Pathogenic susceptibility of cashew (*Anacardium occidentale* L.) to twelve isolates of *Colletotrichum* sp. present on six weeds in cashew orchards in Côte d'Ivoire

Abstract

Cashew nuts are one of the agricultural products that contribute significantly to Côte d'Ivoire's economic development. However, cashew nut production in Côte d'Ivoire is threatened by anthracnose. Reducing anthracnose proliferation factors could reduce its impact on cashew trees. The aim of the study was to assess the virulence of *Colletotrichum* sp. isolated from weeds in cashew trees. It ~~consisted in assessing~~ assessed the pathogenicity ~~on cashew plants~~ of 12 isolates of *Colletotrichum* sp. ~~present on~~ extracted from six weeds on cashew plants ~~in the cashew orchard~~. The inocula were prepared by scraping the mycelium of each fungus in 10 ml of sterile distilled water. The spore suspension was collected and calibrated at 4.6.106 conidia/ml using a Malassez cell. This suspension was applied by spraying to the leaves of 30-day-old cashew plants. The parameters assessed were symptom incidence and severity index. ~~Data were analysed using R 4.4.0 software.~~ All isolates tested induced symptoms characteristic of anthracnose on cashew plants. There ~~results showed~~ a significant difference between isolates in terms of their virulence. The highest incidence of 90% was obtained on plants inoculated with the ColE2 isolate and the lowest incidence of 30% was obtained on plants inoculated with the ColN3 isolate. The highest severity index of 7.66 was obtained on plants inoculated with the ColE3 isolate and the lowest severity index of 2.00 was obtained on plants inoculated with the ColN3 isolate. These results show that *D. oliveri*, *V. paradoxa*, *M. pruriens*, *B. sapida*, *A. zygia* and *P. erinaceus* are hosts of anthracnose in cashew orchards in Côte d'Ivoire. Their integration into cashew pathogen control strategies is necessary.

Key words: inoculation, anthracnose, incidence, pathogenicity and Côte d'Ivoire

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1. INTRODUCTION

The cashew tree (*Anacardium occidentale* L.) is a perennial plant cultivated mainly for its nuts and cashew apples. Belonging to the Anacardiaceae family, the cashew tree has a high commercial value due to the food and industrial value of the cashew kernel. ~~In fact, it~~ It is the only species of the *Anacardium* genus that is of economic interest because of its ability to produce nutritious apples and nuts [1]. As with other crops, abiotic and biotic factors constitute constraints to cashew cultivation. Among the biotic constraints, anthracnose is the major fungal disease of this crop in several producing countries [2, 3, 4]. This disease has a significant impact on the production potential of cashew trees. Anthracnose attacks leaves, inflorescences, nuts, and cashew apples, resulting in huge yield losses of more than 50% [5]. This disease in cashew is caused by the *Colletotrichum gloeosporioides* species complex including *Colletotrichum chrysophilum*, *Colletotrichum fragariae*, *Colletotrichum fructicola*, *Colletotrichum gloeosporioides* sensu stricto, *Colletotrichum queenslandicum*, *Colletotrichum siamense* and *Colletotrichum* tropicale [6]. In Côte d'Ivoire, anthracnose is present on cashew trees in all orchards, with varying degrees of severity from one site to another [7, 8]. It manifests itself through typical symptoms such as patches of necrosis in the form of leaf scorch, scattered black dots on leaf blades, black spots on inflorescences and necrosis on cashew apples [9, 10, 8]. In addition to cashew, the *C. gloeosporioides* species complex attacks other fruit plants [11]. The presence of alternative *Colletotrichum* host plants in a field increases the risk of anthracnose spreading to cultivated plants [12]. The work of [13] showed the presence of *Colletotrichum* fungi on weeds in the Ivorian cashew orchard. However, the pathogenic nature of these fungi on cashew remains to be demonstrated. The aim of this study was to assess the virulence of *Colletotrichum* sp. isolated from weeds in cashew crops. Specifically, the aim was to determine the latency period for the effect of the isolates on cashew plants, and then to assess their incidence and the severity of the symptoms induced in a controlled environment.

