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# Research Article
# Parasitical Fungi in Cashew (*Anacardium occidentale* L.) Orchard of Côte d'Ivoire

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## Abstract

**Background and Objective:** Cashew tree (*Anacardium occidentale* L.) is an important fruit tree mainly culture for the edible nuts providing substantial income to farmers in northern and central regions of Côte d'Ivoire. However, the productivity is threatened by the strategies development and extension of diseases. Therefore, this study aimed to identify pathogens involved in cashew diseases. **Materials and Methods:** Leaf, shoot tip, twig, flower and fruit samples were collected in the most important cashew-growing areas of Côte d'Ivoire. Sections of 4-6 mm diameter from the periphery of lesions where surface sterilized in 70% ethanol and then in 3% sodium hypochlorite followed by abundant rinsing in sterile distilled water. After removing excess of water, fragments were placed on PDA medium. Symptoms associated with the pathogens in the field were described. To test the pathogenicity of isolates obtained, leaves of twenty eight days seedlings were sprayed with a spore concentration of $10^6$ $mL^{-1}$ used as inoculum for each isolate. Data collected were analyzed using analysis of variance (ANOVA 1) with Statistica version 7.1 software. **Results:** The results revealed that the different pathogenic diseases of cashew in Côte d'Ivoire are caused by *Colletotrichum gloeosporioides* (63.45%), *Pestalotia heterocornis* (30.35%), *Lasiodiplodia theobromae* (1.66%) and other unidentified pathogens. Pathogenicity of isolates was verified by the implementation of Koch's postulate. **Conclusion:** This study confirmed the presence of pathogenic fungi on cashew and the fact that some of these fungi can induce symptoms reducing leaf photosynthetic area and the production capacity. Based on pathogenic characterization of fungi, strategies need to be developed in order to identify fungi species and integrated disease management approaches.

Key words: Cashew, pathogens identification, *Colletotrichum*, *Pestalotia*, *Lasiodiplodia*

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Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Cashew tree (*Anacardium occidentale* L.) is one of the important fruit tree in all producing countries worldwide[1,2]. Originated from Northeastern Brazil, it is now a days produced in tropical, sub-tropical and temperate regions[3]. Introduced in Northern and central areas of Côte d'Ivoire for reforestation, cashew is becoming the main exported crop of these regions. The cultivation of cashew won an economic interest in early 1990-1995 by Indian traders[4]. During the last decades, farmers from favorable district even those of the pre-forest regions are interested on it because of its economical rentability[5]. This involved a regular increase of the cultivated area and the production. Thus, since 2008, Côte d'Ivoire is the first African country exporting cashew nuts with 380000 t[6]. Now a days, the production is estimated at 550000 t obtained on 750000 ha cultivated lands[7]. The nut is nutritious and can be eaten raw, fried, salted or sweetened with sugar[8,9].

In spite of this economic importance, some constraints threat the production and then compromising yields which remain low around (300-500 kg $ha^{-1}$)[7]. Several pathogens infect different organs of cashew plant, including stems, leaves, inflorescences, apples and nuts causing substantial economic losses[10] and many of them have been described[11]. In Tanzania, for exemple, *Colletotrichum gloeosporioides*, *Oïdium anacardii*, *Cryptosporiopsis* spp., *Laetiporus* sp. and *Ganoderma* spp., are the main pathogens responsible of serious diseases on cashew trees[12]. Anthracnose caused by *Colletotrichum gloeosporioides* Penz. is the main disease of cashew in Mozambique and Brazil[13,14]. Inflorescence blight and twig dieback caused by *Lasiodiplodia theobromae* are the main factor limiting cashew nuts production in Nigeria[15]. In Benin, *Colletotrichum gloeosporioides*, *Pestalotia heterocornis* and *Oïdium anacardii* are the fungi that infect cashew plants[11]. Nearby Côte d'Ivoire in Burkina Faso, *Colletotrichum gloeosporioides*, *Pestalotia heterocornis*, *Xanthomonas citri* pv. *anacardii* and *Lasiodiplodia theobromae* are the major pathogens responsible of cashew nuts diseases[16]. Cashew is susceptible to over ten diseases caused by fungi[17-19]. These pathogens are reported to be site-specific[20].

