Full Length Article

Assessment of the Pathogenicity of Fungi Associated with Ginger (*Zingiber officinale*) Cultivated in Two Production Areas in Côte d'Ivoire

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Abstract

Ginger (*Zingiber officinale* Roscoe) is considered as one of the world's most important spices. However, its growing faces phytopathogenic fungi attack which are responsible for yield losses around the world. In Côte d'Ivoire, a recent study has isolated and identified fungi associated to ginger cultivation. Among those fungi, some could be pathogenic. Therefore, fungal strains from the genera *Aspergillus* sp., *Colletotrichum* sp., *Curvularia* sp., *Fusarium* sp., *Leposphaeria* sp., *Penicillium* sp., *Pestalotiopsis* sp., *Pythium* sp., *Sclerotium* sp. and *Rhizoctonia* sp. were inoculated into growing ginger plants. Thirty milliliters of fungal inoculum at a concentration of 10^6 spores mL^{-1} were sprayed on the apparently healthy, 30-day-old ginger seedlings. Then, the plants were started to observe from 10th day after inoculation up to two months and finally, ginger pathogenic fungi in culture were identified through a pathogenicity test. Four different symptoms namely, necrosis, leaf spots, chlorosis and wilting were observed. The plants inoculated with the fungus *Leptosphaeria* sp. had the highest disease prevalence (81.67%) and the most severe symptoms (62.85%). The results obtained also revealed that 9 fungi out of the 10 tested were pathogenic for ginger cultivation in Côte d'Ivoire. These included *Leptosphaeria* sp., *Aspergillus* sp., *Colletotrichum* sp., *Fusarium* sp., *Penicillium* sp., *Pestalotiopsis* sp., *Pythium* sp., *Sclerotium* sp. and *Rhizoctonia* sp. This first study on fungal diseases of ginger in Côte d'Ivoire could help to better guide control methods. © 2023 Friends Science Publishers

Introduction

Ginger (*Zingiber officinale* Roscoe) is a yearlyherb cultivated throughout the tropics (Alèdi *et al*. 2018). Its young roots, which are rhizomes, are widely used in groceries, folk medicine and food technology, particularly in juice production (Adou *et al*. 2018).

In Côte d'Ivoire, ginger is produced mainly in the cities of Bongouanou, Divo, Gagnoa, Soubré, Tiassalé and Koun-Fao with an average yield of 10 t ha^{-1} (FIRCA 2020). The Ivorian yield is estimated at 7087.93 t year^{-1} (FAOSTAT 2021). Ginger is considered as sofone of the world's most important spices, thanks to its many virtues and economic importance. It protects deoxyribonucleic acid, it is an anticoagulant, an anti-hypercholesterolemia, in addition to controlling rheumatism, digestive disorders, nausea, asthma, constipation and diabetes (Nandkangre *et al*. 2015; Tene *et al*. 2020). Moreover, it is a source of income for producers, traders and processors (Adou *et al*. 2018). In Côte d'Ivoire, ginger is used in the production of a drink sold in the streets and is also sold in dried form (ginger pastille) (Adou *et al*. 2018).

Despite its importance and its various virtues, ginger is subjected to high pest pressure (Alèdi *et al*. 2018), particularly phytopathogenic fungi's one. Those pathogens are responsible of discoloration, foliage spotting and burning, plant dieback, stem and rhizome rot, plant wilting and defoliation (Gupta and Tennyson 2019). These diseases caused by phytopathogenic fungi on ginger cultivation often result reduced yield and consequently heavy economic losses. In fact, ginger rhizome rot caused by a species of the genus *Pythium* can lead to yield losses of up to 90%, as was the case in Anqiu (China) in 2010 (Mahdi *et al*. 2013). Similarly, according to Gupta and Tennyson (2019), *Fusarium oxysporum*, the causal agent of Fusarium wilt disease can cause yield losses of up to 70%. According to Qin *et al*. (2013), in addition to yield losses, these agents also adversely affect the quality of harvests and are the main factors limiting the production of high-quality ginger. In Côte d'Ivoire, a recent study has isolated and identified several fungal genera associated with ginger cultivation. Among those fungi, some could be pathogenic. It is therefore necessary to assess their pathogenicity. This study was initiated in this context with the aim of identifying

pathogenic genera of fungi associated in ginger cultivation in Côte d'Ivoire.

