Djibo type experiments identified three isolates (N17, A12, and Z15) from N'douci, Agboville, and Azaguié, with severity indices of 100%. There may be a possibility that these isolates are genetically similar, although no genotype studies have confirmed this. Based on scales used by Amrani *et al.* (2014) and Bessadat *et al.* (2014), the accessions can be categorized as resistant types. The Muntok-CNRA accession showed particular sensitivity to isolates from N'douci ($P < 0.05$) compared to other accessions, which may be due to genetic factors or ineffective induced resistance. Although there was no significant difference in disease prevalence by locality, the high prevalence (PM > 33%) observed across all isolates and accessions could impact photosynthesis and reduce crop yield. The discrepancy between pathogenicity-based and morphometric classifications of isolates indicates no relationship between morphological and pathogenic groups, as previously noted by N'Guettia (2015) for *C. gloeosporioides* infecting mango trees.

Conclusion: This study reveals a variety of symptoms affecting leaves, grains, and stems in black pepper production areas in Côte d'Ivoire. The main symptoms include circular necrotic spots with a yellow halo, large brownish lesions arranged in concentric layers, apical necroses forming an inverted "V" shape with a blackish border, and occasionally a yellow halo, apical drying in an inverted "V" shape, and spots or white powdery deposits on leaves. Additionally, over 42% of leaf surfaces exhibited at least one of these five symptoms. The fungal genera *Colletotrichum* and *Fusarium* are identified as the primary pathogens affecting black pepper. Pathogenicity tests on detached leaves have confirmed that most isolates are pathogenic to the black pepper plant.

The observed variability in virulence may have a genetic basis, both among accessions and isolates. To more accurately identify the species present, molecular analyses are recommended. This approach will facilitate the development of quarantine measures for *Colletotrichum* and *Fusarium* species not currently found in the country, thereby safeguarding national production. Further studies should also investigate the efficacy of different fungicides to provide producers with effective chemical control options.

Conflict of Interest: All authors declare that they have no conflicts of interest to report.

Contributions of authors: Massiata DAGNOGO: Wrote protocols, conducted the study, collected data, performed the statistical analysis, and wrote the first draft of the manuscript.

Kouakou Théodore KOUADIO: Wrote the research project, developed research methodology, and read and approved the final manuscript

Eric-Olivier TIENEBO: Wrote protocols, supervised study, developed part of the research methodology, analyzed and interpreted data, and edited manuscript

Adjoa Marie Joséphine KOUADIA, Nahoulé Armond ADJA, Kouadio Paul Martial KOUAKOU, Kouabenan ABO: Performed literature review, edited and finalized the manuscript

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