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RESEARCH ARTICLE

FUNGAL DISEASES OF PAPAYA IN CÔTE D'IVOIRE: INCIDENCE AND DISTRIBUTION

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ABSTRACT

In Côte d'Ivoire, the papaya crop is threatened by fungal pathogens. The etiology, the incidence and the distribution of these fungal diseases have not yet been elucidated. The objective of this study is to generate scientific data to sustainably control fungal diseases of papaya in Côte d'Ivoire. To this end, an inventory of fungal diseases of papaya and responsible agents has been carried out in several production areas in Côte d'Ivoire. Pathogenicity tests have verified the involvement of the agents responsible for the manifestation of the observed symptoms. Several isolates of four fungal species were obtained from fruit and root samples showing symptoms. It is Phytophthora palmivora, Colletotrichum gloeosporioides (agent responsible for anthracnose), of Fusarium sp. and of Pythium aphanidermatum. These fungi reproduced the same symptoms by soft or sudden inoculation on fruits and papaya's roots. The first three species had an average incidence of 32.5 % for severity 3 (on a scale of 1 to 5) in the region of Grands ponts (Great Bridges), Agnéby-Tiassa, Indénié-Djuablin, Gbokle regions. In other localities, no symptoms of fungal diseases was observed.

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INTRODUCTION

Papaya (*Carica papaya* L.) is a plant from South and Central America. It is the third most cultivated tropical fruit in the world and more than half of the production comes from Latin America and the Caribbean. The main producing countries are: India (2,685,900 T), Brazil (1,811,540 T), Mexico (755,000 T), Nigeria (700,000 T) and Indonesia (230,000 T) (FAOSTAT, 2007). In Côte d'Ivoire, the intensive cultivation of papaya, intended for local consumption and export, has been revealed to the public thanks to the Agricultural Export Promotion and Diversification Project (PPDA). The main production area of the papaya was the southern part of the country, mainly the Agnéby-Tiassa region (Azaguié and Anyama), where former banana planters converted to papaya producers are found. The cultivation of papaya in the Agnéby-Tiassa region is now almost impossible with the recrudescence of the virus. The aim of the project was to improve the production of papaya by controlling the viral environment of orchards in Côte d'Ivoire. Now,the production of papaya is, more and more directed to the central regions of the country.

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However, the emergence and recrudescence of new non-viral conditions also threaten new production areas. These emerging diseases are usually fungal. Many fungi infect the papaya and are responsible for fungal diseases. These diseases are found around the world. In Côte d'Ivoire, from the samples taken from infected papaya orchards, the *Fusarium, Colletotrichum, Phytophthora, Oidium* species (Diallo *et al*., 2011) and *Pythium* sp. (Koffi*et al*., 2009 and 2010) were identified. The symptoms are usually characterized at the roots and neck by rots. On the leaves, various spots and colorations manifest the symptoms. On fruits, soft or dry rot stains have been observed on both fruits and leaves (Diallo *et al*., 2011). Thus, to improve the production of papaya and meet the ever-increasing demand of consumers, it is necessary to know and control the health environment of papaya. To this end, the determination of the incidence and distribution of fungal diseases of papaya will therefore better guide the control methods.

METHODOLOGY

The survey was carried out in the papaya plantations in the different areas. All plantations were visited and sampled. Box papayas (growing in towns, behind houses, in the street) were also explored and sampled.

Prospection, symptomatology and collection of plant material: Phytosanitary prospecting missions were carried out in the localities of 14 listed regions;theseareGrands ponts, Agneby, Indenie-Djuablin, Gontougo, Gbokle, Aries, Gbeke, Poro, Guemon, Cavally, Tonkpi, Goh, and Sud-comoe. During these surveys, plant samples (leaves, fruits, trunks and roots) with symptoms of fungal diseases were collected and stored for laboratory work. Similarly, the incidence and severity of symptoms were determined by variety, region, and phenological stage of the plant. Wild papayas, and those growing behind the concessions, were observed and sampled.

