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Evaluation of Anthracnose Disease of Mango (*Mangifera indica* L.) Fruits and Characterization of Causal Agent in Côte d'Ivoire

M. Y. N'Guettia
Laboratoire de Biologie et Amélioration des Productions Végétales, Université Nangui Abrogoua 02 BP 801 Abidjan 02 Côte d'Ivoire
nguettiayah@gmail.com

N. Kouassi
Centre National de Recherche Agronomique (CNRA), 01 BP 1740 Abidjan 01 Côte d'Ivoire

H. Atta Diallo
Laboratoire de Biologie et Amélioration des Productions Végétales, Université Nangui Abrogoua 02 BP 801 Abidjan 02 Côte d'Ivoire

F. R. Y. Y. Kouakou
Laboratoire de Biologie et Amélioration des Productions Végétales, Université Nangui Abrogoua 02 BP 801 Abidjan 02 Côte d'Ivoire

***Abstract* – Mango (*Mangifera indica* L.) anthracnose is the most important postharvest disease limiting shelf life and export of fresh mangoes fruits in Côte d'Ivoire. However, its scientific report of disease is still lacking, primarily on anthracnose disease. Market and field survey were conducted in 2009 and 2010-2011 respectively. Disease Incidence (DI) and Disease Severity (DS) were recorded and causal agent characterized. Results revealed the presence of anthracnose in mango orchards in Côte d'Ivoire. Symptoms were rounds with regular or irregular contours black spots. The incidence and severity of anthracnose recorded on mangoes collected in field were higher in Odienné and Ferkessédougou than those in Korhogo. The incidence and severity of the disease seem to be influenced by climatic parameters and cultural practices. Based on white-grey colony and cylindrical conidia and the pathogenicity test, the pathogen was identified as *Colletotrichum gloeosporioides*. Variability of the pathogenicity of strains of *C. gloeosporioides* also revealed the presence of pathotypes. The PCR performed with the *C. gloeosporioides* and *C. acutatum* species-specific primer *Cg*Int and *Ca*Int2 with the universal primer ITS4 yielded a single band of 450 bp for *C. gloeosporioides*. Nucleotide sequences of the ITS region of the ribosomal DNA of twelve selected isolates showed 100% homology with *C. gloeosporioides* isolates available in the GenBank of NCBI. Thus, present study demonstrated that *C. gloeosporioides* is the pathogen responsible for anthracnose disease of mango produced in Côte d'Ivoire.**

***Keywords* – *Colletotrichum gloeosporioides*, Disease Incidence, Disease Severity, Specific Primer, Côte d'Ivoire.**

I. INTRODUCTION

Mango (*Mangifera indica* L.) is considered as the most popular and commonly eaten fruits by millions of people in the tropical area especially the developed countries. Many countries are shipping large volumes of fruit to markets of the United States and Europe, and they must compete on the basis of price and quality [1]. Developing countries account for about 98 % of the total production while, developed countries account for 80 % of the world import trade [2].

In Côte d'Ivoire, the average of the annual production is more than 100,000 tons of mangoes, and only 10 % of that production is exported [3]-[4]. The production of mango variety Kent and logistical advantages of Côte d'Ivoire make it as the third largest supplier to the European market [5]. However, these production and exportation are limited by several biotic factors such as anthracnose caused by *C. gloeosporioides* Penz. var. *minor* J.H. Simmonds, *C. acutatum* J. H. Simmonds and the teleomorph fungus *Glomerella cingulata* (Stoneman) Spauld & Schrenk) [6]. This disease is the most important and prevalent in all mango growing regions [7]. Anthracnose disease mainly attacks inflorescences and fruit bodies (both during ripening and post harvest) and occasionally affects young leaves [8]. Under wet or very humid conditions, the incidence of the disease on mango can reach almost 100 % [1]. Incidence of infection is favored by temperature between 20 and 30 °C and high relative humidity [9]. Production of the both main commercial mango varieties (Kent and Keitt) from Côte d'Ivoire is severely affected by this disease. The main fructification period of these mango varieties takes place during the long rainy season, from May to August. These seasonal conditions seem to enhance the rapid development of anthracnose disease impacting negatively the exportation of high quantities of Ivorian mangoes

[10]. A first step in the way of finding a solution to this problem is to better characterize the pathogens in term of biology and ecology. Morphological approaches for detection, diagnostic and identification using disease symptoms, isolation, and cultural characterization constitute the first step in plant pathogen studies [11].

However, due to environmental influences on the stability of morphological traits and the existence of intermediate forms, these criteria are not always reliable for the discrimination of the *Colletotrichum* species causing the anthracnose disease. Therefore, several molecular approaches has been used [12].

Specific oligonucleotides have been widely used to differentiate these two species, *C. gloeosporioides* and *C. acutatum* causal agent of mango anthracnose disease [8]-[13]-[14]-[15].

The objective of this research was to (i) study the occurrence and the severity of mango anthracnose disease in the main production zone in northern Côte d'Ivoire and (ii) characterize the causal agent of this disease by pathogenicity test and molecular method.

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II. Materiel and Methods

2.1. Sampling sites

Surveys of mango fruit anthracnose were conducted in 2009, 2010 and 2011 in the urban markets of Abidjan and in the main production zone of mango in Côte d'Ivoire (Fig. 1). These works were done during the fructification periods (i.e. long rainy season). Côte d'Ivoire has 4 mains climatic zones: (i) subequatorial climate located in the south, (ii) mountains climate located in the west, (iii) humid tropical climate located in the center and (iv) tropical in the north of the country. The main production zone of mango is located in the Sudanese section of tropical climate in the north of Côte d'Ivoire. In this area, the annual rainfall varies between 500 and 1000 mm. Two seasons can be distinguished: a dry season (from October to April) and a rainy season (from Mai to September).