Materials and Methods

Biological organ and organisms

The biological organs were of yellow-fleshed ginger rhizomes, which were purchased with ginger producers and used for setting up the plants. As for the biological organisms, these were composed of 10 species of fungi associated with ginger cultivation in Côte d'Ivoire, provided by the Plant Health Unit of NANGUI ABROGOUA University. The fungi included *Aspergillus* sp., *Colletotrichum* sp., *Curvularia* sp., *Fusarium* sp., *Leptosphaeria* sp., *Penicillium* sp., *Pestalotiopsis* sp., *Pythium* sp., *Rhizoctonia* sp., *Sclerotium* sp.

Ginger culture soil preparation

Moist, well-drained soil collected from in UNA forest. This soil was sterilized in an autoclave at 121°C for 45 min to eliminate microorganisms likely to infect the rhizomes. It was put in nursery bags measuring 26 cm × 13 cm. Inside a greenhouse, culture bags, filled with sterilized soil, were placed on benches to avoid direct contact with the ground.

Ginger rhizome preparation

Rhizomes were prepared by cutting them, usinga sterile knife, into explants about 5 cm wide with at least three eyes (usually small green dots). After the rhizomes cut, the explants were kept at room temperature to allow their healing so as to minimize the risk of infection by other pathogens.

Ginger rhizome planting

This step consisted in placing each rhizome explant 5 cm into the soil contained in the bag. The explants were placed in bags to ensure that the eyes were facing upwards. A total of 450 bags were used to set up the nurseries. The seedlings obtained were used for inoculation tests.

Fungal inoculation of ginger plants

Before to plants inoculation, fungal inocula were first prepared. Indeed, for each fungal strain with spores, the spore suspensions were obtained from 7-day-old pure colonies. Fifty milliliters of distilled water were added to the scraped fungal colonies using a sterilized scalpel blade. The resulting suspensions were filtered through filter paper (Whatman) to separate conidia from mycelial fragments. The spore concentration of the suspensions was estimated using a Malassez hematimeter (Malassez blade) and adjusted to 10^6 spores mL^{-1} for a volume of 100 mL of suspension. Finally, a 1% glucose solution was added to the spore suspension to promote spore adhesion to plant surfaces.

For fungal strains not producing spores, inocula were obtained by directly adding the scraped mycelium from the culture media's surface to 100 mL of distilled water. The mixture was vigorously stirred before use. After getting the inocula, a gentle inoculation was carried out on the leaves and stems. Young plants at the 3-leaf stage were used for inoculation. Inoculation was carried out by spraying the surface of the aerial organs (leaves and stems) of the young plants with 30 mL of inoculum. Fifteen plants were inoculated with each fungal strain. The experiment was repeated 3 times.

Symptom observation and description

After inoculation, the plants were observed daily to determine the incubation period. The different symptoms observed on the organs after inoculation were noted and described. Concerning symptom description, their appearance, shape, coloration and evolution according to the different fungi were noted during the observations.

Assessment of symptom prevalence

Symptom prevalence was assessed every two weeks for two months after plant inoculation. Symptomatic and asymptomatic plants were counted. The prevalence was determined by the ratio of the number of symptomatic plants to the total number of plants inoculated with a fungus according to the formula of Ackah *et al.* (2008).

$$P\,(\%) = \frac{NI}{NT} \times 100$$

Where P: Prevalence, NI: Number of symptomatic plants, NT: Total number of plants inoculated by a fungus.

Assessment of symptom severity

Symptom severity was also assessed two months after inoculation. The severity of symptoms caused by each fungus on the leaves of infected plants was determined basing on the proportion of leaf area showing the symptom. Symptom severity was assessed using assessment rating scale (Lebeda and Urban 2004). Leaves were rated from 0 to 4 according to symptom status:

0: Absence of visible symptoms on the leaves
1: less than 25% of leaf area infected
2: 26–50% of leaf area infected
3: 51–75% of leaf area infected
4: more than 75% of leaf area infected

The average severity was then determined according the formula of Kobriger and Hagedorn (1983).

Identification of pathogenic fungi in ginger cultivation

A verification of Koch's postulate was performed in order

to ensure that the observed symptoms were caused by the inoculated fungi. This verification was carried out in several stages.