Identification of fungi: The sick papaya organs collected were cultured in the laboratory for the isolation of the associated fungi. From the leaves, stems, roots and diseased fruits seeded on the PDA medium, the pure fungal strains were isolated for identification. Light microscopic observations of the microscopic characters made it possible to characterize the fungal strains using Barnett's identification keys, Lanier.

Pathogenicity test: The fungal isolates used for the pathogenicity test were cultured on PDA medium to obtain pure cultures of 7 days. These cultures were kept in bins away from light. Isolates of *Colletotrichum gloeosporioides, Phytophthora palmivora* and *Fusarium* sp. isolated from papaya samples and already indexed as potential fungal rots were used. The apparently healthy mature papayas of the solo variety from the locality of Azaguié were used for the pathogenicity test. Indeed, 40 papayas were disinfected by soaking in a solution of sodium hypochlorite 8 ° (2.7% active chlorine) at 10% for 5 minutes. They were then rinsed three times with sterile distilled water and dried at room temperature (27 ± 2 ° C). Five fruits were then inoculated by fungal genus and five other fruits served as a control for each inoculation technique: brutal inoculation and mild inoculation. The brutal inoculation was done on a sample of 15 fruits divided into three lots of five fruits each. Thus, five fruits were inoculated with each fungal isolate. Using a sterile lancet knife, four wounds 5 mm in diameter were made. One on the peduncle, the other on the apical end and the other two on the epicarpto be diametrically opposed. Mycelial disks 5 mm in diameter taken from 7-day old fungal cultures on PDA medium were then deposited on the wounds (opening). The deposition sites of the mycelial disks were covered with sterile hydrophilic cotton moistened according to the modified method of Xiao and Rogers (2004). This modification consisted in the use of hydrophilic cotton instead of sterile blotting paper for fixing the inoculum.

The inoculated fruits were put in plastic containers at a rate of five fruits per tray and fungal genus. Five other fruits injured as before and inoculated with non-fungal PDA media discs served as a control during this experiment. Forty-eight hours after inoculation, the hydrophilic cotton pieces were removed and the fruits were daily observed for 6 days to evaluate certain parameters. The experiment was repeated three times. Gentle inoculation was also done on a batch of five fruits for each of the three fungal genera. Mycelial disks 5 mm in diameter were directly deposited on the fruits at different points. Fruit inoculation points were also covered with moistened cotton wool. These fruits were kept in plastic tubs and incubated at laboratory temperature (27 ± 2º C). The control fruits were treated as previously described. The pathogenicity test with the strain of *Pythium* sp. was carried out by gentle inoculation according to the method of Haware and Nene (1982) on 5 healthy papaya seedlings 21 days old. Forty-eight hours after inoculation, the hydrophilic cotton pieces were removed and the fruits were daily observed for 6 days to evaluate the growth parameters. The experiment was repeated three times. The fungi were then re-isolated and the macroscopic and microscopic characteristics of the reproductive structures were compared with those previously observed.

Assessment of infection's development: After inoculation, the development of the fungal infection is assessed not only by the area covered by the infection but also by the depth. The volume of infection is determined according to the formula of Masher and Defago (2000) as follow: $Vp = Si \times Pf = \pi r2h$

RESULTS

Incidence and severity of fungi associated with papaya rots
From the 13 regions where the survey took place, symptoms of papaya diseases were observed in 12 regions. In these areas, several symptoms were observed. At the fruit level, dry rot is found in the furrows of the fruit, with soft rots causing circular depressions and soft rots covering the entire fruit (Figure 1). At the root level, crown rot and / or papaya roots have been found (Figure 1). From these symptoms and from the rotting samples, four main potentially pathogenic fungal strains were isolated. It is *Phytophthora palmivora, Colletotrichum gloeosporioides*. (Agent responsible for anthracnose), *Fusarium* sp., and *Pythium aphanidermatum* (Figure 1).

The incidence of diseases ranged from 7% to 44% with a severity index ranging from 2 to 3. The most important incidences were noted in the region ofAbidjan(44%), Agnéby-Tiassa (38%) and Indenier-Djuablin (35%). Fungal diseases of papaya are moderately present in the southern regions of Comoé (28%), Gbokle (25%), Belier (21%) and Cavally (21%). The lowest incidences of fungal diseases of papaya were recorded in Goh (12%), Gbêkê (9%), Guemon (7%) and Tonpki (7%). The severity of fungal attacks varies from level 2 to level 3. The attacks were more severe in the regions of Abidjan, Agnéby-Tiassa, Indénié-Djuablin, Belier, Gbèkè, Gémon and South comoé (Table I).