2.2. Assessment of disease occurrence and severity

Field survey of fruit anthracnose was carried out in the five selected mango-growing areas (Korhogo, Sinématiali, Napié, Ferkessédougou and Odienné). Twenty mango fruits from each surveyed-orchard (Three orchards per area) were randomly sampled to assess the frequency and/or occurrence and the severity of the disease. A total of 300 and 60 mature and green fruits belonging to the Kent variety were respectively collected in 2010 and 2011. In 2011 only orchards located at Korhogo were sampled to determine fruit anthracnose frequency and/or occurrence and severity and to compare the main parameter over the two years in that area. Samples were putted into clean bags, labeled and carried to the laboratory where, they were conserved at 25± 2 °C for 10 days. Mangoes were examined every day for rotting fruits. Symptoms were described based on lesions color, shape and arrangement on the surface of the mango fruit bodies as described by Arauz [1]-[16]-[17]. Fruit anthracnose was assessed using the standards assessment protocol suggested by [7]. Scale 1 to 5 was used (Table 1). Disease incidence (percentage of diseased fruits), and disease severity (percentage of area affected on the fruit on average) were then obtained by the following formula.

$$DI = \frac{Xi}{Xt} \times 100 \qquad (1)$$

$$(DS) = \left(\frac{\sum ni}{Xt \times 5}\right) \times 100 \qquad (2)$$

DI: disease incidence; Xi: number of infected fruits by location; Xt: total number of fruits collected

DS: disease severity; ni: infected fruits and their corresponding score scale; 5: the highest score of the scale.

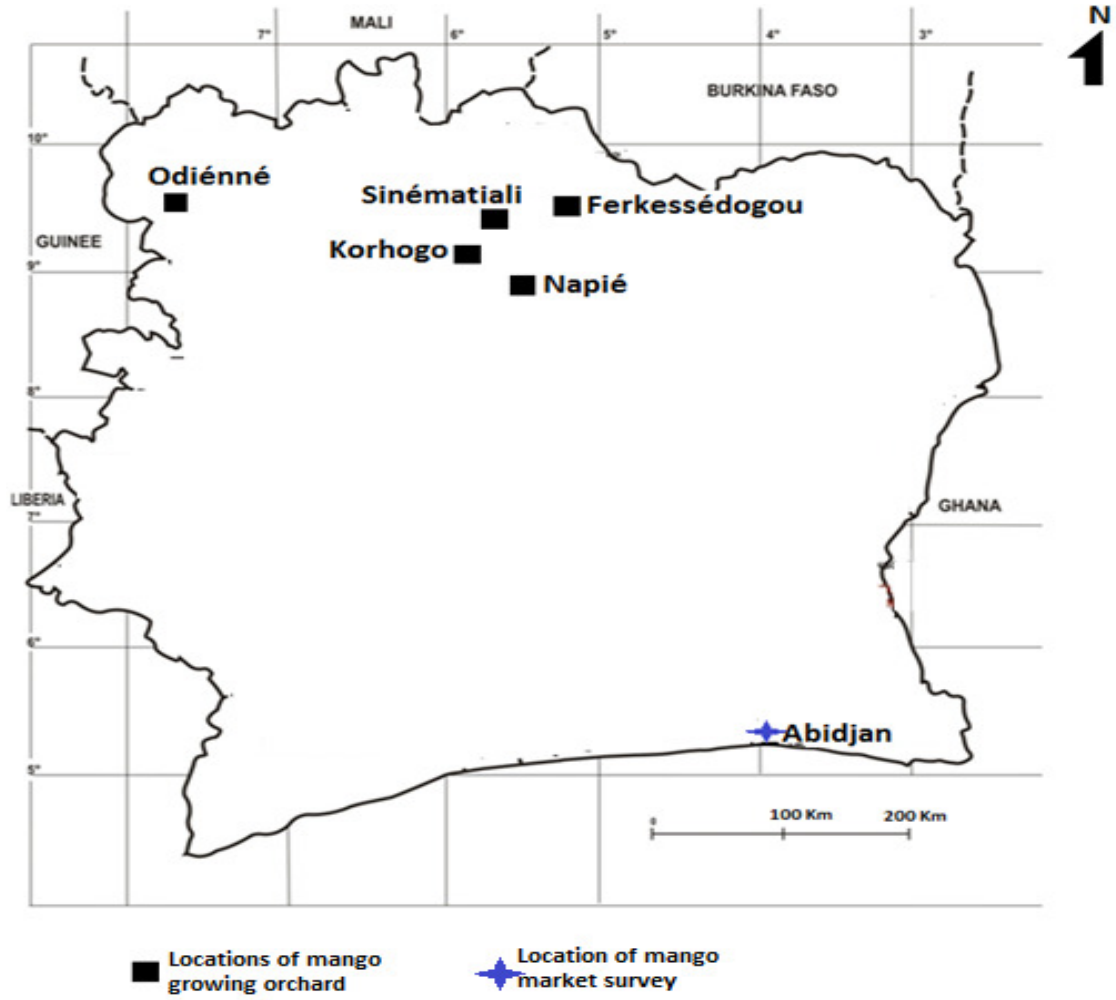

Fig.1. Location of mangoes surveyed

Table 1: Scale rating used for disease severity evaluation on mango fruits collected in field.

