Scientific Research Publishing

American Journal of Plant Sciences, 2023, 14, 448-463
https://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

Fungal Flora on Weeds in the Cashew (*Anacardium occidentale* L.) Orchard in Côte d'Ivoire

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How to cite this paper: Aboulaye, T., Sibirina, S., Emmanuella, A.A.R., Karidia, T.-O., Abo, K. and Daouda, K. (2023) Fungal Flora on Weeds in the Cashew (*Anacardium occidentale* L.) Orchard in Côte d'Ivoire. *American Journal of Plant Sciences*, **14**, 448-463.
https://doi.org/10.4236/ajps.2023.144029

Received: February 2, 2023
Accepted: April 14, 2023
Published: April 17, 2023

Abstract

Since 2015, Côte d'Ivoire has been the world's largest cashew producer. However, cashew orchards in Côte d'Ivoire are infected by fungal diseases that weaken production. And the contribution of weeds to the spread of these diseases is not yet understood. This study was initiated with the aim of establishing the role of weeds in the proliferation of pathogenic fungi in orchards. It consisted of a survey of weeds showing disease symptoms in cashew orchards in Côte d'Ivoire from February 2021 to July 2022. The itinerant method was used for the weed inventory. Symptomatic leaves were collected and sent to the laboratory for diagnosis on PDA (Potatoes Dextrose Agar) medium. In total, 50 species in 46 genera and 23 families were recorded. Laboratory diagnosis of the samples showed that 80% of the weeds identified harboured pathogenic fungi. The highest infection rates were obtained on *Danielia oliveri* R. (99.33% to 100%), *Vitellaria paradoxa* G. (100%), *Pterocarpus erinaceus* P. (83.91% to 99.33%), *Micuna pruriens* L. (98.33% to 100%) and *Isoberlinia doka* C. et S. (56.33% to 100%). The diagnosis revealed the presence of *Lasiodiplodia* sp, *Colletotrichum* sp, *Pestalotia* sp, *Alternaria* sp and *Curvularia* sp on weeds in the cashew orchard in Côte d'Ivoire.

Keywords

Weed, Infection Rate, Symptoms, Cashew, Côte d'Ivoire

1. Introduction

Cashew (*Anacardium occidentale* L.) is an Angiosperm in the class Dicotyledonous in the order Sapindaceae, which contains 73 genera and about 600 species [1]. Native to Brazil, the cashew is currently cultivated in more than 32 countries around the world. However, the vast majority of marketed production is concentrated in four major areas, namely Southeast Asia, West Africa, East Africa and Brazil [2]. Africa produces about 40% of the total raw nuts in the world and 80% of the production is obtained in West Africa with over 2,901,825 ha of plantations [3] [4]. Côte d'Ivoire has been the world's leading producer and exporter of raw cashew nuts since 2015. Indeed, Ivorian cashew nut production has undergone a spectacular evolution. Production has risen from 235,000 tonnes in 2006 to 968,676 tonnes of raw cashew nuts in 2021, an increase of 14% compared to 2020 [5].

Cashew tree cultivation and exploitation contribute to the socio-economic development of several countries in the world [6] [7]. In rural areas, the cashew sector is a powerful lever in the fight against poverty and unemployment. This crop has become the main source of income for the population and facilitates the schooling of children in rural areas. In addition, cashew products are full of nutritional and therapeutic benefits. Cashew kernel consumption is an excellent way to reduce the risk of cardiovascular disease [8]. Cashew apples are very rich in vitamin C polyphenolic compounds [9] [10] [11] [12] and have a very diverse carotenoid profile [13]. It is also used for wine and vinegar production [14].

Unfortunately, the productivity of cashew orchards is compromised by numerous phytosanitary problems. In addition to pests, more than a dozen diseases have been described on cashew [15]. Among these diseases, anthracnose (*Colletotrichum gloeosporioïdes*), pestalotiose (*Pestalotia heterocornis*) and bud rot (*Lasiodiplodia theobromae*) are responsible for significant damage in the cashew orchard in Côte d'Ivoire [16]. He found that symptoms of these diseases are also present on weeds in the orchard. These weeds could be alternative hosts of fungal pathologies in the orchard. Despite the importance of cashew in the Ivorian economy, there is very little data on the presence of alternative weed hosts of cashew fungal diseases in the orchard. However, knowledge of these alternative host weeds can help to implement a sustainable control strategy against cashew fungal diseases. This study was initiated to establish the role of weeds in the spread of pathogenic fungi in cashew orchards. The aim was to characterize symptomatic weeds and then to identify the pathogenic fungi they harbour.

2. Material and Method

2.1. Study Areas

Figure 1 illustrates the different study zones, namely the Northern agro-ecological zone, the Central agro-ecological zone, the Eastern agro-ecological zone and the Central-Western agro-ecological zone.