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2. MATERIALS AND METHODS

2.1. STUDY AREA

The study was carried out at the Agricultural Production Improvement Laboratory of the Jean Lorougnon Guédé University. This university is located in the town of Daloa in central-western Côte d'Ivoire. The town of Daloa is located at latitude 6°53 north and longitude 6°27 west. Daloa is 139.1 km from

Yamoussoukro, the political capital of Côte d'Ivoire, via the A6 motorway. It is the capital of the Haut-Sassandra Region, with a surface area of 15,200 km^2 ~~and an estimated population of more than 1,396977~~ [14]. The climate is Sudano-Guinean, with four seasons. The main rainy season lasts from April to mid-July, while the short dry season lasts from mid-July to mid-September. ~~The climate is Sudano-Guinean, with four seasons. The main rainy season runs from April to mid-July, while the short dry season lasts from mid-July to mid-September.~~ The short rainy season runs from mid-September to mid-November and the long dry season from December to March. The dry and wet seasons alternate, with temperatures ranging from 24.65°C to 27.75°C on average. Daloa's soil substrate is made up of old Precambrian granite. The region's soils are predominantly ferralitic. They are generally very deep, with high levels of organic matter. They have good agricultural potential for all types of crop [15].

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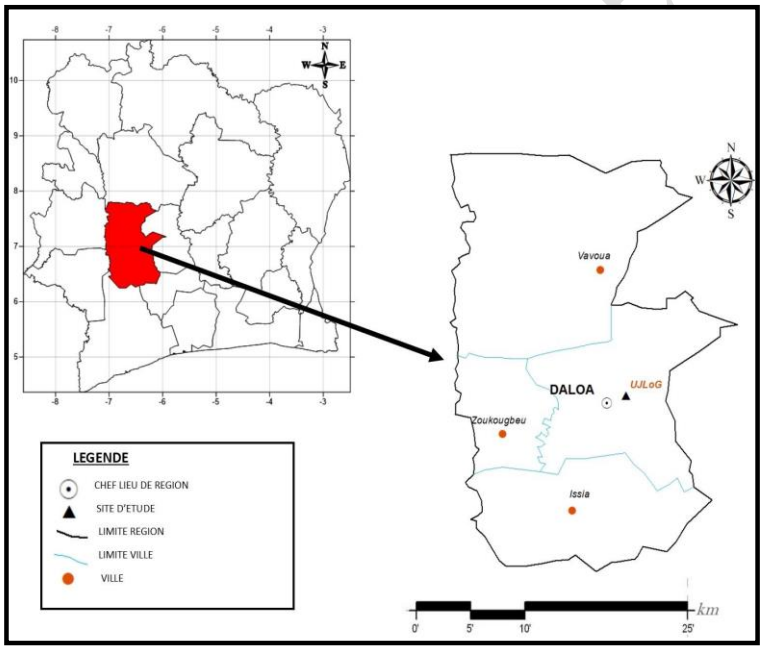

Figure 1: Map of the study area (Kouadio, 2015 [16]).

2.2. MATERIAL

This work required the use of a set of materials comprising plant material,

technical material and fungal material. The plant material consisted of 30-day-old cashew seedlings. These plants were produced on sterile substrate in nursery bags and pots. The substrate consisted of 50% soil and 50% wood soot. The substrate was steam sterilised for 60 minutes in a hermetically sealed metal bucket. The equipment used included an autoclave, a laminar flow hood, a Malassez cell, an electronic balance and an electron microscope. PDA (Potato Dextrose Agar) culture medium was used to grow the fungi. The fungal material consisted of 12 isolates of *Colletotrichum* sp. present on weeds in the cashew orchard (Table I).