Grade	Fruit area infected
0	No infection
1	< 5 %
2	6-10 %
3	11-20 %
4	21-50 %
5	> 50 %

2.3. Isolation and identification of pathogens

Isolation and identification of the causal agents were performed on 65 symptomatic mango fruit bodies with 30 of them from urban markets and 35 from fields. Pathogens were isolated from each single rotting fruit. Mangoes were first soaked in 70 % ethanol solution for 5 minutes and then soaked in 0.5 % hypochlorite solution 5 minutes. The fruits were finally rinsed two times in sterile distilled water. Portion of peeled epicarp of the infected fruit was removed at the point of progression of disease symptom and cut into small pieces. The pieces were placed (or cultured) in Petri dishes containing potato dextrose agar (PDA) medium amended with 5 % citric acid. Incubation was performed at 25±2 °C for three days under a photoperiod of 12 hours/day. Isolated colonies were, sub-cultured into fresh Petri dishes until pure cultures were obtained and identified by visual examinations under optic microscopes. Then they were described and classified based on conidia and colony morphology as described by [18]-[19]-[20]. Cultures of *Colletotrichum* sp. obtained were purified using single spore technique according [21].

2.4. Pathogenicity Tests

The pathogenic evaluation of the disease was done by inoculating mature and unripe mango fruit bodies with the isolates using the wound method [22]. The fruit bodies were first washed with water, soaked in 3 % hypochlorite solution for 5 minutes and then rinsed two times in sterile distilled water, air-dried before the inoculation. The inocula consisted of mycelial disks (4 mm in diameter) removed from seven-day-old pathogen colonies grown on PDA culture medium. Inoculation was performed by scratching and depositing inoculums in three equidistant points on the fruit peel surface. Control fruits were inoculated with PDA disks without pathogens. Inoculated points were then sealed with parafilm and fruits were placed in plastic bag and incubated for 5 days at 25±2 °C. Anthracnose symptoms were measured 5 days after inoculation in two opposite directions.

The pathogenicity of *Colletotrichum* strains was estimated according to the grading scale presented in the table 2 varying from 0 to 4 [23].

Percentage of strains belonging to each class of pathogenicity was assessed according the following formula:

$$Pi = \frac{\sum ni}{Nt} \times 100 \qquad (3)$$

Pi: percentage of fungi belonging to pathogenicity class i
Ni: number of fungi producing lesions of class i
Nt: Total number of tested fungi

Fungi of *Colletotrichum* with anthracnose symptom after inoculation were re-isolated on PDA plate and comparison was made with the origin strains

Table 2: Grading scale used for evaluation of the pathogenicity of *Colletotrichum* sp. isolates in mango fruits.

Grade	Symptoms characteristics (lesions Ø)	Classification of the isolates
0	Absence of lesions	Non-pathogenic
1	from 1 to 5 mm	Weakly pathogenic
2	Lesions from 6 to 15 mm	Mildly pathogenic
3	from 16 to 25 mm	Pathogenic
4	Lesions over 25 mm	Highly pathogenic

2.5. Molecular characterization of Colletotrichum species

2.5.1. DNA Extraction

The isolated *Colletotrichum* were grown on 100 ml of PDA medium containing citric acid (1 %) for 7 days. Thereafter, the mycelium was collected by vacuum filtration in Eppendorf tubes (2 mL). DNA was extracted and purified as previously described by [24]. DNA extraction was conducted using New Glucanex (Novo Nordisk Ferment Ltd, Bern, Switzerland – 18 mL H_2O, 42 mL NaCL 1 M, 1.8 g New Glucanex adjusted to pH 6·0). Digestion of cell walls was conducted in Eppendorf tubes (2 mL) for 2 h at room temperature (25±2 °C), and DNA was precipitated with isopropanol followed by a centrifugation for 7 min. Pellets were washed with 70 % ethanol. The dried DNA samples were resuspended in 100 µl of 1× TE buffer at 37 °C for 60 min. DNA concentration was evaluated on agarose gels (1 % in TBE) using constant voltage (80 V) for 1 h.

2.5.1. PCR reactions

C. gloeosporioides *Cg*Int (5´-GGCCTCCCGCCTCCGGGCGG-3´) and *C. acutatum* *Ca*Int2 (5´-GGGGAAGCCTCTCGCGG-3') specific primers, were combining with ITS4 universal primer, to allow identification of the species [14]-[19]. The amplification reaction was performed in a final volume of 50 mL, containing 10 ng of genomic DNA, 25 mM each primer, 2 mM of dNTPs, 1.5 or 3.75 mM $MgCl_2$ (for *C. gloeosporioides* and *C. acutatum* respectively), 1U Taq DNA polymerase (Invitrogen™) and molecular grade water to complete the final volume [19]. Amplification was carried out under the following conditions: one cycle at 95 °C for 5 min; 34 cycles at 95 °C for 45 sec, 75 sec at 55 °C (for CgInt) or 69 °C (for CaInt), 2 min at 72 °C, and one final cycle at 72 °C for 5 min. The amplification products were separated in 1 % agarose gel in Tris–acetate–EDTA buffer, at 80V for 60 min. The gel was visualized in ethidium bromide solution at 0.5 mg/L under ultraviolet light. Each individual analysis was replicate twice.

2.5.3. Sequencing of ITS PCR products

PCR products obtained from twelve selected isolates tested positive were purified and sequenced by the DNA sequencing service of Eurofins Genomic (France). Sequencing of the PCR product was performed in both

directions. Multiple sequence alignments and comparisons of nucleotide sequences were performed. Nucleotide homology searches were performed with the nucleotide program BLAST (http:www.ncbi.nih.gov) to confirm the identity of the pathogen. The ITS sequences of *C. gloeosporioides* isolates FB2; FD4; OT6; M12; FZ2; Yr9; OV5; Kr2; OV4; OV3 and Ko7 were deposited in the NCBI as accession numbers KF773852, KF773853, KF773854 ; KF773855 ; KF773856 ; KF773857 ; KF773858 ; KF773859 ; KF773860 ; KF773861 ; KF773862 and KF773863

2.6. Data Analysis

Statistical analyses were performed with the software STATISTICA version 7.1. Data from disease incidence and severity were compared using chi-square test comparing. Mean lesion diameter development in inoculated fruit bodies were subjected to analysis of variance (ANOVA). Comparison of means was made by the LSD test. DNAMAN 7.1 was used to obtain the sequence consensus. Alignment of nucleotide sequences was performed with the mega 5 muscle alignment tool and compare to GenBank accession with the nucleotide program BLAST of NCBI.