In Côte d'Ivoire, reports on cashew pathogens and diseases are very scanty. So the aim of this study was to isolate and identify the pathogens associated with cashew diseases symptoms. This work will give a better understanding of organisms involved in the diseases expression and serves as pointer towards diseases management.

## MATERIALS AND METHODS

Samples were collected from October -November, 2015 in 19 areas which are the main areas where cashew is grown in Côte d'Ivoire then pathogens isolation and their characterization where carry out in Plant Physiology Laboratory of Félix Houphouët-Boigny University.

**Collection of plant organ exhibiting diseases symptoms:** The cashew trees showing diseases symptoms were selected from farmer field in all growing areas of Côte d'Ivoire. It concern nineteen districts divided in five areas (Bondoukou, Bouaké, Odienné, Korhogo and Séguéla) by the Cashew and Cotton Council (CCC). Samples of leaves, inflorescences, fruits (nuts and apples) and twigs with typical symptoms were collected for pathogens isolation as describe by Afouda *et al.*[11].

**Pathogens isolation:** Isolation of pathogens from samples was carried out on Potato Dextrose Agar (PDA) medium. Sections of 4-6 mm diameter were cut from the periphery of the symptoms using sterilized scalpel. Explants were sterilized firstly in ethanol (70%) for one minute followed by immersion in 3% of sodium hypochlorite solution for 3 min. The infected section was then washed three times in Sterile Distilled Water (SDW). Sterilized explants were dried on sterile blotter paper and grown on PDA medium. Cultures were incubated at 25±2°C. After 48-72 h, colony growth was hyphal-tip transferred on a new PDA medium to obtain pure cultures. Colony morphology was described after 7 days. Conidial sizes were recorded through their length and Width. Identification of pathogens were done by microscopic examination and compared with an identification key[21].

**Pathogenicity test:** Cashew nuts were sown in plastic plots containing a sterilized soil and put under a green house at 12 h of photoperiod. The plots were watered every 2 days. After 28 days, seedlings were inoculated with a suspension of conidia when they reached 6-8 leaves stage. Inoculum was prepared from 30 days old cultures by flooding plates with 10 mL sterile distilled water. Conidia were collected with micropipette and counted in a Malassez haemacytometer. Conidia concentration was adjusted to $10^6$ $mL^{-1}$ conidia per ml and one ml of an agar agar and glucose solution (1%) was added to it. The conidia suspension (12.5 mL per plant) was applied on leaves inferior surface with a hand-held garden sprayer. Control plants were sprayed with agar agar and glucose solution. Before and after inoculation, plants

Table 1: Pathogens associated with cashew plant parts

| | Associated microorganisms | | | |
|---|---|---|---|---|
| Plant parts | *Colletotrichum* sp. | *Pestalotia* sp. | *Lasiodiplodia* sp. | Indeterminated |
| Leaves | + | + | - | + |
| Inflorescences | + | - | - | - |
| Twigs | - | + | + | - |
| Fruits | + | - | - | - |
| Shoot tip | + | - | - | + |

+: Present, -: Absent

were covered with a clear plastic to maintain high humidity (96-100%) and plants were regularly sprayed with water. Eight isolates of *Colletotrichum gloeosporioides* and five isolates of *Pestalotia heterocornis* were used. Ten plants are been inoculated for each isolate.

**Statistical analysis:** The experimental design was completely randomized with three replications for each treatment. Data collected were analyzed using analysis of variance (ANOVA 1) one way. $p \leq 0.05$ and averages were compared through Newman-Keuls multiple range test with statistica software 7.1 version StatSoft[22].

## RESULTS AND DISCUSSION

Isolates of pathogens and their pathogenicity: Fungi associated with cashew leaves, inflorescences, twigs and fruits diseases are *Colletotrichum gloeosporiodes*, *Pestalotia heterocornis*, *Lasiodiplodia theobromae* and those undeterminated. This study was limited to diseases that affect different parts of cashew. *Colletotrichum* species were isolated from leaves, inflorescences and fruits (nuts and apples). *Pestalotia* was isolated from infected twigs and leaves, while *Lasiodiplodia* was only associated with twigs of cashew (Table 1).