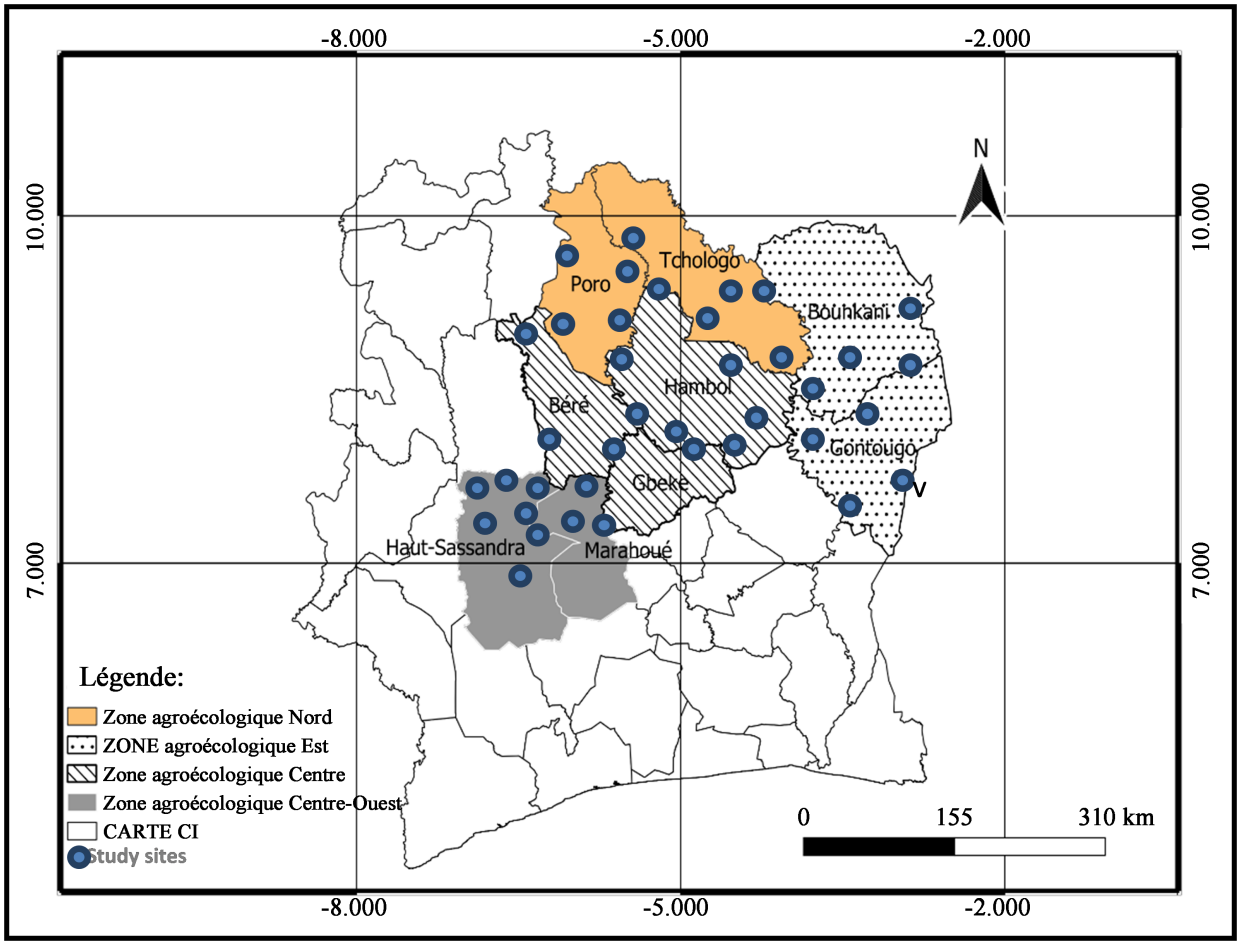

Figure 1. Study areas.

The Northern agro-ecological zone is characterized by a Sudanese climate with two seasons. The dry season runs from November to April and the rainy season from May to October. Average temperatures vary between 24˚C and 33˚C. The average annual rainfall is between 1100 and 1600 mm. The vegetation in this zone is savannah.

The eastern agro-ecological zone is characterised by a tropical climate with a very hot and dry period from November to February and a rainy period from March to October. The average annual temperature in this zone is 26.4˚C and rainfall averages 850.8 mm. The vegetation is essentially tree and shrub savannah with gallery forests.

The climate of the Central agro-ecological zone is of the Baulean type, characterised by a very hot and dry period from November to February and a rainy period from March to October. The average annual temperature varies between 26˚C and 34˚C. The average annual rainfall varies between 745.4 mm and 1580 mm. The vegetation is dominated by savannah trees.

The Centre-West agro-ecological zone is characterised by a mountain climate with four seasons. The long rainy season starts in April and ends in 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. Almost the entire basin is in the tropical rainforest zone with dense forest vegetation.

2.2. Material

The plant material used in this study consisted of leaves of weeds showing disease symptoms in the cashew orchard. The technical equipment consisted of a GPS, a camera, pruning shears and sterile bags. In the laboratory, PDA medium, an autoclave, a laminar flow hood and an electronic balance were used.

2.3. Methods

2.3.1. Collection of Samples

Surveys were conducted in orchards in four agro-ecological zones of the Ivorian cashew basin from February 2021 to July 2022. In each agro-ecological zone, ten orchards of one hectare in size were randomly selected. The itinerant method was used for the inventory of weeds showing disease symptoms in the orchard. The method consisted of walking the orchard in a diagonal direction. Weeds with disease symptoms found elsewhere in the orchard were added to the list. During this survey, the name and morphological type of weeds showing disease symptoms were determined. Attacked organs were collected with pruning shears which were immediately cleaned with 70% alcohol after sampling. The samples collected consisted mainly of leaves. These samples were stored in envelopes and coded and sent to the laboratory for diagnosis on PDA (Potatoes Dextrose Agar) medium.

2.3.2. Isolation and Purification of Fungi

In the laboratory, samples showing the characteristic symptoms of anthracnose, desiccation and pestalotiose were selected for diagnosis. Riviera's method has been modified and used [17]. Each sample was thoroughly washed with tap water and dried on blotting paper. After drying, the samples were cleaned with 70% alcohol. Then, 3 - 4 millimetre explants were taken from the growth front of the symptoms using a sterile scalpel. The sampling equipment was automatically cleaned with 70% alcohol after each sample. Explants from the same sample were then soaked in 5% sodium hypochlorite for 3 minutes before being rinsed three times in succession with sterile distilled water and dried on blotting paper in an aseptic environment. Seeding of the explants was done under a laminar flow hood near the flame of the benzene burner. It consisted of placing four explants of the same sample equidistantly in a Petri dish containing frozen PDA medium. The Petri dishes were sealed with para film, coded (reference and date) and then incubated at a temperature of 27˚C ± 2˚C until proliferation of the fungal colonies.