III. RESULTS

3.1. Characteristic of the anthracnose symptoms

On ripe fruits, anthracnose symptoms appeared on pericarp 5 to 7 days after their harvest. These symptoms were in some case just one dark brown necrotic and sunken lesion. In other fruits, symptoms were black irregular lesions with several sizes recovering rapidly the surface of the fruit body. At the advanced state, symptoms coalesced to give rotten fruit.

Symptoms of some fruit bodies presented typically tear-stain black lesions that run from the stem-end to the basal of the fruit. According to distribution of lesions on fruits, several types of symptoms were observed:

- Lesions like small spots distributed on all fruits surface
- Large necrotic spots giving depressed spots on fruits
- Tear-stain lesion, beginning from the stem-end to the basal of fruits

3.2. Occurrence and Severity of anthracnose disease of mango fruit in field

The anthracnose disease incidence on the 300 mangoes fruits collected in field surveyed in 2010, was 26 % and 24 % from Ferkessédougou and Odienné respectively. Anthracnose symptoms were not observed on the fruits collected in Korhogo, Sinematiali and Napié. Disease severity was higher on fruits collected at Odienné than those from Ferkessédougou (Table 3). Scale of disease severity on mangoes collected varied from 2 to 5 (Fig. 2). Disease severity was slightly less on fruits collected in Ferkessédougou where disease severity grades varied from 2 to 3 and with mean severity of 1.45.

3.3. Influence of the collection period on the prevalence of anthracnose

No symptoms of anthracnose were observed on fruit collected in 2010 in Korhogo. However, 5 % of fruits collected in this locality in 2011 have shown anthracnose severity varying from 2 to 3. The chi-square test for comparison of means has shown that there are no significant differences (P = 0.083) between the incidence of anthracnose on the fruits collected during these two different years The developed symptoms were similar in the different areas.

Table 3**:** Incidence and severity of anthracnose in different mangoes growing zones in Côte d'Ivoire

Zones	Incidence (%)	Severity
Korhogo	0b	1±0.00c
Ferkessédougou	24±10.88a	1.45±0.10b
Odienné	26±16.61a	2±0.23a
F	50.69	11.75
P	< 0.001	< 0.001

Means followed by the same letter are not significantly different from one another at LSD ($\alpha = 0.05$; $P < 0.001$).

3.4. Isolation and identification of anthracnose disease pathogen on mango fruits

Colonies were characterized by dense, aerial mycelium, cottony texture to covered texture. Coloration of colonies ranged from white to grey (Fig. 3). All isolates presented hyaline and septate mycelium with cylindrical conidia characteristics of *Colletotrichum gloeosporioides*. All conidia presented both ends rounded or one end rounded and other pointed.

3. 5. Pathogenicity of strains of Colletotrichum sp. on cv Kent fruits

Pathogenicity test on Kent mango fruits showed that only *Colletotrichum gloeosporioides* with white and grey colonies reproduced anthracnose disease symptom typical of those observed on field survey. Strains of *Colletotrichum* sp. with pink and yellow colonies did not induce symptoms on mango fruits. Symptoms caused by strains of *Colletotrichum gloeosporioides* were black lesions and lightly circular in shape around the points of inoculation. Five days after inoculation, all strains induced lesions. Distribution of lesions severity on fruits allows classification of strains into 3 pathogenic groups (Fig. 4). The first group was strains with low pathogenic level (6.45 %). Diameter of these lesions was less than 5 mm.

The second group represented by strains with moderately pathogenic level (87.09 %). They caused lesions with diameters varying from 6 to 15 mm. The last group represented strains with high pathogenic level (6.45 %). Lesions diameters caused by these strains varied from 16 to 25 mm.

Statistical analysis showed significant difference in the frequency of pathogenicity of *C. gloeosporioides* strains. Cultures re-isolated from the inoculated fruits with anthracnose symptoms were similar to those of the original isolates used for the inoculations.