This study showed that all parts of the cashew plant are susceptible to pathogens attack. *Colletotrichum* is the most serious pathogen affecting many parts of the cashew plants, a part from twigs. *Pestalotia* species were isolated from leaves and twigs while *Lasiodiplodia* species were only found on twigs. *Colletotrichum* species are considered as one of the most economically important plant pathogens affecting a wide range of plant species such as banana, ginger, soybean, longan, mango[23]. The results of this study are in agreement with those of some authors who have isolated *Colletotrichum* species from different organs of cashew such as leaf, pseudo-fruit and nut[13,14,24,25]. *Lasiodiplodia theobromae* responsible of gummosis in Côte d'Ivoire is the causal agent of twig dieback and inflorescence blight of cashew in Nigeria[2,26].

A summary of the key disease symptoms and morphological structures of the pathogens associated is presented in Fig. 1.

Blackish necrotic lesions observed on apples and nuts (Fig. 1a) were the effect of *Colletotrichum gloeosporiodes*. *Colletotrichum gloeosporioides* was also isolated from necrotic, reddish brown areas on leaves of adult trees or seedlings (Fig. 1b). Inflorescence drying up (Fig. 1d) was also attributed to *Colletotrichum gloeosporioides*. *Colletotrichum gloeosporioides* and *Pestalotia heterocornis* were frequently isolated from leaves tissue exhibiting the same symptoms as shown on Fig. 1c and g. Nevertheless, *Pestalotia heterosornis* necrotic black spots were relatively small than those of *Colletotrichum gloeosporioides* on leaves. A complex consisting of *Lasiodiplodia theobromae* in association with *Pestalotia heterocornis* was isolated from crack blackened lesions on twig (Fig. 1j).

Conidial size recorded showed significant differences $p \leq 0.05$ in length and width between the three genera pathogens isolated from cashew trees when compared by Newmann-Keuls test at 0.05 level. For these two measures, *Lasiodiplodia theobromae* recorded the higher value, respectively 20.13 and 11.16 µm for the length and width while *Colletotrichum gloeosporioides* showed the lowest size 13.94 and 4.81 µm, respectively for length and width.

Mycelia growth of *Colletotrichum*, *Lasiodiplodia* and *Pestalotia* species did not show statistical differences during the experimental conditions. However, *lasiodiplodia theobromae* presented the high value in terms of mycelial growth diameter with 3.9 mm difference. So, alternating photoperiod of 12/12 h light and darkness at (25±2°C) is suitable for maximum growth of these fungi species (Table 2).

In all the districts where cashew is grown in Côte d'Ivoire, *Colletotrichum gloeosporioides* is the most important fungi affecting cashew followed by *Pestalotia heterocornis*. *Lasiodiplodia theobromae* is the least important and has been observed in only one district which is Bouaké (Table 3).

The analysis of Fig. 2 showed that *Colletotrichum gloeosporioides* is the most important pathogen isolated