The purification of the fungal colonies was done under the same aseptic conditions as the inoculation. A fragment of the mycelium was removed from the outgrowth zone of the fungal colony and transplanted into the centre of a new

Petri dish containing frozen PDA medium. Pure fungal isolates were obtained from successive purifications [18]. The fungal isolates obtained from the diagnosis were identified according to their macroscopic characteristics on PDA medium and microscopic characteristics according to the identification key of [19].

2.3.3. Weeds Infection Rate

A weed is said to be infected if the diagnosis reveals the presence of at least one fungus on a sample from one of its organs showing disease symptoms. The infection rate was calculated according to the following formula:

$$\mathrm{Ti}(\%) = (\mathrm{NEp}/\mathrm{NtE}) * 100 \quad (1)$$

With:

Ti: Infection rate of a weed,

NEp: Number of samples testing positive and

NtE: Total number of weed samples showing disease symptoms.

2.3.4. Frequency of Fungi

Walder's formula was used to calculate the isolation frequency of fungi [20]:

$$\mathrm{Fi}(\%) = (\mathrm{Ni}/\mathrm{Nti}) * 100 \quad (2)$$

With:

Fi: Frequency of isolation in percentage.

Ni: Number of isolations of one fungal genus in all samples.

Nti: Total number of isolations of all fungal genera.

2.3.5. Data Processing

Microsoft Excel 2013 spreadsheet software was used for data entry and graph construction. Statistica version 7.1 was used for statistical analysis of the data. Normality was checked before the data were subjected to analysis of variance (ANOVA). When differences were significant at the 5% level, comparison of means by the Newman-Keuls test was performed.

QGIS software version 3.28.0 was used to produce the Study Area Map.

3. Results and Discussion

3.1. Results

3.1.1. Observed Symptoms

The study conducted on weed symptomatology in the cashew orchard in Côte d'Ivoire identified 50 species divided into 46 genera and 23 families. The most represented family was the Fabaceae. Weeds showing disease symptoms in the cashew orchard belonged to two classes, namely Dicotyledons (76%) and Monocotyledons (24%). The main symptoms identified were of four types. These included necrosis on the leaves, beach-like spots in the form of burning, desiccation and deposits of red or whitish powder on the leaves (**Figure 2**). Laboratory diagnosis of the samples showed that 80% of the weeds identified harboured cashew pathogenic fungi in the orchard in Côte d'Ivoire. The infected weeds were

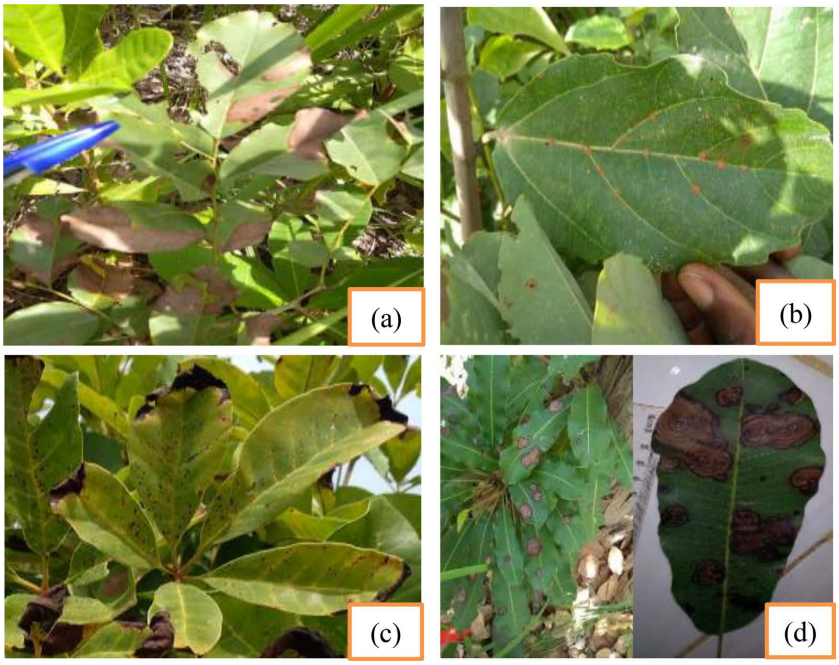

Figure 2. Symptoms observed on weeds in the cashew orchard. (a) Necrosis patch (Anthracnose) on *Pterocarpus erinaceus* Poir. (b) Rust on *Ficus sur* leaf on F. (c) Withering on leaves of *Vitex doniana* S. (d) Necrosis spots evolving in concentric circles on leaves of *Vitellaria paradoxa* G.

distributed among three morphological types, namely shrubs (65%), lianas (25%) and herbaceous plants (10%).

3.1.2. Weed Infection Rate in Cashew Orchard According to Agro-Ecological Zones

Figure 3 shows the infection rate of weeds showing disease symptoms in the cashew orchard in Côte d'Ivoire. The weed infection rate varies according to the agro-ecological zones. ANOVA tests showed that there was no significant difference ($F = 1.96$ and $P = 0.12$) between weed infection rates in the different agroecological zones surveyed. However, the highest infection rate (79.12%) was obtained in the Central agroecological zone. In contrast, the lowest weed infection rate was obtained in the Eastern agro-ecological zone. Intermediate infection rates of 63.51% and 60.15% were obtained in the North and Centre-West agroecological zones respectively.

3.1.3. Weed Infection Rate in the Agro-Ecological Zones According to Species

The infection rate within the agro-ecological zones varies according to the weeds identified. The ANOVA test performed at the 5% level showed that there was a highly significant difference ($F = 14.35$ and $P = 0.000$) between the weed infection rates within each agroecological zone.