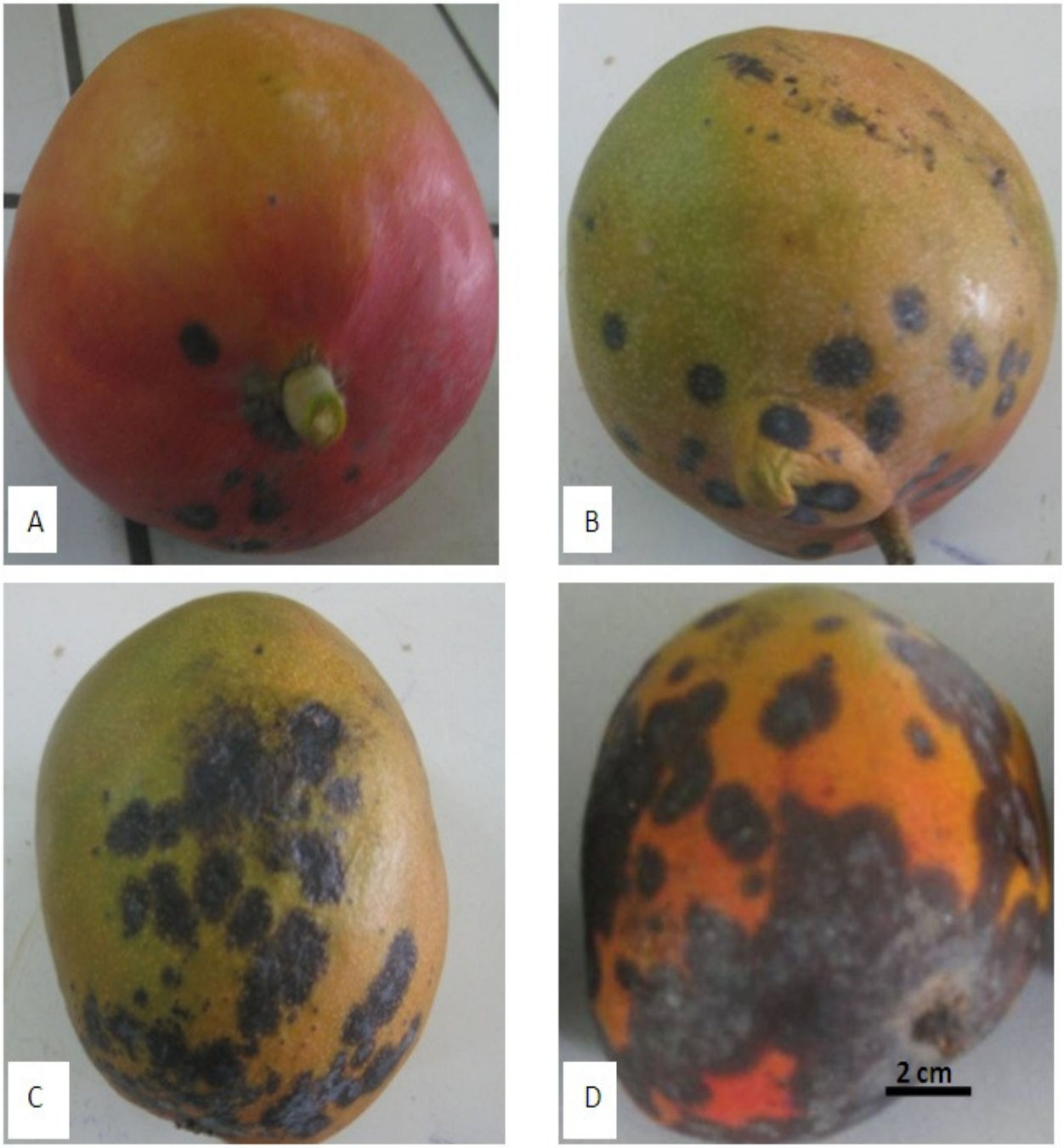

Fig.2. Mango fruit bodies of the variety Kent presenting anthracnose symptoms with several grades of severity. A: Severity grade 2; B: severity grade 3; C: severity grade 4 and D: severity grade 5.

3.6. Molecular identification of Colletotrichum sp. with specific primers

DNA from 83 *Colletotrichum* sp. strains was amplified with specific primer CaInt2 for *C. acutatum* and CgInt from *C. gloeosporioides*. Result of PCR reaction showed reaction with *C. gloeosporioides* specific primer CgInt and no reaction with *C. acutatum* specific primer CaInt2 and confirmed identification of *C. gloeosporioides*. Presence of *C. gloeosporioides* species revealed presence of 450 bp band on agarose gels (Fig. 5). All grey and white colonies identified as *C. gloeosporioides* had positive reaction with CgInt/ITS4 primers with presence of 450 bp fragment. Yellow and pink strains had negative reaction to both primers.

A comparison of a nucleotide sequence of *Colletotrichum* isolates to NCBI was used to confirm the identity of the pathogen as *C. gloeosporioides*. The results showed 99 to 100 % homology with DNA sequences from other *C. gloeosporioides* isolates deposited in the GenBank. Comparisons of nucleotide sequences of *C. gloeosporioides* the isolates FB2 (KF773852) and NCBI accession numbers DQ45400 is presented in figure 6.