Collection of ginger leaf and stem samples

Ginger plants showing symptoms after inoculation were sampled. Leaves and stems from symptomatic plants were collected, wrapped in paper towels and labeled according to the symptom and the fungus used as inoculum. These collected samples were sent to the Laboratory, cleaned and disinfected using 70% at 96°C alcohol.

Isolation of fungi associated with symptoms

Fungi were isolated on Potato dextrose agar (PDA) medium. To do this, small pieces (explants) of leaves and stems were taken from the growth front of the symptoms on the organs collected. After culture medium solidification in Petri dishes, the explants were seeded on the culture medium. In each dish, 4 explants were placed at the two ends of dish diameters. Each Petri dish was incubated at ambient laboratory temperature for 2 days. The different fungal colonies developed were sub-cultured separately on other culture media until homogeneous fungal colonies were obtained. These fungal colonies isolated were then identified.

Identification of fungi associated with symptoms

Fungi identification was carried out macroscopically from the cultural characters observed in a Petri dish and microscopically by observation under an optical microscope (OPTIKA). The cultural characters were assessed according to coloring, mycelium aspect, colony development mode. As for the microscopic characters, they essentially concerned the type of propagules (mycelium or spore). For each type of propagule, the coloring, shape, appearance, partitioning or not were observed. Identification of the fungal genus was made using the identification key of Botton *et al.* (1990).

Comparison of isolated and inoculated fungi

The cultural and microscopic characters of each fungus from the samples were compared with those of the fungi inoculated into ginger plants. If the induced symptom was similar to that initially observed and the re-isolated fungus showed the same characteristics as those of the inoculated fungus, then the inoculated fungus was said to be responsible of the symptom observed in the field.

Statistical analyses

The data obtained were analysed by using Statistica version 7.1 software. Test for homogeneity of variances was performed to determine future tests. When there was no significant difference (*P > 0.05*), the nonparametric test was used. In the event of a significant difference (*P < 0.05*), the parametric test was used to compare the means, then the post-hoc test was performed to see where the difference was. Disease prevalence and symptom severity indexes were subjected to statistical analyses.

Results

Diversity of symptoms observed after inoculation of ginger plants

All the fungi inoculated to the ginger plants caused symptoms. Four different types of symptoms were observed. These were necrosis, leaf spots, chlorosis and wilting.

Necroses appeared in 2 different aspects: apical leaf necrosis the leaves (Fig. 1A and B) and marginal leaf necrosis (Fig. 1C). Leaf spots appeared as small oval to elongated fusiform spots on the plant's leave with a papyraceous spindle-shaped spots that appeared on the leaves of the plant with a papery center (Fig. 1D). Chlorosis was characterized by a yellowing along leaf margins with a relatively accentuated presence at the apical part (Fig. 1E). As for the wilting first appeared as yellowing of the lower leaf margins, then it gradually expanded to cover all of the leaves (Fig. 1F). Symptoms such as necrosis and chlorosis were caused by all inoculated fungi. Wilt was caused by the fungi *Curvularia* sp., *Fusarium* sp., *Penicillium* sp., *Pestalotiopsis* sp. and *Rhizoctonia* sp. Leaf spots were induced solely by the fungus *Pestalotiopsis* sp.

Incubation period of different fungi inoculated into ginger plants

The incubation period varied from 7 to 13 days depending on the fungus inoculated. Indeed, the fungus *Leptosphaeria* sp. recorded the shortest incubation period. These periods were relatively average for the fungi *Aspergillus* sp., *Pythium* sp., *Pestalotiopsis* sp., *Penicillium* sp., *Fusarium* sp., *Sclerotium* sp., *Rhizoctonia* sp. The longest incubation period was obtained with fungi *Colletotrichum* sp. and *Curvularia* sp. (Fig. 2).