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Table I: Fungal isolates used for pathogenicity testing on cashew seedlings

Fungal genus	Code	Host weed	Agro-ecological zone
Colletotrichum	ColN1	*Daniellia oliveri*	North
	ColN2	*Pterocarpus erinaceus*	North
	ColN3	*Vitellaria paradoxa*	Nord
	ColC1	*Daniellia oliveri*	Centre
	ColC2	*Blighia sapida*	Centre
	ColC3	*Vitellaria paradoxa*	Centre
	ColE1	*Daniellia oliveri*	East
	ColE2	*Vitellaria paradoxa*	East
	ColE3	*Albizia zygia*	East
	ColCO1	*Blighia sapida*	Central West
	ColCo2	*Pterocarpus erinaceus*	Central West
	ColCo3	*Micuna pruriens*	Central West

2.3. METHODS

2.3.1. Study design

The ~~system used was~~ study was conducted in a completely randomised block with 13 treatments and 3 replicates. Each *Colletotrichum* sp. isolate corresponded to a treatment that was applied to 05 cashew plants for one replication. Control plants were treated with tap water.

2.3.2. Preparation of fungal inocula

The inoculum was prepared according to the method of Silué et al. [17], which was

modified and adapted to the conditions of our study. The pure fungal cultures used for inoculum production were aged for 15 days on a PDA medium. Preparation consisted of scraping the mycelium from each mushroom in 10 ml of sterile distilled water using a sterile scalpel. The spore suspension was collected using a sterile 20 ml syringe and filtered using filter paper to remove any residual mycelium. The concentration of conidia was calibrated at 4.6.106 conidia/ml using the Malassez cell. A drop of tween 20 was added to the spore suspension to facilitate its adhesion to the inoculated leaves.

2.3.3. Application of inoculum to cashew seedlings

The inoculum was applied to 30-day-old cashew plants. One day before inoculation, the plants were covered with transparent plastic to maintain humidity. The virulence of each fungus was tested on 05 healthy cashew seedlings. The inoculum was applied to the leaves of each cashew plant. After inoculation, the plants were covered with transparent plastic, depending on the isolate, for 48 hours to maintain humidity (95-100%). The plants were regularly sprayed with tap water. The experiment was repeated three times.

2.3.4. Evaluation of parameters

2.3.4.1. Incidence of attack

Young cashew plants showing symptoms of disease after contact were counted manually. The incidence was calculated using the following formula suggested by Kranz [18]:

$$I = (NPm / NP) \times 100$$

~~With :~~

Where I: Incidence of attacking plants–; **NPm:** Number of diseased plants; **NP:** Total number of plants.

2.3.4.2. Severity of attack

The severity of the disease on inoculated plants was determined using a visual rating scale from 0 to 9 [19, ~~[~~20].

Table II: Anthracnose severity rating scale ~~according [19, 20]~~.

Grade	Surface area of infected	Interpretation
0	0 %	no symptoms
1	1-5 %	Moyenne
3	6-10 %	Medium

5	11-25 %	mildly severe
7	26-50 %	Severe
9	>50 %	Very severe

The severity index was calculated using the following formula given by Abu et al.~~from~~ [21] :

$$Is = \sum (Xi*ni) / (N*Z)$$

~~With :~~

Where Is : Severity index ; **Xi** : Score assigned to the diseased plant ; **ni** : Number of diseased plants with the same score **xi** ; **N** : Total number of plants and **Z** : the highest score.

2.3.4.3. Statistical analysis of the data

The data were ~~entered using Excel,~~analysed using the software (R 4.4.0) ~~was used for statistical analysis of the data~~. Following tests for normality and homogeneity of variance, the data were subjected to an analysis of variance. In the event of significant differences between the means, the Tu~~r~~key's test was used to compare the means.