The highest weed infection rates in the eastern agro-ecological zone were obtained on *Daniellia oliveri* R. (100%), *Micuna pruriens* L. (100%), *Vitellaria paradoxa* G. (100%) and *Albizia zygia* M. (99%). Intermediate infection rates were

obtained on *Bridelia ferruginea* B. (87.13%), *Terminalia schimperiana* H. (75.25%), *Diospiros mespiliformis* H. (45.33%) and *Calepogonium mucunoides* Desv. In contrast, *Centroseuma pubescens* B. and *Ficus sur* F. had the lowest infection rate (25.75%) in the eastern agro-ecological zone. The infection rate was zero for the weeds *Anchomanes diformis* Bl., *Stylochiton hypogaeus* Lepr. and *Synedrella nodiflora* L. (Figure 4).

Weed infection rates in the Central agro-ecological zone varied from 25.66%

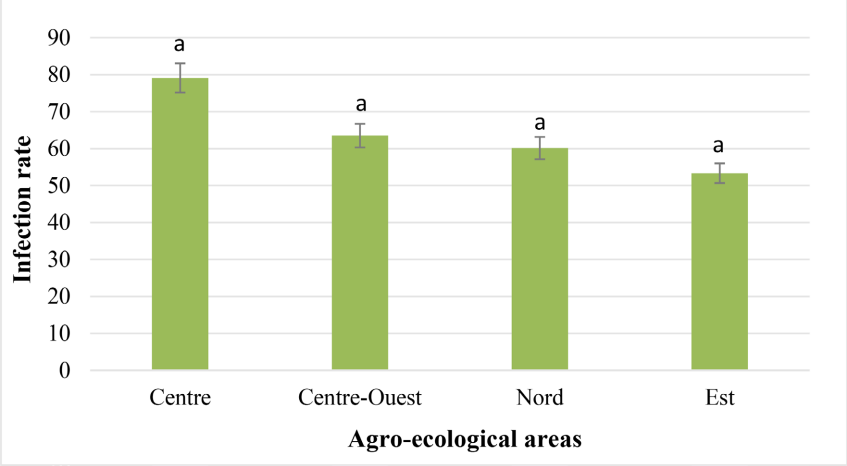

Figure 3. Weed infection rates according to agro-ecological zones. Bands with the same letter are not significantly different according to the Newman-Keuls test at the 5% threshold.

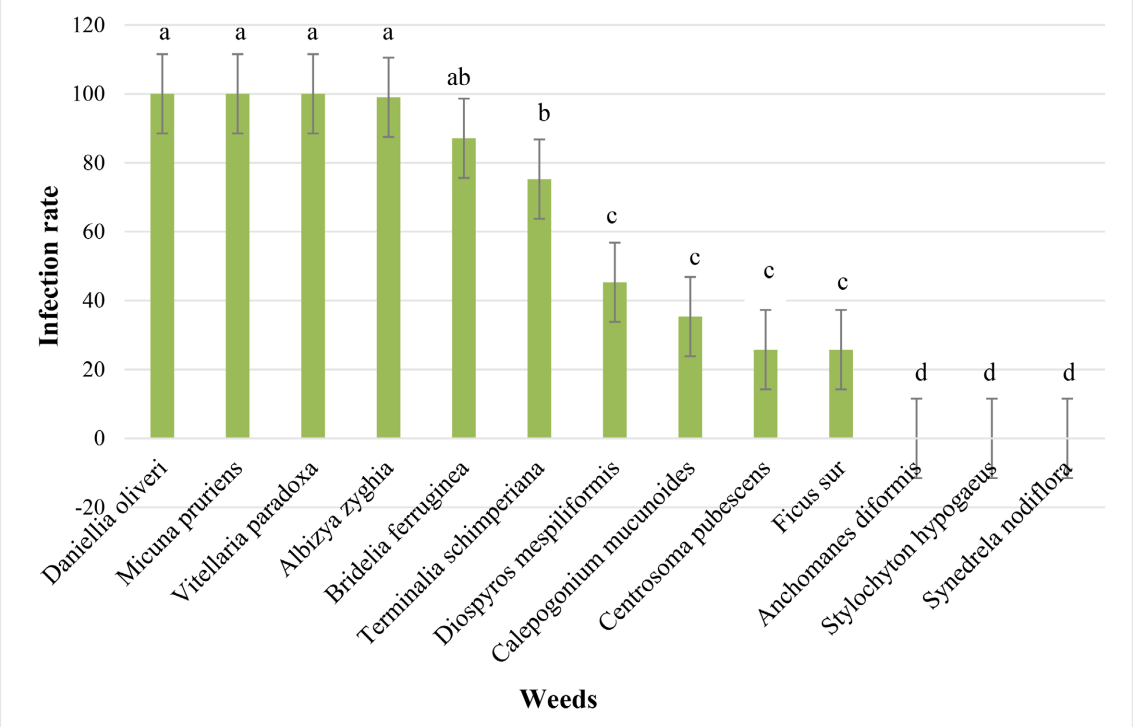

Figure 4. Weed infection rate according to species in the agro-ecological zone East. Bands with the same letter are not significantly different according to the Newman-Keuls test at the 5% threshold.

to 100%. The highest infection rates were obtained on *Daniellia oliveri* (100%), *Isoberlinia doka* C. et S. (100%), *Vitellaria paradoxa* G. (100%), *Pterocarpus erinaceus* P. (99.33%) and *Micuna pruriens* L. (99.26%). While *Calepogonium mucunoides* Desv. had the lowest infection rate (25.66%) in the Central agroecological zone. In this zone, no fungi were isolated from the weeds *Pseudocedrela kotchyi* Sch. and *Tacca leontopetaloides* L. (Figure 5).