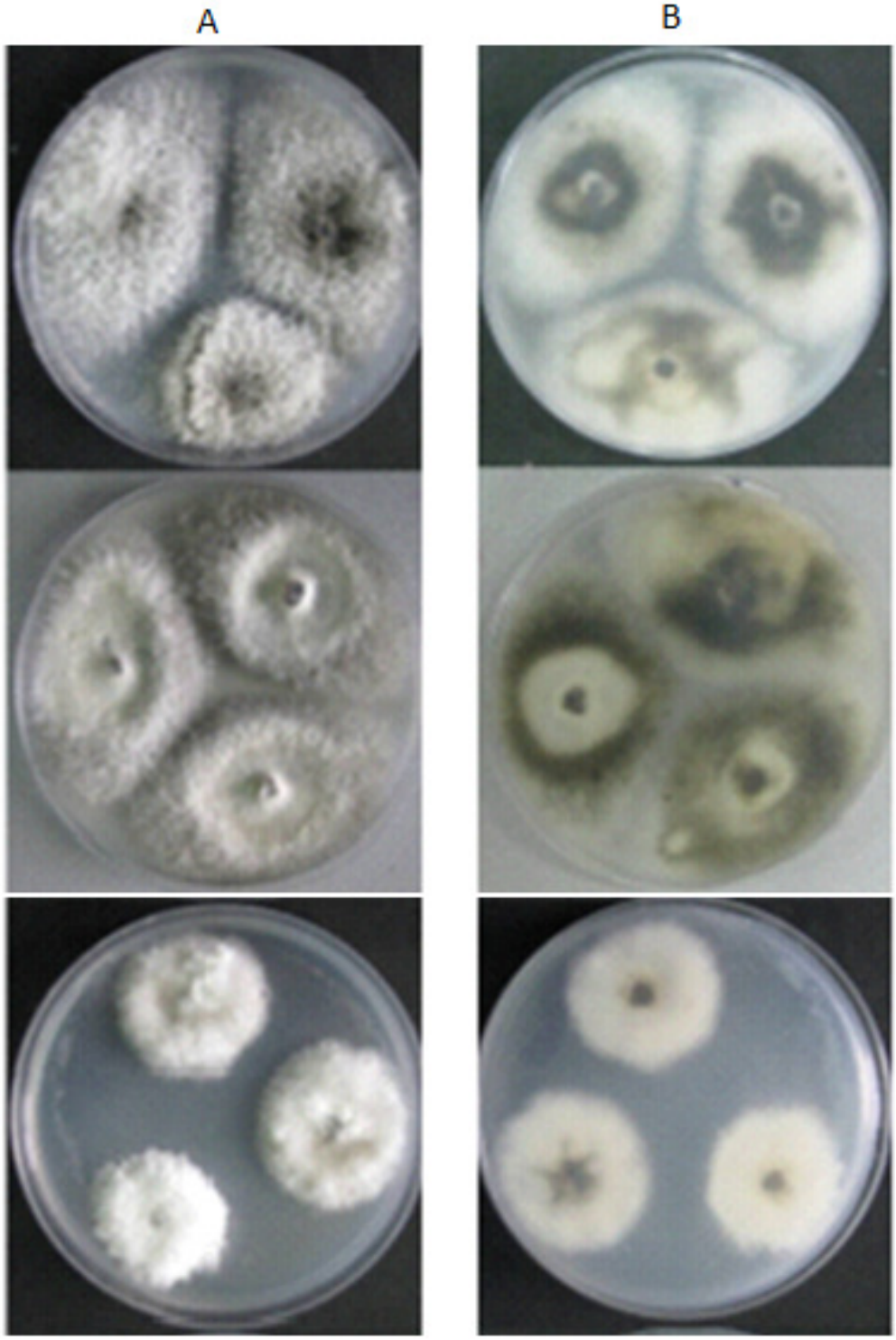

Fig.3. *Colletotrichum gloeosporioides* colonies 10 days after incubation in PDA
(A): top view of colony in a Petri dish; (B): reverse view

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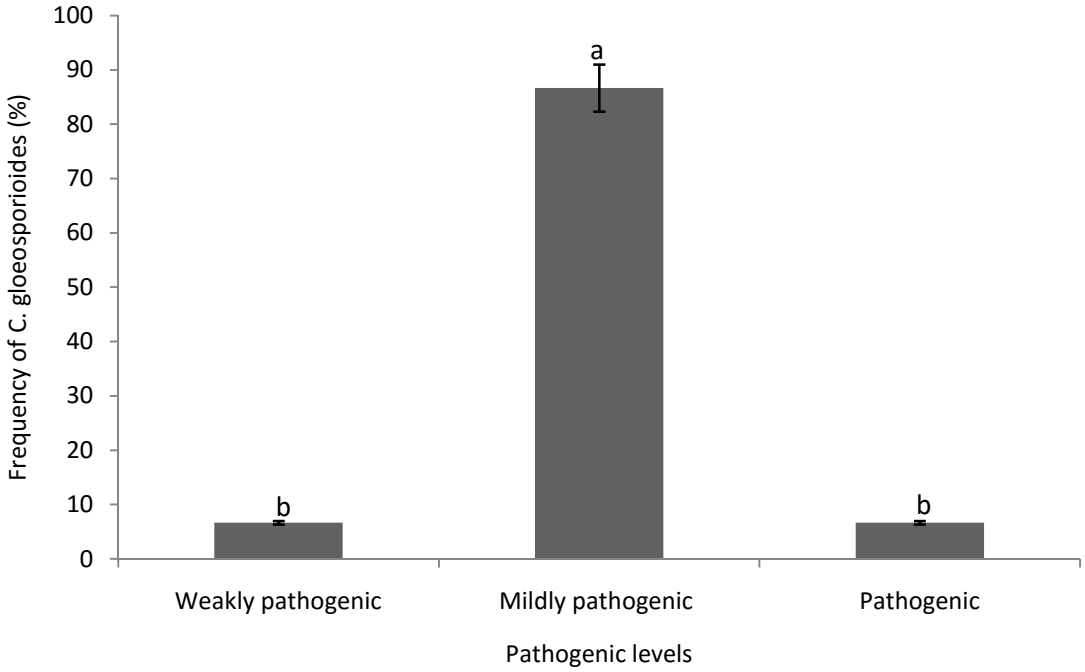

Fig.4. Distribution of *C. gloeosporioides* according pathogenic levels 5 days after inoculation on mango fruits, cv Kent