3. Results and discussion

3.1. Results

3.1.1. Symptoms induced by isolates on young cashew trees

The *Colletotrichum* isolates identified on the weeds induced two types of symptoms on the inoculated young cashew seedlings. These included black dots scattered across the leaf blades and patches of greyish necrosis in the form of burning on the leaves. However, no disease symptoms were observed ~~on~~ in the control plants (Figure 2). Microbiological analysis of the infected leaves taken from the inoculated cashew trees enabled the initial fungi to be re-isolated.

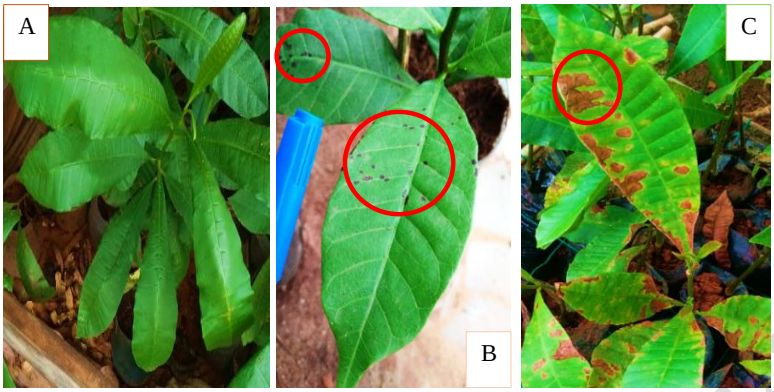

Figure 2: Symptoms induced on inoculated cashew plants

(A): Control cashew plants; (B): Black dotted lines on inoculated cashew leaves and (C): Patches of necrosis on inoculated cashew leaves.

3.1.2. Incidence of symptoms

Figure 3 shows the results relating to the incidence of symptoms induced by *Colletotrichum* sp. isolates on inoculated young cashew plants. The results showed that all inoculated cashew seedlings showed disease symptoms, in contrast to non-inoculated seedlings. Analysis of variance revealed a highly significant variation in incidence depending on the origin of the fungal isolates ($P < 0.001$). The highest incidence (90%) was obtained on plants inoculated with the ColE2 isolate from the east. The lowest incidence (30%) was obtained on plants inoculated with the ColN3 isolate from the north.

The results also showed a highly significant variation in the incidence of symptoms induced on young cashew seedlings by isolates from the same agroecological zone ($P < 0.001$). For isolates from the Centre agro-ecological zone, the highest incidence (73.33%) was obtained on plants inoculated with the ColC1 isolate. The lowest incidence (50%) was found in plants inoculated with the ColC3 isolate. For isolates from the Centre-West agro-ecological zone, the highest incidence (43.33%) was obtained in plants inoculated with the ColCO1 isolate and the lowest incidence (36.66%) was obtained in plants

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inoculated with the ColCO2 isolate. For isolates from the eastern agro-ecological zone, the highest incidence (90%) was obtained on plants inoculated with the ColE2 isolate. The lowest incidence (56.66%) was obtained in plants inoculated with the ColE1 isolate. For isolates from the Northern agro-ecological zone, the highest incidence (66.66%) was obtained on plants inoculated with the ColN2 isolate. The lowest incidence (30%) was obtained in plants inoculated with the ColN3 isolate.

3.1.3. Symptom severity index

Figure 4 shows the results of the evaluation of the symptom severity index induced by *Colletotrichum* sp. isolates on young cashew seedlings. The results showed that the incidence was high in the inoculated plants. Analysis of variance showed highly significant variation in symptom incidence according to the origin of the isolates ($P < 0.001$). In fact, the highest severity index of 7.66 was obtained in plants inoculated with the ColN3 isolate from the North. The lowest severity index was 2.00 for plants inoculated with the ColE3 isolate from the east.