The infection rate of the weeds in the northern agro-ecological zone varied from 25.75% to 100%. The highest infection rates were obtained on *Daniellia oliveri* R. (100%), *Vitellaria paradoxa* Gaertn. (100%), *Isoberlinia doka* Craib. et Stapf. (98.86%) and *Piliostigma thonningii* Schumach (87.22%). The species *Vitex doniana* S. had the lowest infection rate (25.75%) in the Northern agro-ecological zone. In this zone, intermediate infection rates were recorded for the species *Terminalia schimperiana* H. (78.66%), *Bridelia ferruginea* B. (77.54%), *Erythrina senegalensis* DC. (75.66%), *Nauclea latifolia* Smith. (70.60%) and *Saba senegalensis* A. DC. (67.66%). The infection rate was zero for the weeds *Anona senegalensis* Pers., *Hymenocardia acida* Tul. and *Setaria barbata* Lam (Figure 6).

In the Centre-Ouest agro-ecological zone, infection rates of weeds showing disease symptoms in orchards varied from 20.56% to 99.33%. The highest infection rates were obtained on *Daniellia oliveri* R. (99.33%), *Micuna pruriens* L. (95.33%) and *Vitex doniana* S. (95%). On the other hand, the species *Millettia zechiana* Harms. had the lowest infection rate (20.56%) in the Centre-Ouest agro-ecological zone. The infection rate was zero for the weeds *Calepogonium*

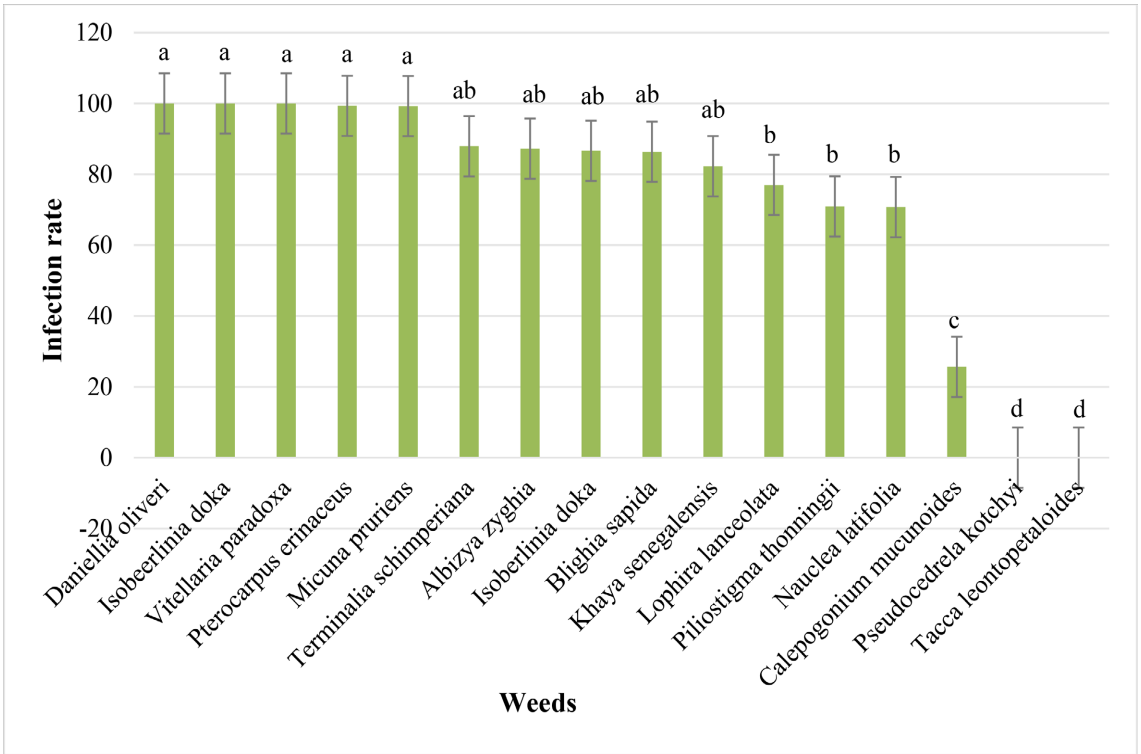

Figure 5. Weed infection rate according to species in the Centre agro-ecological zone. Bands with the same letter are not significantly different according to the Newman-Keuls test at the 5% threshold.