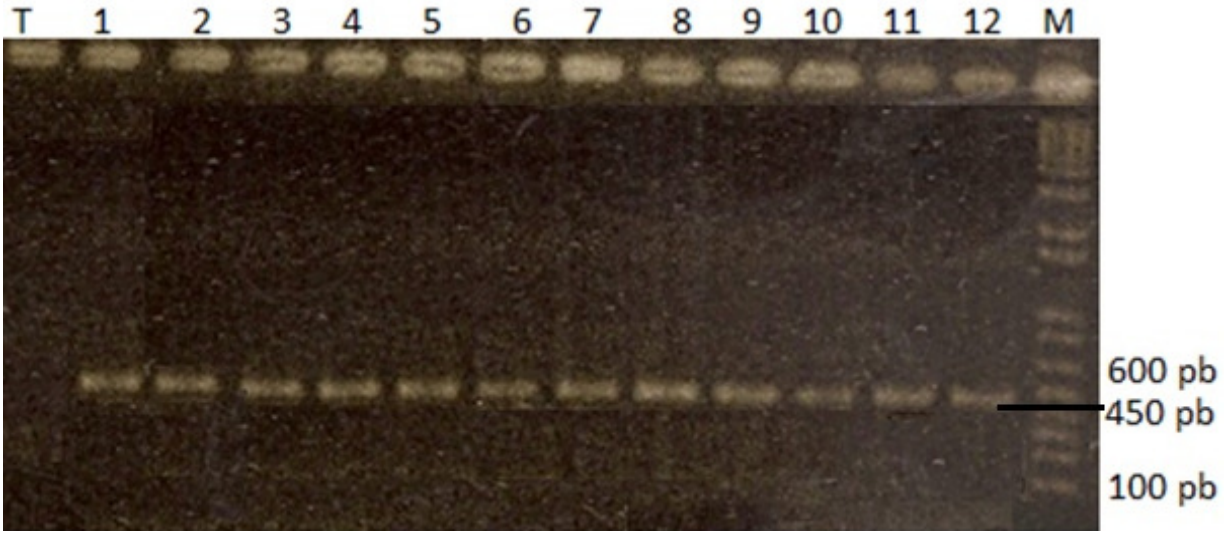

Fig.5. Amplification of DNA of *Colletotrichum* sp. with ITS4 and CgInt specific primers of *C. gloeosporioides*
M: DNA marker; T: negative control; 1 to 12: *C. gloeosporioides* isolates

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KF773852  1    TTCCTCCGCTTATTGATATGCTTAAGTTCAGCGGGTATTCCTACCTGATCCGAGGTCAAC  60
                |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
DQ454005  536  TTCCTCCGCTTATTGATATGCTTAAGTTCAGCGGGTATTCCTACCTGATCCGAGGTCAAC  477

KF773852  61   CTTTGGAGAATTGGGGGTTTTACGGCAAGAGTCCCTCCGGATCCCAGTGCGAGACGTAAA  120
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
DQ454005  476  CTTTGGAGAATTGGGGGTTTTACGGCAAGAGTCCCTCCGGATCCCAGTGCGAGACGTAAA  417

KF773852  121  GTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCCACTACCTTTGAGGGCCTACATCAGC  180
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
DQ454005  416  GTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCCACTACCTTTGAGGGCCTACATCAGC  357
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KF773852  181  TGTAGGGCCCCAACACCAAGCAGAGCTTGAGGGTTGAAATGACGCTCGAACAGGCATGCC  240
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
DQ454005  356  TGTAGGGCCCCAACACCAAGCAGAGCTTGAGGGTTGAAATGACGCTCGAACAGGCATGCC  297

KF773852  241  CGCCAGAATGCTGGCGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGC  300
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
DQ454005  296  CGCCAGAATGCTGGCGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGC  237

KF773852  301  AATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCC  360
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
DQ454005  236  AATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCC  177

KF773852  361  GTTGTTAAAAGTTTTGATTATTTGCTTGTACCACTCAGAAGAAACGTCGTTACATCAGAT  420
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
DQ454005  176  GTTGTTAAAAGTTTTGATTATTTGCTTGTACCACTCAGAAGAAACGTCGTTACATCAGAG  117

KF773852  421  GCTGGTTATCCTCCGGCGGGCGCCGACCCGCCCGGAGG-GGGAG  463
               |||||||||||||||||||||||||||||||| || |||||
DQ454005  116  TTTGGTTATCCTCCGGCGGGCGCCGACCCGCCCGGGGGCGGGAG  73
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Fig.6. Comparison of nucleotide sequences of *C. gloeosporioides* isolates FB2 (KF773852) from Côte d'Ivoire and NCBI accession number DQ45400.

IV. Discussion

This study revealed specific symptoms of anthracnose disease on mango fruits from the north of Côte d'Ivoire. Symptoms were found as small or large necrotic spots with depression [25]-[1]-[6]. Incidence and severity of this disease was also found varying among mango production areas of this country. This incidence was high in Odienné and Ferkessédougou, while no incidence was observed in Korhogo, Sinématiali and Napié. These results can be explained by orchard sanitation. Indeed, although sanitation aspect of orchards was not evaluated in our study, general observations showed that orchards from Korhogo, Sinématiali and Napié were cleaned than those from Ferkessédougou and Odienné.

Post-harvest mango infection is influenced by orchards sanitation and particularly cleaning and pruning that are useful to decrease the rate of disease infection [16]. He was also found on studies of coffee anthracnose, that disease was present in orchards where good agricultural practices are not respected [26]. These results can also be explained by the fact that orchards visited in Ferkessédougou and Odienné are important source of inoculums. Absence of hygiene was observed in the orchards of Ferkessédougou and Odienné, that might favored the accumulation of inoculums from one crop cycle to another and contributed to enhance the rate infection. Long humid periods and dense vegetation associated to the difference in the inoculums concentration may explain the significant differences in the disease incidence in orchards of the same region [27].

It was observed that higher level of fungal infection can explained by higher primary inoculums level in the field resulted higher infection in the field [28].