Prevalence and severity of symptoms observed on inoculated ginger plants

Depending on fungi: The average prevalence of symptoms caused by fungi varied from 52.37 to 81.67%, respectively. The highest average prevalence was caused by the fungus *Leptosphaeria* sp. while the lowest one was caused by *Penicillium* sp. (Table 1). Despite those variations, mean symptom prevalence was statistically similar (*P > 0.05*).
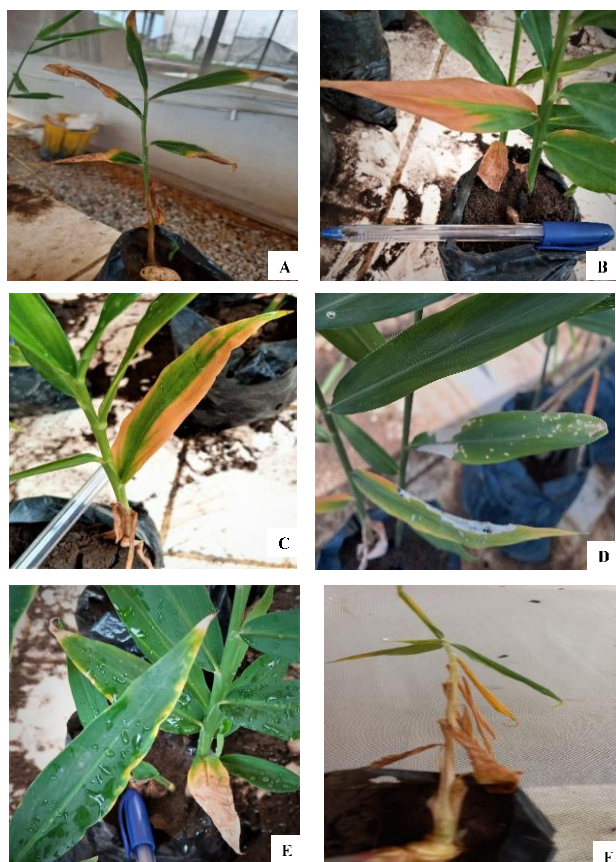
Fig. 1: Symptoms observed on ginger plants inoculated with fungi

A and **B**: apical necrosis on leaves; **C**: marginal necrosis on leaves; **D**: leaf spots; **E**: marginal leaf chlorosis; **F**: plant wilting

Regarding the average severity of symptoms caused by fungi range from 28.11 to 62.85%. The fungus *Leptosphaeria* sp. induced the most severe symptoms, while the least severe symptoms were caused by *Colletotrichium* sp. Statistical analysis showed that there was a significant difference between the severities of the symptoms caused by the different fungi (Table 1).

Depending on the different symptoms

Average prevalences of necrosis, chlorosis, wilt and leaf spots varied. Thus, the prevalence of necrosis varied from 42.85 to 61.42%. The fungus *Rhizoctonia* sp. caused the highest prevalence and the lowest one was caused by *Penicillium* sp. As for chlorosis, its prevalence varied from 42.25 to 60.57%. The highest prevalence was caused by *Penicillium* sp. while the lowest one was caused by *Pythium* sp. With regard to wilt its prevalence ranged from 49.32 to 55.57%. The fungus *Fusarium* sp. caused the highest prevalence while the lowest one was obtained with *Curvularia* sp. As leaf spot was caused solely by the fungus *Pestalotiopsis*, its prevalence was 58.57% (Table 2). Despite these variations between the different prevalence values,

Table 1: Average prevalence and severity of symptoms caused by fungi inoculated into ginger plants

Fungi	Average prevalence	Average severities
Leptosphaeria sp.	81.67±4.10[a]	62.85±0.29[a]
Colletotrichium sp.	60.00±4.66[a]	28.11±0.39[c]
Aspergillus sp.	75.00±4.29[a]	42.28±0.35[b]
Fusarium sp.	79.00±4.30[a]	40.00±0.31[b]
Rhizoctonia sp.	71.42±4.70[a]	57.13±0.30[a]
Pythium sp.	63.33±4.72[a]	44.66±0.34[b]
Sclerotium sp.	69.99±4.30[a]	39.90±0.36[b]
Penicillium sp.	52.37±4.32[a]	31.42±0.40[c]
Pestalotiopsis sp.	66.00±4.36[a]	23.80±0.41[c]

Values with the same letters in the same column are statistically identical according to Fisher's LSD test at 5% threshold. F: Fisher statistic; P: probability value

Table 2: Prevalence of the different symptoms observed on ginger plants depending on the inoculated fungi