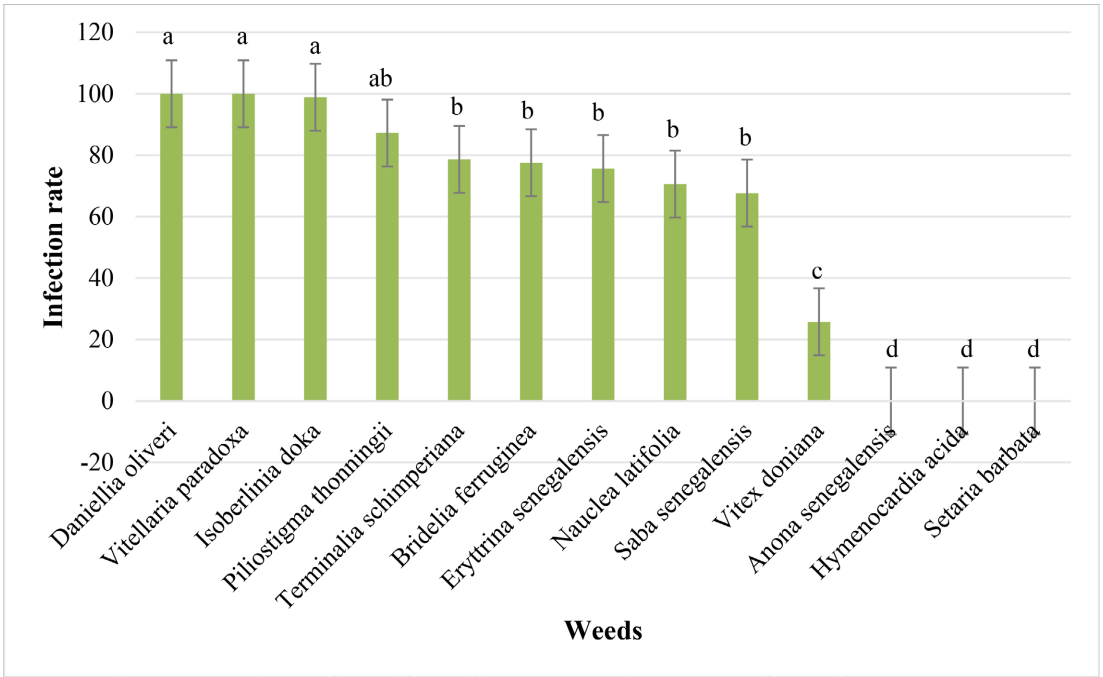

Figure 6. Weed infection rate according to species in the Northern agro-ecological zone. Bands with the same letter are not significantly different according to the Newman-Keuls test at the 5% threshold.

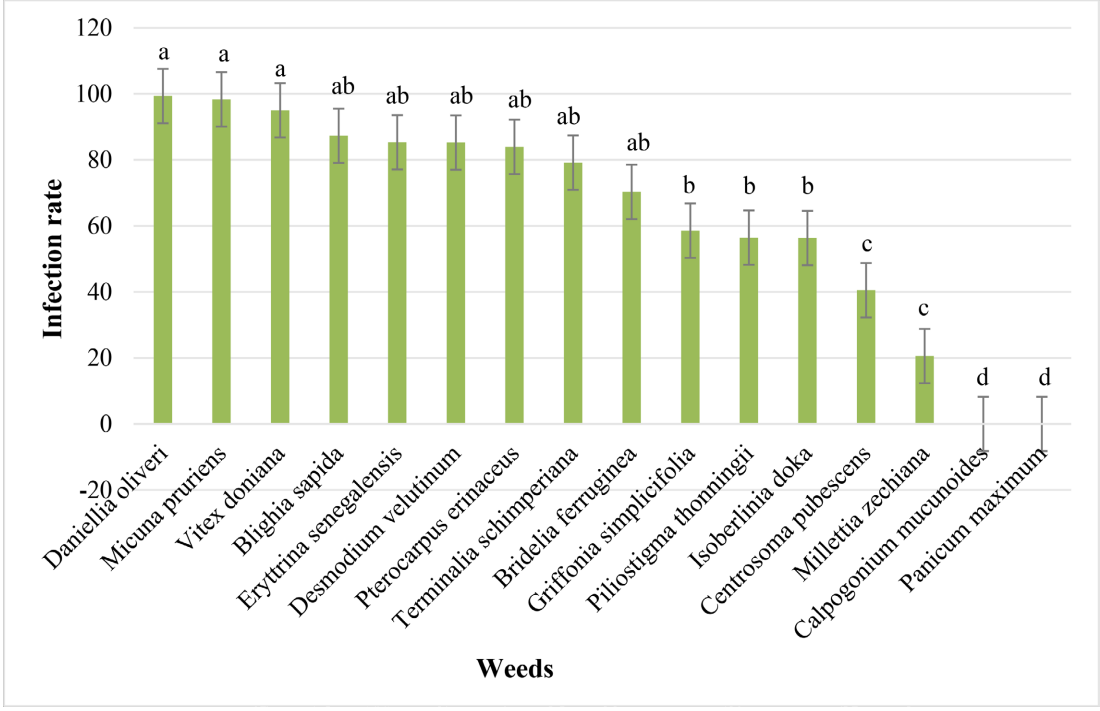

Figure 7. Weed infection rate according to species in the Centre-Ouest agro-ecological zone. Bands with the same letter are not significantly different according to the Newman-Keuls test at the 5% threshold.

mucunoides Desv. and *Panicum maximum* Jacq. (Figure 7).

3.1.4. Fungal Flora on Weeds

Samples taken from weeds showing disease symptoms in the cashew orchard

were subjected to laboratory diagnosis. This diagnosis revealed that five species of fungi, namely *Lasiodiplodia* sp, *Colletotrichum* sp, *Pestalotia* sp, *Alternaria* sp and *Curvularia* sp are present on weeds in the cashew orchard in Côte d'Ivoire. These mycopathogens were identified on the basis of morphological characters on the PDA culture medium and under the microscope. Three of the species identified were common on shrubs and vines in the four agroecological zones surveyed (**Figure 8**).

The other two, *Alternaria* sp and *Curvularia* sp, were occasionally found on shrubs and grasses in the Central-Western and Northern agro-ecological zones of Côte d'Ivoire (**Figure 9**).

3.1.5. Frequency of Fungi

Figure 10 shows the frequency of isolation of the three most frequent fungi on weeds in the cashew orchard in Côte d'Ivoire. The graph shows that the species *Colletotrichum* sp was the most frequent in all agro-ecological zones. Furthermore, the frequencies of *Colletotrichum* sp (47.5% to 55.25%), were statistically identical for all agro-ecological zones. In contrast, the isolation frequencies of *Pestalotia* sp (10.5% to 22.5%) were the lowest in all agro-ecological zones. The isolation frequencies of *Lasiodiplodia* sp (30% to 40.33%) were intermediate in all agro-ecological zones.