The results also showed a highly significant variation in the severity index between isolates from the same agro-ecological zone ($P < 0.001$). In the Centre agro-ecological zone, the highest severity index (6.66) was obtained on plants inoculated with the ColC1 isolate. The lowest severity index (5) was obtained on plants inoculated with the ColC2 isolate. In the Centre-Ouest agro-ecological zone, the lowest severity index (3.66) was obtained on plants inoculated with the ColCO1 isolate. ~~And the~~The lowest severity index (2.66) was obtained on plants inoculated with the ColCO3 isolate. In the eastern agro-ecological zone, the highest severity index (7.66) was obtained on plants inoculated with the ColE3 isolate and the lowest severity index (4.00) was obtained on plants inoculated with the ColE1 isolate. In the northern agro-ecological zone, the highest severity index (6.66) was obtained on plants inoculated with the ColN2 isolate and the lowest severity index (2.00) was obtained on plants inoculated with the ColN3 isolate.

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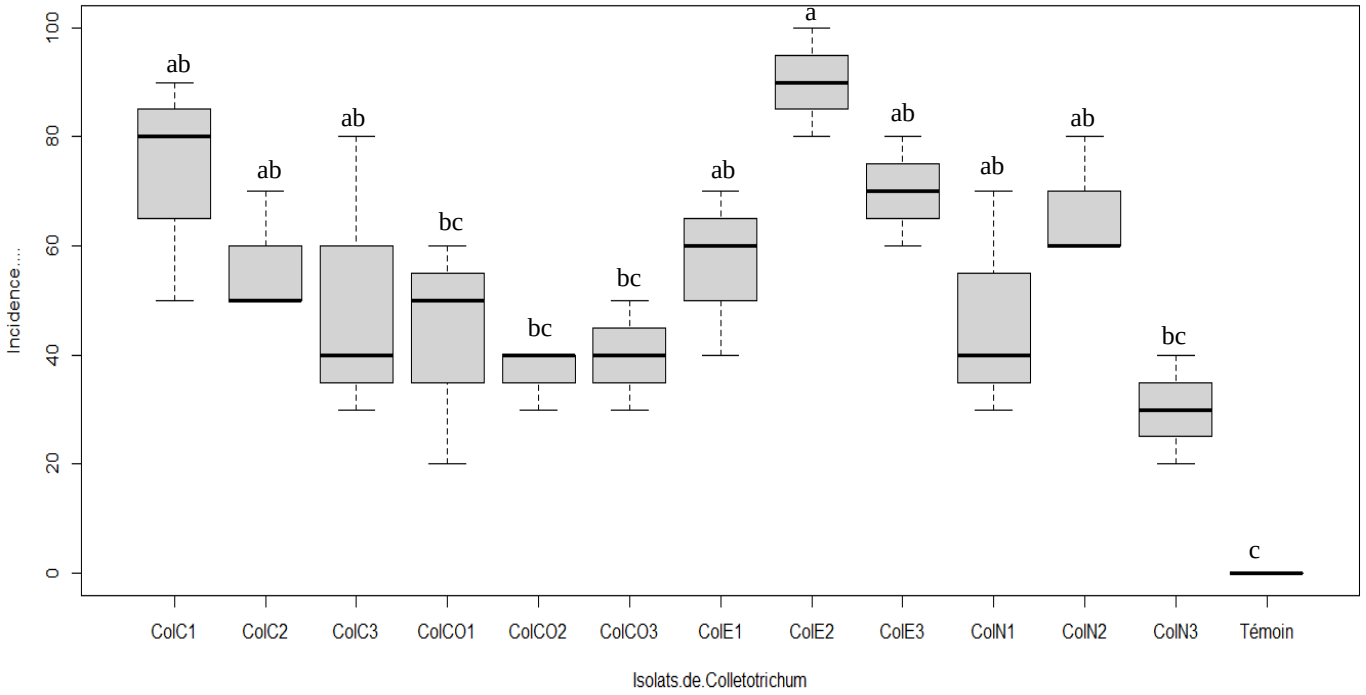