Absence of anthracnose symptoms in mangoes collected in Korhogo, Sinématiali and Napié orchards in 2010 does not mean that the disease is not present. However, the incidence of the disease on fruit collected in June 2011 was 5 %. These results show that this area is not free of the disease. Distribution of anthracnose of mango in all areas of production of mango in the world has been demonstrated by various studies [6]-[19]-[29]. Application of good sanitary conditions can explained the low incidence of the disease in Korhogo area. According to [16], orchard sanitation can reduce the infection rate of post harvest even during the rainy season. The difference in disease incidence can be explained by collect period of fruits in the two years. Indeed the collection period from June corresponds to a period of heavy rainfall in Côte d'Ivoire.

The incidence of disease in the two years is not significantly different in Korhogo area. Similar results have been observed by [30]. These authors have shown that once the fungal are established in an orchard, their relative frequencies appear to be stabilizing from year to year and throughout the growing season.

Several strains of *Colletotrichum* were isolated from lesions on fruits. Findings show that mango fruits would be hosts for this pathogen. Indeed, several studies showed that *Colletotrichum* causes anthracnose on cultivated crops, particularly on tropical fruits [13]-[1]-[31]. Frequency of isolation of *Colletotrichum* sp. in

Ferkessédougou and Odienné areas were 59.52 % and 40.47 % and not statistically different. Findings demonstrated the highest rate of implication of anthracnose on mango in Côte d'Ivoire.

Strains were characterized by colony coloration ranging from white to grey and from yellow to pink with cylindrical and fusiform conidia. Grey and white strains have characteristic of *Colletotrichum gloeosporioides* described on mango [13]-[19] and others hosts plants [32]-[33]-[8]. Other strains presented yellowish and pinkish coloration with fusiform conidia could be *Colletotrichum dematium* according to the description by [34]. Variations in coloration and texture of strains colonies were similar in all mango production areas. Findings are in accordance with those of [22]. Authors observed that there was no geographical specificity concerning colonies coloration of *Colletotrichum gloeosporioides* isolated from mango and avocado fruits in South Africa.

Inoculation of strains on mango Kent fruits showed that only fungi identified as *C. gloeosporioides* cause lesions on mango fruits. These ones reproduced characteristic lesions of anthracnose. Symptoms are similar to those on collected fruits in orchard. Re-isolated strains presenting the same characteristics like those inoculated. Findings are in agreement with results of several authors that showed that *Colletotrichum gloeosporioides* is the causal agent of mango anthracnose [17]-[8]-[2]. Strains produced lesions on all inoculated mango fruits. This demonstrates the highest rate of implication of *C. gloeosporioides* in the development of mango anthracnose in Côte d'Ivoire. Similar result showed that only inoculation of *C. gloeosporioides* reproduced specific symptoms of anthracnose, whereas strains with fusiform conidia will not reproduced symptoms [2]. These results confirm that only *C. gloeosporioides* species is associated to mango fruits post-harvest anthracnose [29].

In this study, *Colletotrichum gloeosporioides* strains responsible of anthracnose were identified by specific primers CgInt/ITS4. Findings show that theses primers are sufficient for *Colletotrichum gloeosporioides* identification. According to [13], a specific primer CgInt and CaInt help to discriminate *Colletotrichum* species but is not adapted to distinguish sub-populations. All strains identified by morphological characteristics of *C. gloeosporioides* were confirmed by molecular identification. These results are in accordance with those of [35]. Authors separated strains of *C. acutatum* from those *C. gloeosporioides* using morphological characteristics and confirmed by specific primers.

Pink and yellow strains were not amplified by primers used. These strains did not belong to *C. gloeosporioides* and *C. acutatum* species. No reaction was obtained with specific primers of *C. acutatum*. Findings demonstrate that *C. acutatum* is not implicated in post-harvest mango infection in Côte d'Ivoire. It has been found that *C. acutatum* is not implicated in post-harvest infection of mango but can be found on leaves and panicles infection [19]. Indeed, several studies showed that *C. acutatum* would be linked to infection in field, particularly causing foliar spots, flowers, stems, immature fruits infection. *C. acutatum* is associated to pre-harvest infection on lemon and no post-harvest infection of this fruit [36]. Identification of causal agent of anthracnose on three crops in Colombia showed that only *C. gloeosporioides* species was associated to mango. However on the two other crops, *C. gloeosporioides* and *C. acutatum* were identified [8].

The BLAST similarity search confirmed the results obtained by the species-specific primers PCR analysis as the ITS sequences obtained from *C. gloeosporioides* shared 100 % sequence similarity with sequence of *C. gloeosporioides* at NCBI.

V. Conclusion

This study showed that anthracnose is prevalent in all the mango-growing areas surveyed and mangoes sold in markets in Côte d'Ivoire. The incidence and severity of anthracnose on mangoes collected in the fields were higher in Odienné and Ferkessédougou than those from Korhogo. Symptoms were black spots with regular or irregular contours. In addition, the causative agent of the disease was identifying like *C. gloeosporioides*. The pathogenicity test and molecular analysis confirmed that *C. gloeosporioides* is responsible for anthracnose disease of mango in Côte d'Ivoire.

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AUTHOR'S PROFILE

Ms. Marie Yah N'Guettia
was born on February 28, 1977 in koun Fao city of Côte d'Ivoire and holds a Bachelor degree in plant Protect and M. S. degree in Biology and Plant Protect, option Plant Pathology from University Nangui Abrogoua (Côte d'Ivoire).
She is currently registered in Ph.D degree Plant Pathology from University Nangui Abrogoua (Côte d'Ivoire). She has one peer reviewed publications in an International journal.
N'Guettia M. Y., Atta H. D., Kouassi N., Coulibaly F., 2013. Diversité morphologique et pathogénique des souches de *Colletotrichum* sp. responsables de l'anthracnose de la mangue en Côte d'Ivoire Journal of Animal &Plant Sciences, 2013. Vol.18, Issue 3: 2775-2784.