In Gontougo and Poro no visible symptoms of disease were observed in the areas visited. The anthracnose of papaya caused by *Colletotrichum gloeosporioides* is present in almost all Papaya producing regions in Côte d'Ivoire. In fact, this pathogenic fungus of papaya was isolated in 86% of the areas producing papaya. The second most common strain in the production areas is *Fusarium* sp. This strain was observed in about 54% of the areas visited. It has been encountered in the South, Southeast, East, West and Center of the country. Regarding the strain of *Phytophthora palmivora*, it was found only in the southern zone (Grands ponts, Agnéby-Tiassa) and the East. It is present in 23% of the zones studied. The last strain responsible for diseases on papaya is *Pythium aphanidermatum*. This pathogenic fungus was isolated from papaya root rot in the Agnéby-Tiassa region (Table I).

Pathogenicity of fungal species isolated from papayas: Fruits inoculated by the soft method developed three types of symptoms. These are brown rot caused by *Colletotrichum gloeosporioides* (Figure II, E) and white rot caused by *Phytophthora palmivora* (Figure II, G) on fruit and root rot caused by *Pythium aphanidermatum* (Figure II, I).

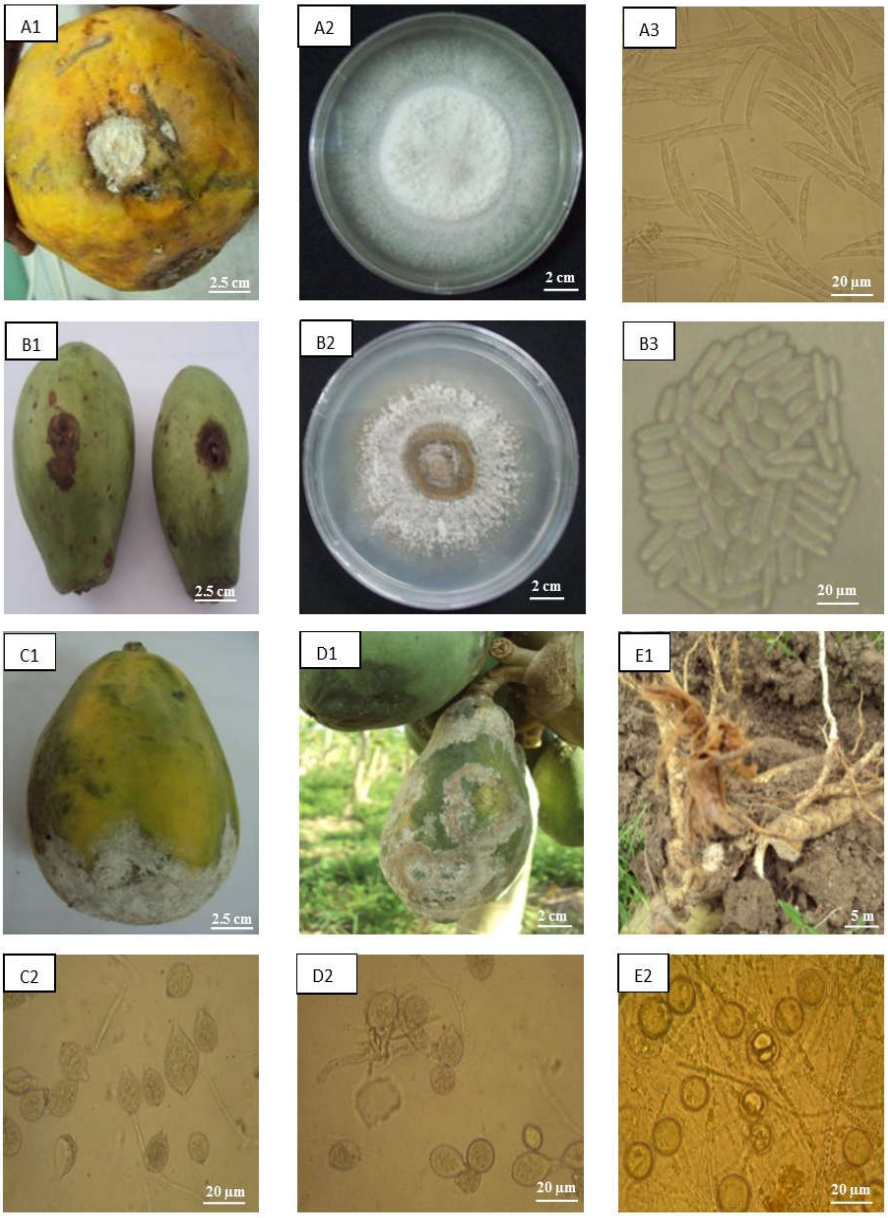

Figure 1 : Illustrations of some symptoms of the fungal diseases of papaya in Cote d'Ivoire and some associated pathogens