Figure 3 : Incidence of symptoms induced on young cashew plants

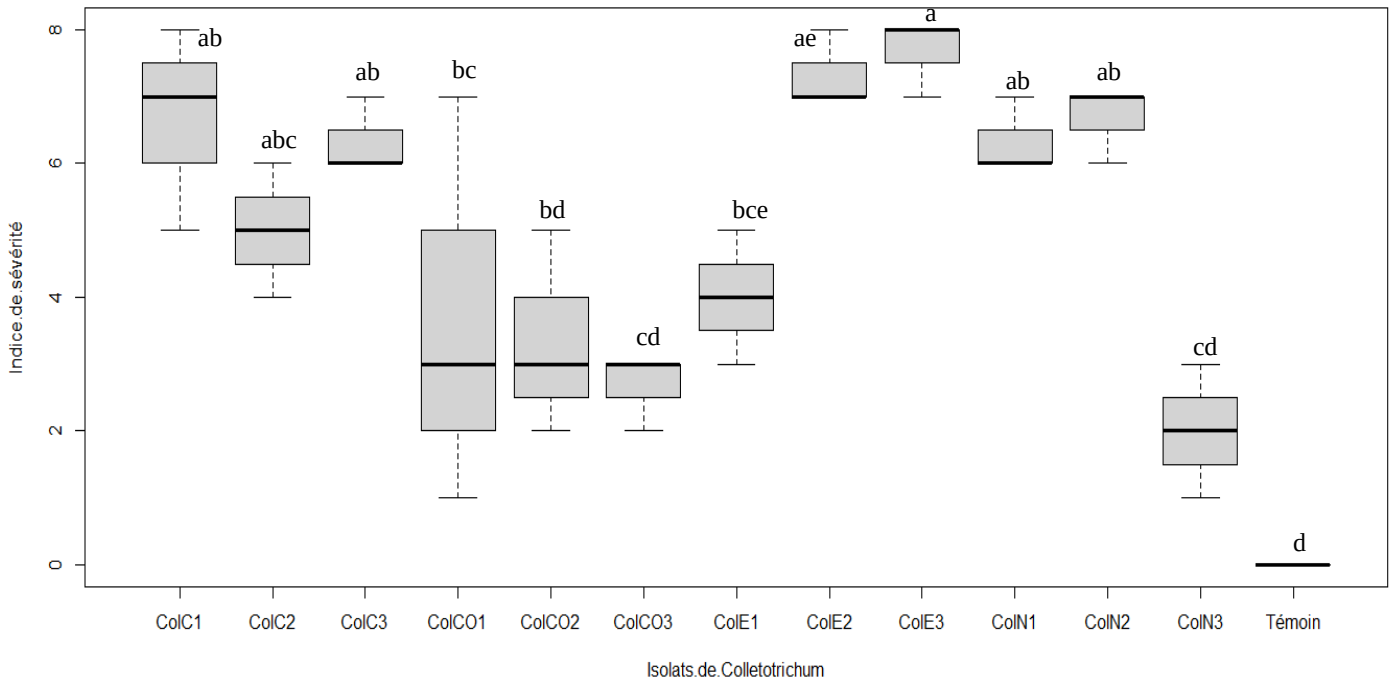

Figure 4 : Symptom severity index on young cashew plants

3.2. Discussion

The results showed that all isolates induced symptoms characteristic of anthracnose ~~on~~ in young cashew seedlings. This means that the isolates tested are pathogenic to cashew. Consequently, the weed hosts of these fungi would be alternative hosts of anthracnose in the cashew orchard. The symptoms identified on the weeds were black or grey dots scattered across the leaf blades and patches of reddish necrosis developing in the form of scorch marks on the weed leaves. These symptoms are similar to the anthracnose symptoms described on cashew in several previous studies [22, 10, 8]). These authors reported that *Colletotrichum gloeosporioides* is responsible for cashew anthracnose in Benin and Côte d'Ivoire. The twelve isolates tested belonged to the *gloeosporioides* complex. These results corroborate those of Muntala et al. [3], who reported ~~the presence of~~ anthracnose symptoms on young cashew seedlings inoculated with a sporal suspension of *Colletotrichum gloeosporioides*. A difference in virulence was noted between the isolates. The ColE2 and ColE3 isolates present on *V. paradoxa* and *A. zygia* respectively in the eastern agro-ecological zone and the ColN1 isolate on *D. oliveri* in the northern agro-ecological zone were more virulent and aggressive than the others. The difference in aggressiveness observed could be explained by the fact that the isolates come from different species and belong to different agroecological zones. These results are similar to those of Kouamé et al. [23], who reported a difference in virulence between two isolates of *Colletotrichum gloeosporioides* inoculated into mangoes belonging to the keitt variety. These results could also be linked to the phenotypic characteristics of the isolates, which can vary according to the environmental conditions of the medium or the physicochemical composition of the host. This result is in agreement with those of Photita et al. [24] and Martínez et al. ~~[24,~~ [25] who reported that a variation in the colour of the mycelium from white to grey or from pink to orange is possible and ~~that~~ these variations are due to the host plant, the nature of the strain and the environmental conditions. The fungi re-isolated from infected cashew leaves are identical to the original isolates. Koch's postulate has therefore been verified. The fungi found to be pathogenic to cashew trees in this study came from *D. oliveri*, *V. paradoxa*, *M. pruriens*, *B. sapida*, *A. zygia*, and *P. erinaceus*. These results attest to the presence of weed hosts of anthracnose in the cashew orchard. The presence of these weeds in the orchard would not be a problem if they were fully taken into account in anthracnose control strategies.