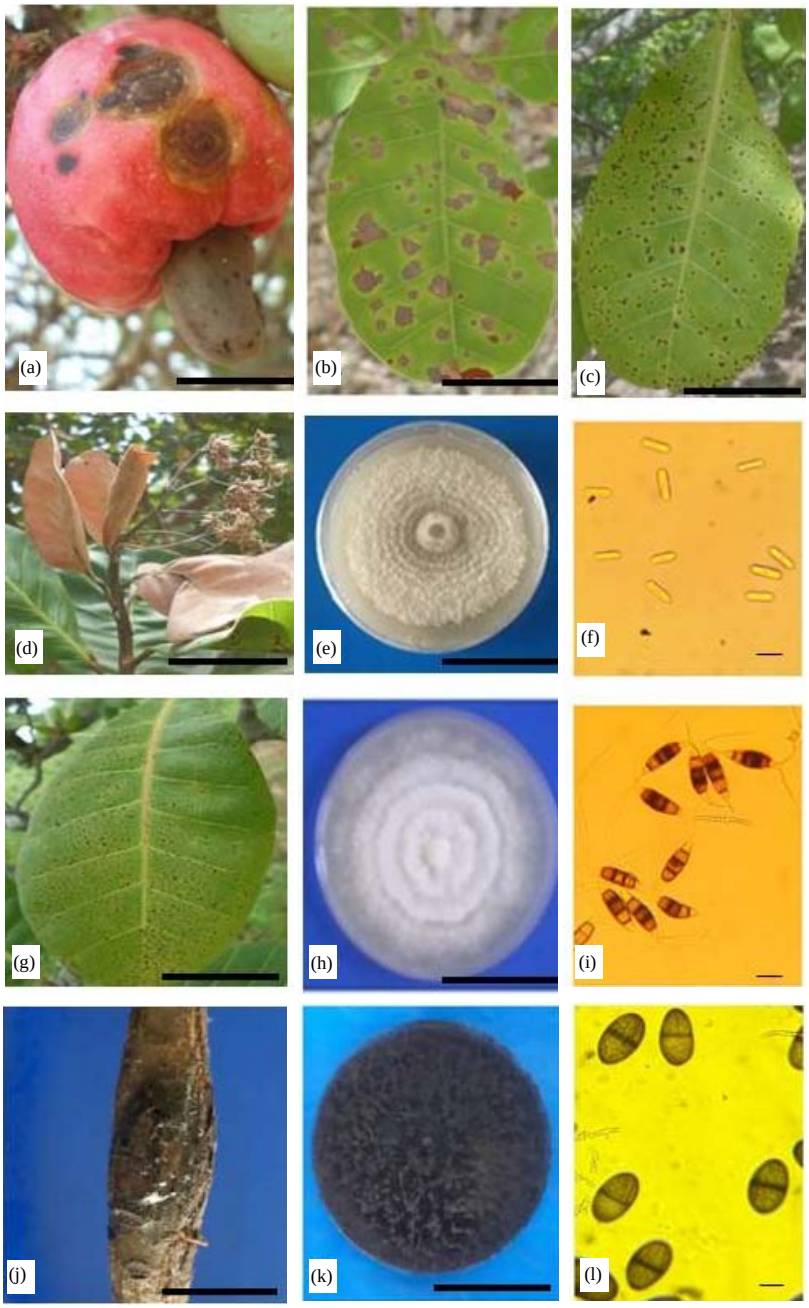


Fig. 1(a-l): Fungal structures and different field symptoms of commonly found fruit, leaf, inflorescence and twig diseases of cashew (*Anacardium occidentale*) in Côte d'Ivoire. (a-d) *Colletotrichum* sp. symptoms, (e) Mycelial, (f) Conidia, (g) Symptoms, (h) Mycelial, (j) Conidia of *Pestalotia* sp., *Lasiodiplodia* sp., symptoms, (k) Mycelial and (l) Conidia
GX400 =10 µm, = 5cm. Source: (Silué Nakpalo)

Table 2: Morphological characteristics of cashew fungi isolates

| | Conidia characteristics | | | |
|---|---|---|---|---|
| Fungi | Length (µm) | Width (µm) | Shape | Mean mycelial growth (mm) |
| *Colletotrichum gloeosporioides* | 13.94[c] | 4.81[c] | Cylindrical | 77.35[a] |
| *Pestalotia heterocornis* | 15.97[b] | 5.96[b] | Ellipsoid to fusoid | 81.10[a] |
| *Lasiodiplodia theobromae* | 20.13[a] | 11.16[a] | Cylindrical | 85.00[a] |

Means followed by the same letter in the same column are not significantly different according to Newman-Keuls test (5%)