A1 : soft rot; A2 : synthetic planting of *Fusarium sp* ; A3 : Conidials of *Fusarium* sp.; B1 : brown rot ; B2 : *Colletotrichum gloeosporioides* cultural aspect; B3 : conidials of *C. gloeosporioides* ; C1/D1: soft rots and whitened aspect ; C2/D2 : *Phytophthora palmivora'sporangium* ; E1 : root rot ; E2 : *Pythium aphanidermatum'* spores.

Table I: Incidence, severity and main strains causing fungal diseases of papaya in various areas of Cote d'Ivoire

Areas	Impact (%)	Severity (1 à 5)	Isolated fungal strains
Grands Ponts	44	3	*Fusarium* sp., *Colletotrichum gloeosporioides*, *Phytophthora palmivora*
Agnéby-Tiassa	38	3	*Fusarium* sp., *Colletotrichum gloeosporioides*, *Phytophthora palmivora*, *Pythium aphanidermatum.*
Indénié-Djuablin	35	3	*Fusarium* sp., *Colletotrichum gloeosporioides*, *Phytophthora palmivora*
Gontougo	0	0	
Gbokle	25	2	*Fusarium* sp., *Colletotrichum gloeosporioides*
Bélier	21	3	*Fusarium* sp., *Colletotrichum gloeosporioides*
Gbekè	9	3	*Fusarium* sp., *Colletotrichum gloeosporioides*
Poro	0	0	
Guemon	7	3	*Colletotrichum gloeosporioides*
Cavally	21	2	*Colletotrichum gloeosporioides*
Tonkpi	7	2	*Colletotrichum gloeosporioides*
Gôh	12	2	*Fusarium* sp., *Colletotrichum gloeosporioides*
Sud-Comoé	28	3	*Fusarium* sp., *Colletotrichum gloesporioides*, *Phytophthora palmivora*

Severity Index from 1 to 5. **1:** Absence of visible symptoms; **2:** 1-25 % of leaves or attacked fruits; **3:** 26-50 % of leaves or attacked fruits ; **4 :** 51-75 % of leaves or attacked fruits; **5 :** more than 75 % of leaves or attacked fruits