But some of them, notably *V. paradoxa* and *B. sapida*, are deliberately kept in the orchards for their fruit, which is used for human consumption. Others are kept for their therapeutic properties [26]. These plants thus escape cleaning operations and are not taken into account in fungicide treatments of orchards. The ability of fungi from these weeds to induce anthracnose in cashew trees remains a concern. The work of Aboulaye et al. [13] showed that *D. oliveri*, *V. paradoxa*, *M. pruriens*, *B. sapida*, *A. zygia* and *P. erinaceus* have high infection rates in ivorian cashew orchards. In addition to cashew, *Colletotrichum gloeosporioides* attacks other plants such as mango and avocado [26, 27]. The ability of weeds to harbour fungal crop pathogens has been demonstrated in previous studies [28, 29, 30, 31, 32]. The work of Alvarado-Huaman et al. [31] showed that *Cercospora coffeicola* isolated from the weeds *Cyathula achyranthoides* (Kunth) and *Chromoleana laevigata* is responsible for iron spot of coffee. The same authors reported that *Colletotrichum* sp. isolated from *Anthurium croatii* is responsible for the anthracnose of coffee.

4. Conclusion

The ~~study showed that~~ isolates of *Colletotrichum* sp. present on weeds in the cashew orchard in Côte d'Ivoire are pathogenic to cashew. All twelve isolates tested induced symptoms in inoculated plants. The symptoms induced on young inoculated plants were typical of anthracnose. The weeds,~~The results show that~~ *D. oliveri*, *V. paradoxa*, *M. pruriens*, *B. sapida*, *A. zygia*, and *P. erinaceus* are alternative hosts of anthracnose in cashew orchards in Côte d'Ivoire. ~~These weeds are found throughout the ivorian cashew basin.~~ Their presence in the orchard certainly increases the risk of spreading anthracnose in the cashew orchard. However, some of them are permanently maintained in the orchard for their economic, nutritional, and therapeutic role. These trees should be regularly monitored in the same way as the cashew tree in order to reduce the risk of the disease spreading. For ~~the~~ others, choosing a good cleaning period could help to control them better.

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