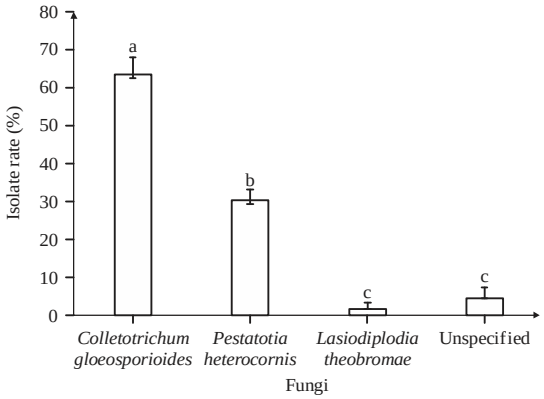


Fig. 2: Isolate mean rate of fungi genera met in cashew orchards of Côte d'Ivoire

Column diagrams with the same letter are not significantly different using Newman-Keuls test. Vertical bars represent the Standard Error (SE)


Fig. 3(a-b): Comparison of inoculated and non-inoculated cashew seedlings. (a) Necrotic black spot of *Colletotrichum* sp. on leaves inoculated and (b) Control plant leaves with any symptom

Source: (Silué Nakpalo)

with 63.45% mean rate from samples collected in farmer's field. It's followed by *Pestalotia heterocornis* (30.35%), *Lasiodiplodia theobromae* (1.66%) and the indeterminated (4.52%). As a function of isolate rate, statistical analysis showed three groups. *Colletotrichum gloeosporioides* form the first group which isolate mean rate is significantly higher ($p<0.05$) than the others according to Newman-Keuls test with a difference of 33.10% compared to *pestalotia*. *Pestalotia heterocornis* constitute the second group and is halfway between *Colletotrichum gloeosporioides* and the third group form by *Lasiodiplodia theobromae* and the indeterminated. *Pestalotia heterocornis* isolation rate were 25.83% higher than those of the third group. Isolation mean rate of *Lasiodiplodia theobromae* fungi are lowest than previous as shown by Freire *et al.*[13]. The results of this study are in conformity with those reported by Wonni *et al.*[16]. The researchers showed that anthracnose caused by *Colletotrichum gloeosporioides* is the most important disease in cashew orchards of Burkina Faso. On the contrary, while gummosis (*Lasiodiplodia theobromae*) seems to be less important according to this study, it is the main pathogen responsible of inflorescences and shoot dieback in Nigeria[27].

**Pathogenicity tests:** After 3 weeks, cashew seedlings inoculated with $1\times10^6$ conidial suspension of selected isolates developed small black spot on seedlings leaves. 2-3 months later, symptoms are well view with a yellowish area around the necrotic black spot. All the isolates were pathogenic on cashew seedlings. The control did not develop any symptom during probation period (Fig. 3). The pathogen was consistently re-isolated from infected cashew leaves on PDA to fulfil Koch's postulates.

Table 3: Mean rate (%) of pathogens isolate associated with cashew diseases in different district of Côte d'Ivoire

| | Fungi species | | | |
|---|---|---|---|---|
| Growing area | *Colletotrichum gloeosporioides* | *Pestalotia heterocornis* | *Lasiodiplodia theobromae* | Indeterminated |
| Bondoukou | 62.8 | 29.20 | - | 8.30 |
| Bouaké | 58.33 | 34.65 | 2.33 | 4.68 |
| Korhogo | 64.28 | 35.71 | - | - |
| Odienné | 75.00 | 25.00 | - | - |
| Séguéla | 71.42 | 28.57 | - | - |

-: Non isolated

## CONCLUSION

This study revealed that cashew fields in Côte d'Ivoire are susceptible to diseases occurring on various plant organs mainly leave, nut and inflorescence. Among these pathogens, *Colletotrichum* genera seem to be the most important fungus in Côte d'Ivoire cashew orchards. These findings create bases for the morphological and molecular characterization of these pathogens for better understanding of their biology, identification and classification in order to integrate efficient diseases management approaches.

## SIGNIFICANCE STATEMENT

This is the first study which was undertaken in cashew orchard of Côte d'Ivoire in order to determine pathogens associated with cashew diseases. The results of this study create base for *in vitro* screening of fungicides to efficient control of diseases.

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