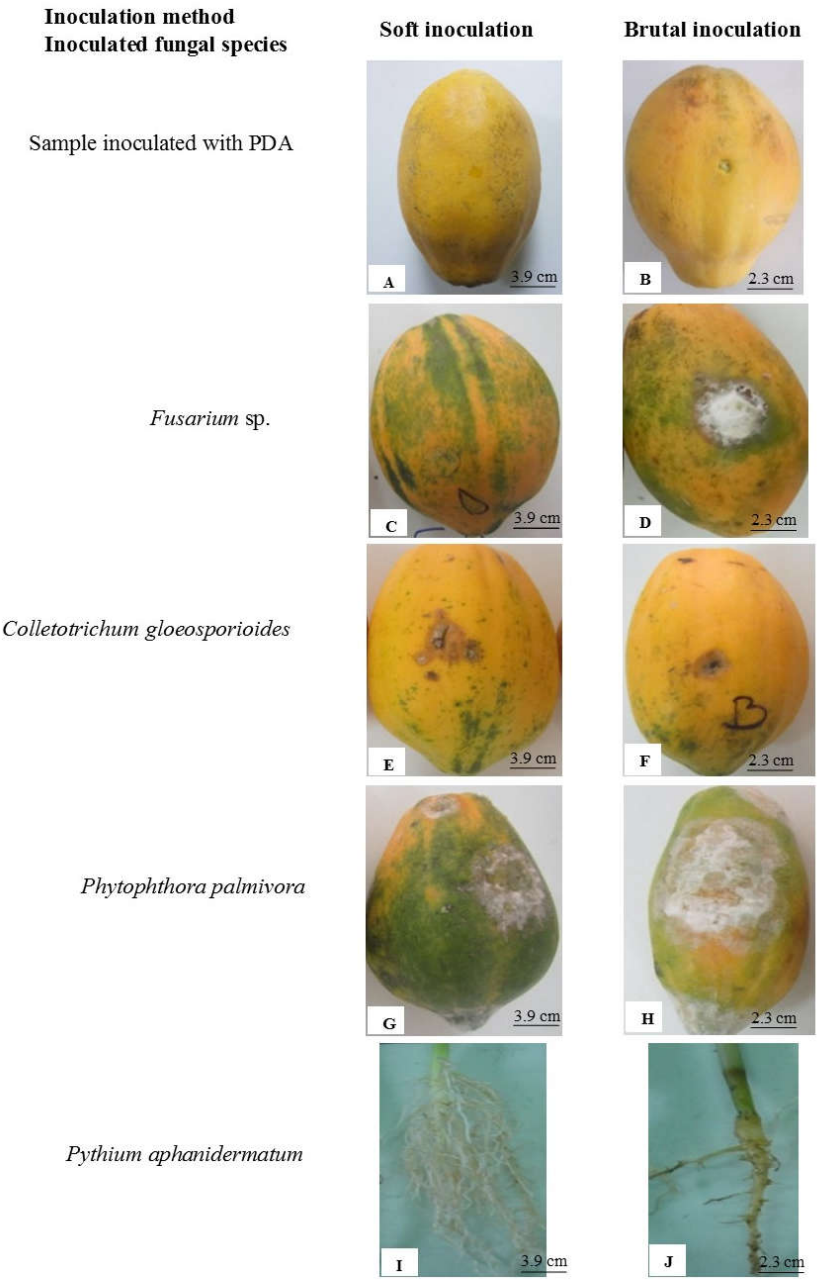

Figure 2 : Rots caused by fungal species six days after a soft and brutal

A et B : Fruits inoculated with PDA; C and D : soft and brutal inoculation with *Fusarium* sp.; E and F : soft and brutal inoculation with *Colletotrichum gloesporioides*, G and H : soft and brutal inoculation *Phytophthora palmivora;* I and J : soft and brutal inoculation with *Pythium aphanidermatum*

Table II: Average volume of rots on papayas caused by fungal species in soft and brutal inoculation after six days of incubation

Fungal species	Soft inoculation		Brutal inoculation	
	Incubation Time (j)	Rot's Volume (cm^3)	Incubation Time (j)	Rot's Volume (cm^3)
Phytophthora palmivora	4	12.72 ± 1.47^a	3	18.28 ± 2.29^a
Fusarium sp.	4	0.00 ± 0.00^c	3	4.99 ± 0.82^b
Colletotrichum gloeosporioides	5	4.64 ± 0.94^b	4	6.65 ± 1.39^b
H		34.23		25.32
P		0.000		0.000

In column, affected figures of various letters are significantly different according to the U test of Mann-Whitney at the limit of 5%.

However no symptoms, was observed after inoculation of *Fusarium* sp. (Figure II, C). Papayas injured during inoculation developed different symptoms for all fungal types tested. Three types of symptoms were observed contrary to the control (Figure II). These are whitish circular soft rot, circular brown rot and root rot. Brown rot was induced by *Colletotrichum gloeosporioides* (Figure II, F). Whitish circular soft rot was caused by *Phytophthora palmivora* (Figure II, H) and *Fusarium* sp. (Figure II, D). Regarding root rot, it was caused by *Pythium aphanidermatum* (Figure II, I).