The analysis within each agro-ecological zone shows that there is a significant difference between the isolation frequencies of the different fungus species. In fact, in the Northern agro-ecological zone, the highest frequency (47.5%) was obtained by *Colletotrichum* sp. and the lowest frequency (22.25%) was obtained by *Pestalotia* sp. In the Central agro-ecological zone, the highest frequency

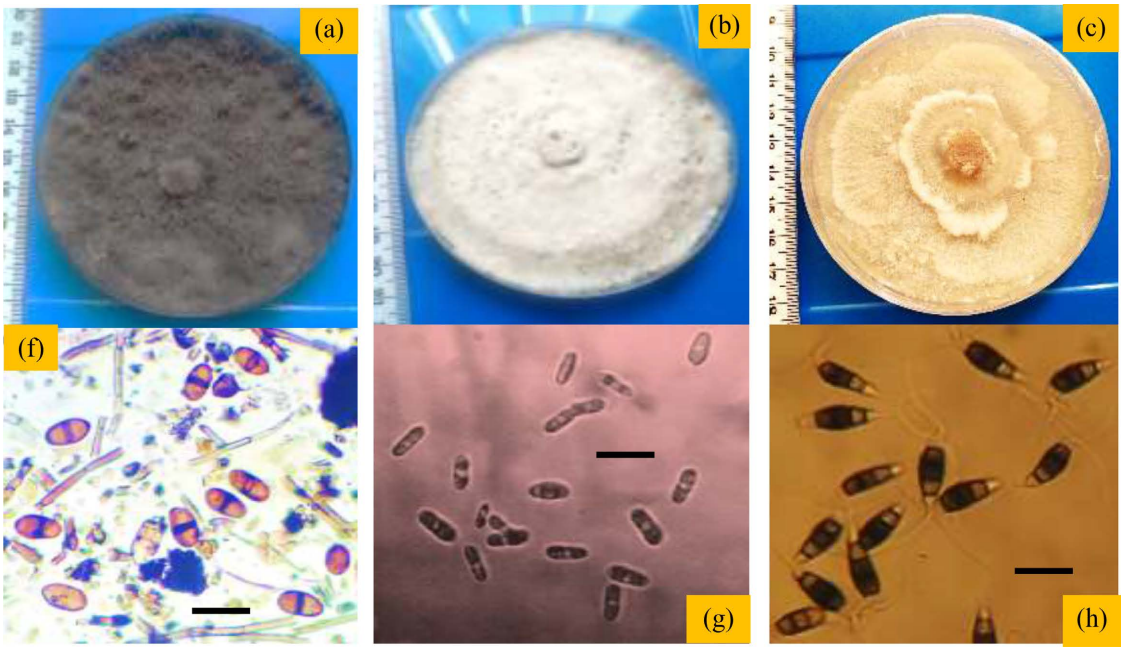

Figure 8. Main fungi identified on weeds in cashew orchard. (a) and (f): *Lasiodiplodia* sp; (b) and (g): *Colletotrichum* sp; (c) and (h): *Pestalotia* sp. (Observation au microscope optique, grossissement 40)

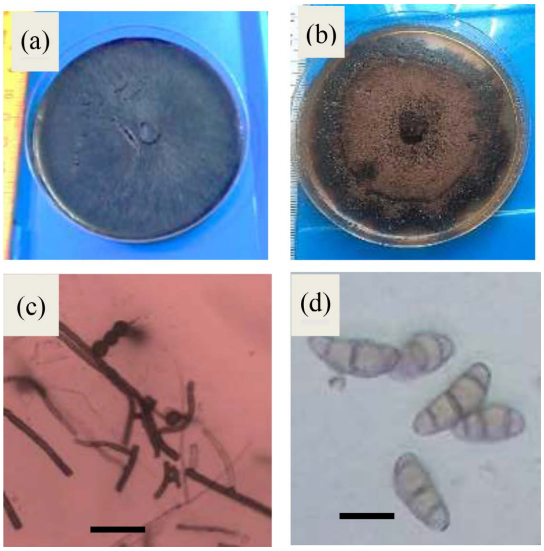

Figure 9. Fungi occasionally found on weeds. (a) and (c): *Alternaria* sp. (b) and (d): *Curvularia* sp. (Observation au microscope optique, grossissement 40)

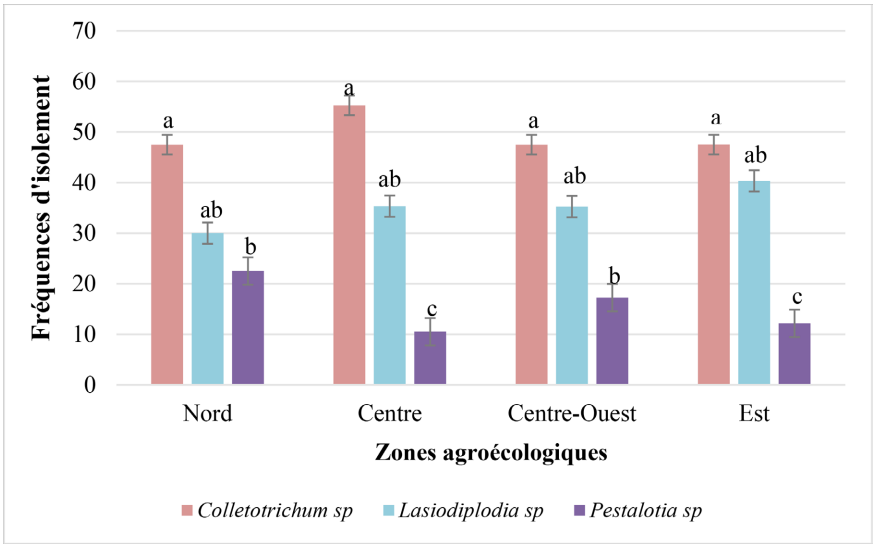

Figure 10. Frequency of species according to agro-ecological zones. Bands with the same letter are not significantly different according to the Newman-Keuls test at the 5% threshold.