Fungal strains	Average prevalence (%)			
	Necrosis	Chlorosis	Wilting	Leaf spots
Aspergillus sp.	46.12±4.82[a]	60.50±5.86[a]	0	0
Curvularia sp.	47.22±4.04[a]	44.16±6.44[a]	49.32 ± 3.80[a]	0
Fusarium sp.	55.71±6.92[a]	42.25±4.85[a]	55.57 ± 6.43 [a]	0
Colletotrichum sp.	44.75±6.42[a]	50.37±6.69[a]	0	0
Leptosphaeria sp.	61.37±6.38[a]	48.00±5.59[a]	0	0
Penicillium sp.	42.85±4.85[a]	60.57±5.86[a]	55.71 ± 6.98 [a]	0
Pestalotiopsis sp.	56.57±2.98[a]	48.85±3.87[a]	50.57 ± 3.62 [a]	58.57±4.02[a]
Rhizoctonia sp.	61.42±6.40[a]	48.57±3.82[a]	52.37 ± 6.82 [a]	0
Pythium sp.	53.87±6.24[a]	42.25±5.87[a]	0	0
Sclerotium sp.	55.12±6.43[a]	54.75±6.08[a]	0	0
F	1.12	1.22	7.16	18.08
P	0.36	0.31	0.000 5	0.000

In the same column, the figures with identical letters are statistically identical according to the ANOVA test at 5% threshold. The letters are in descending order

Table 3: Severity of the different symptoms observed on ginger plants depending on the inoculated fungi

Genera	Average severity			
	Necrosis	Chlorosis	Wilting	Leaf spots
Aspergillus sp.	2.85±1.39[b]	2.85±1.39[b]	4.00±0.52[a]	0 ± 0[c]
Colletotrichum sp.	3.06±1.31[a]	3.52±1.83[a]	4.00±1.37[a]	0 ± 0[c]
Curvularia sp.	3.06±1.15[a]	3.67±1.20[a]	0.81±0.52[c]	0 ± 0[c]
Fusarium sp.	3.66±0.52[b]	0.00±0[c]	3.24±0.88[a]	0 ± 0[c]
Leptosphaeria sp.	4.00±2.22[a]	4.00± 1.74[a]	4.00±1.15[a]	0 ± 0[c]
Penicillium sp.	2.96±2.14[b]	2.04± 1.35[b]	4.00±1.50[a]	0 ± 0[c]
Pestalotiopsis sp.	2.03±1.81[b]	0.00± 0[c]	3.48±0.97[a]	2.85±1.86[b]
Pythium sp.	3.71±0.88[a]	4.00± 0.57[b]	4.00±1.63[a]	0 ± 0[c]
Rhizoctonia sp.	4.00±1.48[b]	2.84± 1.35[b]	4.00±1.39[a]	0 ± 0[c]
Sclerotium sp.	2.45±1.20[b]	3.80± 1.45[b]	3.26±1.15[a]	0 ± 0[c]
F	4.79	5.10	1.28	23.08
P	0.000 07	0.000 04	0.26	0.00000

In the same column, the numbers assigned different letters are significantly different according to the ANOVA test at 5% threshold. The letters are in descending order

whatever the different symptoms, the average prevalence of symptoms types according to fungi were statistically identical (*P > 0.05*).

Symptom severity varied according to the symptoms and the fungi inoculated. Thus, the most severe necrosis and chlorosis symptoms were caused by the fungus *Leptosphaeria* sp. with respective severities of 4 each.
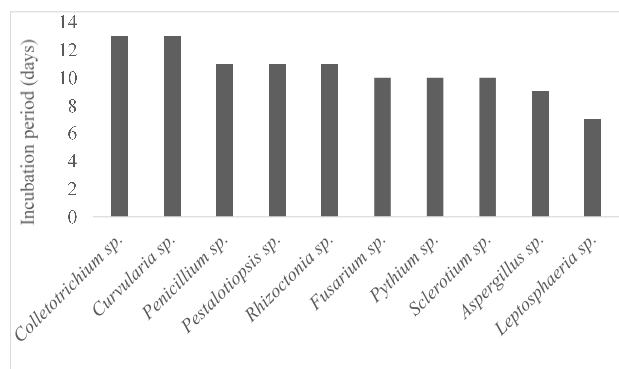
Fig. 2: Incubation period of fungi that produced symptoms on inoculated ginger plants

Pestalotiopsis sp. and *Penicillium* sp. caused the least severe symptoms in terms of necrosis (2.03) and chlorosis (2.04) respectively. For wilting, symptom severity depending on the inoculated fungi varied from 0.81 to 4. The fungi *Aspergillus* sp., *Leptosphaeria* sp., *Penicillium* sp., *Pythium* sp., *Rhizoctonia* sp. and *Colletotrichum* sp. caused the most severe symptoms while the least severe symptom was caused by *Curvularia* sp. The severity of leaf spot symptoms caused by the fungus *Pestalotiopsis* sp. was 2.85. Statistical analyses revealed a significant difference between the severities of necrosis, chlorosis and wilting symptoms caused by the different inoculated fungi (Table 3).