Incubation period of fungal species: The appearance of the first on fruits was delayed a day in soft inoculation unlike brutal inoculation and no matter the fungal type. It varied from 4 to 5 days in soft inoculation and from 3 to 4 days in sudden inoculation (Table II).

Average volumes of rots: The average rot volumes caused by the different fungal species in mild inoculation ranged from 4.64 to 12.72 cm^3. The highest average volume was obtained with *Phytophthora palmivora* (12.72 cm^3) followed by *Colletotrichumgloeosporioides* (4.64 cm^3). Statistical analyses showed a significant difference between the decay volumes of the different fungal species ($H = 34.23$, $P < 0.05$). Thus, three homogeneous groups were determined (Table II). In sudden inoculation, average volumes of rots ranged from 4.99 to 18.28 cm^3. The highest average volume was obtained with *Phytophthora palmivora* (18.28 cm^3) followed by *Colletotrichum gloeosporioides* (6.65 cm^3). The smallest average volume was obtained with *Fusarium* sp. (4.99 cm^3). Statistical analysis showed a significant difference between decay volumes ($H = 25.32$, $P < 0.05$) (Table II). Regarding the strain of *Fusarium* sp., the incubation time was 4 days only when it was inoculated abruptly. Symptoms of crown rot of papaya seedlings started 7 days after inoculation.

DISCUSSION

The survey conducted on fungal diseases of papaya in Côte d'Ivoire shows that fungal diseases of papaya are more important and more severe in the South and East compared to the north of the country. This unequal distribution of fungal diseases of papaya in Côte d'Ivoire could be explained by the unequal distribution of rainfall in the different areas. In fact, humidity is more important in the southern and eastern areas than in the northern areas. However, the development of most of these fungi is linked to a high hygrometry. Pathogenicity tests have shown that strains of *Phytophthora palmivora, Colletotrichum gloeosporioides*are pathogenic fruit and *Pythium aphanidermatum* pathogenic roots and / or collar. The strain of *Fusarium* sp. could only cause damage to the fruit after injury. It is a post-harvest fungal disease of many fruits such as tomato, banana and papaya. Strains of *Fusarium* sp. cause enormous damage when fruit is injured at harvest, during transport or storage. Strains of *Phytophthora palmivora* and *Pythium aphanidermatum* are responsible for seedling melting and losses of young papaya plants. However, with *Colletotrichum gloeosporioides*, they cause huge damage in post-harvest. The research work carried out by Kouadio*et al*. (2017) showed that most of these strains are involved in postharvest crop rot of dessert bananas in Côte d'Ivoire. Fungi associated with papaya diseases in Côte d'Ivoire attack roots and fruits. The fungal disease highlighted are known for devastating actions in papaya orchards around the world. The PIP in 2010 reported the importance of *Colletotrichum gloeosporioides, Phytophthora palmivora* and *Pythium aphanidermatum* in field papaya and post-harvest fruit losses. The incubation time of the fungal species varied from 4 to 5 days regardless the incubation method. The absence of a difference in incubation time is due to the low physical barriers that the epidermal cells represent. The present results confirm those of Bujung (1990); Allou (1992) and Ouattara (2003). These authors claimed that the incubation time of the fungal species depends on the anatomy of the fruit.The diameter of the lesions induced by the fungal genera on the fruits grows with the phenological stage of the fruit. This progression could be attributed to fruit ripening, which also results in the gradual reduction of the elements involved in the fruit defense mechanism. These results corroborate those of Zainuri *et al*. (2003) and Jinyoung *et al.* (2002) on the progression of the anthracnose symptom on mango.

Conclusion

The survey of papaya orchards in Côte d'Ivoire revealed that the main fungal diseases of papaya are present in Côte d'Ivoire. The main strains responsible for these fungal diseases are: *Phytophthora palmivora, Pythium aphanidermatum* and *Colletotrichum gloeosporioides*. Their damage is perceptible in both plantation and post-harvest. In addition, this study has shown that the main risk areas are South, South-East and East of Côte d'Ivoire.

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