(55.25%) was obtained by *Colletotrichum* sp. While *Pestalotia* sp. had the lowest frequency of isolation (10.5%). The highest frequency of isolation (47.5%) in the agroecological zone Centre-West was obtained by *Colletotrichum* sp. While, the lowest frequency (17.25%) was obtained by *Pestalotia* sp. In the agroecological zone East, the highest frequency of isolation (47.51%) was obtained by *Colletotrichum* sp. And *Pestalotia* sp had the lowest frequency of isolation (12.16%).

3.2. Discussion

The results showed that fungi are present on weeds in the cashew orchard in Côte d'Ivoire. The infection rate of the weeds surveyed was 80%. This high in-

fection rate indicates that the majority of the symptoms observed on weeds in the cashew orchard are due to fungal attacks. The main symptoms identified were necrosis spots on the leaves, beach spots in the form of burning, desiccation and red or white powdery deposits on the leaves. The high presence of these symptoms on weeds in the cashew orchard is thought to be related to the presence of *Colletotrichum gloeosporioides*, *Lasiodiplodia theobromae*, *Pestalotia heterocornis*, *Cephaleuros virescens* or *Oïdium anacardii*. These symptoms are identical to those described on cashew by several authors in previous studies. The necrosis spots evolving into a beach-like burn on cashew leaves are caused by *Colletotrichum gloeosporioides*. And the red powdery deposits on cashew leaves were described as a symptom of red rust caused by *Cephaleuros virescens* on cashew [21]. Similarly in Cameroon, cashew powdery mildew is manifested by the appearance of whitish colonies on the upper leaves [22]. In Burkina Faso, *Lasiodiplodia theobromae* is responsible for mango tree desiccation [23]. *Pestalotia heterocornis* causes leaf necrosis spots in cashew in Côte d'Ivoire [24].

At the level of morphological type, results showed that shrubs with an infection rate of (65%) were the most infected in the cashew orchard. This could be explained by the fact that the shrubs have a tissue structure similar to that of cashew. These plants, which are essentially made of lignin, would be compatible to the same fungal pathogens. This high rate for shrubs would also be due to the fact that the latter benefit from a relatively longer presence time than the other morphological types in the orchard. This time would be sufficient for the pathogenic fungi to establish a compatibility relationship with the shrubs. Some shrubs in the cashew orchard have food or medicinal functions [25].

Diagnosis of symptomatic samples identified five species of fungi, namely *Colletotrichum* sp, *Lasiodiplodia* sp, *Pestalotia* sp, *Curvularia* sp and *Alternaria* sp. According to several authors the fungal genera identified are responsible for cashew tree diseases in different producing countries [26] [27] [28]. They are said to be capable of attacking all cashew organs. In Côte d'Ivoire, *C. gloeosporioides* has been identified as responsible for anthracnose on all cashew organs [26]. The species *L. theobromae*, is involved in the drying of cashew buds bitten by *helopeltis* sp in Côte d'Ivoire [27]. In Burkina Faso, *C. gloeosporioides*, *P. heterocornis* and *Alternaria* sp have been identified on cashew leaves [28]. Furthermore, these authors reported that *Curvularia* sp is associated with disease symptoms on cashew nut and cashew apple.

C. gloeosporioides and *L. theobromae* were the most frequent species in all agroecological zones. This could be explained by the diversity of host plants of these two species in the cashew orchard. This frequency would also reflect the ability of these fungi to adapt to different agroclimatic conditions. *C. gloeosporioides* and *L. theobromae* are associated with mango desiccation in four provinces belonging to different agroclimatic zones in Burkina Faso [29].

The presence of alternative weed hosts of anthracnose and desiccation in the orchard is a threat to cashew production in Côte d'Ivoire. Indeed, in Mozambique, the yield losses due to cashew anthracnose are between 50% and 70% [30].

The bud desiccation alone can cause cashew yield losses of 70% and the death of more than 50% of vegetative shoots [31]. Furthermore, the results highlight the indirect harmfulness of weeds in the cashew orchard in Côte d'Ivoire. A similar study showed that weeds maintain phytoviruses in Solanaceae crops in Côte d'Ivoire [32].

4. Conclusion

At the end of this study, it was found that the cashew orchard harbours a diversity of weeds hosts of cashew pathogenic fungi in Côte d'Ivoire. The symptoms observed were anthracnose, desiccation, mildew/oidium rust and pestalotiosis. The overall infection rate of weeds showing these symptoms in the orchard was 80%. And the most infected weeds in the cashew orchard were *Daniellia oliveri*, *Vitellaria oliveri*, *Pterocarpus erinaceus*, *Micuna pruriens*, *Albizia zygia* and *Bridelia ferruginea*. Furthermore, the study revealed the presence of *Colletotrichum* sp, *Lasiodiplodia* sp, *Pestalotia* sp, *Curvularia* sp and *Alternaria* sp on weeds. The results of this study are interesting and deserve to be deepened by a molecular characterization of the fungi obtained and the realization of transmission tests of the fungi between the infected weeds and the cashew trees.

5. Significance Statement

This is the first study carried out on alternative weed hosts of cashew pathogenic fungi in the cashew orchard in Côte d'Ivoire. The results of this study show that the cashew orchard in Côte d'Ivoire harbours alternative weed hosts of pathogenic fungi.

Acknowledgements

The authors would like to thank the Fonds Interprofessionnel pour la Recherche et le Conseil Agricoles (FIRCA), the Projet de Promotion de la Compétitivité de la Chaîne de valeur de l'Anacarde (PPCA), the Conseil du Coton et de l'Anacarde (CCA), the Programme National de Recherches sur l'Anacarde (PNRA), for their financial support. We also thank the Agricultural Production Improvement Laboratory of the University Jean Lorougnon Guédé of Daloa for the equipment.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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