Fungi responsible for the symptoms observed

Nine of the inoculated fungi caused the same symptoms as those from which they were initially isolated. Moreover, after their re-isolation, they showed the same cultural and microscopic characters as the inoculated fungi, thus satisfying Koch's postulate. These fungi are *Leptosphaeria* sp., *Fusarium* sp., *Aspergillus* sp., *Pythium* sp., *Penicillium* sp., *Pestalotiopsis* sp., *Colletotrichum* sp. *Sclerotium* sp. and *Rhizoctonia* sp. These 9 fungal strains are therefore considered pathogenic for ginger in Côte d'Ivoire.

Discussion

Fungi inoculated into ginger plants caused a diversity of symptoms in the aerial organs. This diversity of symptoms could be linked to different modes of action of fungi. Indeed, phytopathogenic fungi have various methods of infection and these different methods can promote the development of different symptoms. The variation in the mode of action in phytopathogenic fungi has been confirmed by several authors, including Ritz (2005). According to him, the pathogen's mode of action takes many forms between different pathogens, ranging from necrotrophic attack, through cortical invasion, to blockage of vascular tissue, thus causing a diversity of symptoms. Similarly, other authors

have demonstrated the responsibility of fungi in the expression of different symptoms on cultured ginger. Indeed, Claire and Mikaël (2004) claimed that fungi could cause discoloration and necrotic spots on the foliage of ginger plants.

During this study, the fungus *Leptosphaeria* sp. showed the shortest incubation period compared to other fungi. These results are in agreement with those of several authors. Indeed, Huang *et al.* (2006) and Haddadi *et al.* (2016), confirmed that the biotrophy/necrotrophy transition appears only 5 to 9 days after inoculation in *Leptosphaeria maculans*.

Of all the fungi tested the fungus *Leptosphaeria* sp. caused the highest prevalence and the most severe symptoms on inoculated ginger plants, thus proving to be the most virulent. This increased virulence of this fungus in relation to the other fungi on ginger, would explain its high prevalence and severity. Indeed, Egesi *et al.* (2009) showed that the virulence of a pathogen can have a negative influence on the agronomic performance of varieties and therefore allow severe symptom.

The virulence of *Leptosphaeria* sp. could be explained by its short incubation period. Indeed, this short incubation period could limit ginger plants in triggering an effective defense mechanism. Among the fungi tested for pathogenicity on ginger, *Leptosphaeria* sp., *Aspergillus* sp., *Colletotrichum* sp., *Fusarium* sp., *Penicillium* sp., *Pestalotiopsis* sp., *Pythium* sp., *Sclerotium* sp. and *Rhizoctonia* sp. were found to be pathogenic to ginger. Among these, *Sclerotium* sp. especially *S. rolfsii* is responsible to cause diseases in hundreds of plant species including chili (Javaid *et al.* 2020), bell pepper (Jabeen *et al.* 2022), chickpea (Khan and Javaid 2015), pea (Nafisa *et al.* 2013) and others. Species of Penicillium cause various post-harvest diseases in fruits and vegetables namely apple, lemon, tomato, garlic etc. (Khan and Javaid 2021; 2022a, b; 2023). The pathogenicity of these fungi could be explained by the sensitivity of ginger to these fungi. Ginger is a host for these fungi. Recent studies have shown that some of these fungi are responsible for diseases associated with ginger cultivation. Indeed, during their work on ginger diseases, Gupta and Tennyson (2019) confirmed the pathogenicity of *Pythium* sp. and *Aspergillus* sp. for ginger cultivation.

Conclusion

Four main symptoms were caused by the fungi inoculated into cultured ginger plants. These were necrosis, chlorosis, wilting and leaf spots. *Leptosphaeria* sp. caused the most severe symptoms and highest symptom prevalence of all the inoculated fungi. This study revealed that the fungi *Aspergillus* sp., *Colletotrichium* sp., *Fusarium* sp., *Leptosphaeria* sp., *Penicillium* sp., *Pestalotiopsis* sp., *Pythium* sp., *Rhizoctonia* and *Sclerotium* sp. are pathogenic for ginger cultivation in Côte d'Ivoire.

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