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Antifungal Potential of Three Plant Extracts in the Control of Some Major Fungi Associated with Cashew Seeds (*Anacardium occidentale* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Cashew (*Anacardium occidentale* L.), is an economically export oriented crop that could plays an important role in the Cameroonian economy. Owing to its importance, a study was carried out to evaluate the potential antifungal activities of three plants extracts against three major fungal pathogens associated to its seeds. To achieve this objective, cashew seeds samples were collected from Cameroon and Chad, and fungal pathogens isolated on Potato Dextrose Agar (PDA) culture medium. In vitro antifungal activities of *Callistemon viminalis*, *Cupressus lusitanica* and *Lantana camara* were assessed on three major fungi; *Cercospora* sp., *Colletotrichum gloeosporioides* and *Pestalotia heterocornis*. Results showed that cashew seeds harbour a diversity of fungal species; the most frequent are *Cercospora* sp. (26%), followed by *Aspergillus niger* (17.78%) and *P. heterocornis* (15.6%). Aqueous extracts at 28 mg/ml and ethanolic extracts at 16 mg/ml of *C. lusitanica* and *L. camara*, inhibited at 100%, the radial growth and sporulation of all tested fungi. This preliminary work has opened up a possibility of the use of these extracts in the treatment of seed-borne fungi of cashew nuts. However further studies are still on-going under in vivo field conditions to practically evaluate on their potential in cashew seeds disease management.

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1. INTRODUCTION

Cashew (*Anacardium occidentale* L.) is a shrub of the Anacardiaceae family, native to Brazil [1], extensively cultivated nowadays in all tropical areas, notably in India and East and West Africa for the multiple assets it offers [2]. Its cultivation plays three main functions: an environmental management function focused on the protection and conservation of natural resources, an economic function of wealth creation and employment, and a medicinal and food function [3]. The cashew fruit is rich in carbohydrates, vitamins (vitamin A, B1, C, D, K and PP), minerals (calcium, phosphorus and iron), fibre, proteins and essential fatty acids [4]. This fruit is composed of an upper part called apple and a lower part representing the seed, called nut or cashew nut, which is the main commercial product of *Anacardium occidentale* [5]. It is processed and used in several countries including food, medicine, cosmetology and the automobile industry [3].

The world production of the nuts in 2019 was estimated at 3.66 million tons and the African production accounted for 60% of the world production. Ivory Coast with a production of 900 thousand tons was the world's largest producer [6]. Cameroon, with a production of 108 tons, is not among the main nuts producing countries despite its low production potential and its cultivation that dates back to 1975 [7,8]. More recently, the Cameroon government has integrated the crop in its strategic plan, as an important cash crop to diversify its production, and as a new source of income for rural populations [3].

Like other crops, cashew production requires good seeds quality that can germinate and produce vigorous seedlings. Seeds health therefore is an important factor in the success of the crop production. As reported by [9] and [10], seeds health is the most important parameter of seeds quality as most of the pathogens initially present in the seeds can give rise to progressive disease development in the field and affect crop production. Among the various factors that affect seeds health, the most important are seed-borne pathogens that do not only lower seeds germination, but also reduce seeds vigour resulting in low yield [11,12]. Amongst those pathogens, fungi are the most important that can cause up to 40% yield losses. Some are reported to cause dieback (*Lasiodiplodia* sp.), anthracnose (*Colletotrichum gloeosporioides*), *pestalotia* leaf spot (*Pestalotia heterocornis*) and powdery mildew (*Oïdium anacardii*) [13,14].

Seed treatment has been used to protect seeds and future plants from diseases and insect attacks from the moment they are sown. The use of chemical fungicides based on Carbendazim and Prochloraz has been advocated by some authors treatment to control cashew seed-borne fungi [15,4]. These chemicals pose problems of toxicity for consumers linked to the presence of residues in agricultural products [16] and environmental pollution [17]. Faced with these adverse effects of chemical fungicides, considerable efforts are being directed towards exploring plants extracts as alternative control methods. Plants extracts have the advantage of being not only available to farmers, but also being non-toxic and easily biodegradable and therefore environmentally friendly [18,19].

In Cameroon, several works have been reported on the identification of the main pathogens of cashew fruits [20,21]; the observation is that there is no data on seed-borne fungi of cashew. However, such information is necessary for the development of effective control measures against these fungal species. The present study was therefore initiated with the aims of isolating fungi associated with cashew seeds and evaluating the antifungal potential of three plant extracts against major fungal pathogens.

2. MATERIALS AND METHODS

2.1 Isolation and Identification of Fungal Pathogens from Cashew Seeds

Seeds of *Anacardium occidentale* L. were collected from Cameroon (Ngaoundéré, Garoua and Yagoua) and Chad (Koumra, Moundou and Sahr). These seeds were put in plastic bags, labelled and transported to the Plant Pathology and Agricultural Zoology Research Unit (UR_PHYZA), University of Dschang for isolation. Seed fragments of about 2 mm², were surface sterilized with 5% sodium hypochlorite solution for 5 minutes, rinsed in sterilized distilled water and aseptically plated in Petri dishes containing 20 ml Potato Dextrose Agar (PDA) medium amended with Chloramphenicol (1 g/l) to prevent bacterial contamination and then incubated at 24 ± 2°C.

After 5 days of incubation, the growing mycelium was sub-cultured on fresh PDA medium until pure cultures were obtained. Fungi identification was carried out based on colony characteristics and morphology of fruiting bodies under a compound microscope with the help of identification keys of mycology [22,23]. Isolation frequencies (IF) of the different fungi were determined using the following formula:

$$IF\ (\%) = \frac{\text{Number of specific fungal which are isolated}}{\text{Total number of fungi which are isolated}} \times 100$$

2.2 Preparation of Plant Extracts

Aerial parts of *Callistemon vminalis* and *Lantana camara* (leaves and flowers), as well as the young leaves of *Cupressus lusitanica* collected in the locality of Dschang, were washed with tap water, dried separately in the shade for a fortnight and finely ground [24]. 100 g of powder of each plant was macerated separately in 500 ml of solvent which was distilled water or ethanol. The mixture was filtered through Whatman paper N°1 after 48 hours of maceration. The filtrates from the ethanolic maceration were introduced into the flask of a Buchner rotary evaporator at 67°C for partial evaporation of the extraction solvent and then dried in an oven at a temperature of 40°C. The aqueous filtrates were dried in the same oven at 40°C [25].

2.3 Antifungal Activity Assay of Plant Extracts on the Growth of Major Fungi

Evaluation *in vitro* of the antifungal activity of plant extracts on the growth of fungi was done by the solid-state dispersion method. The fungal species chosen for this test were *Colletotrichum gloeosporioides*, *Pestalotia heterornis* and *Cercospora* sp. Ethanolic extracts were dispersed in the medium using Dimethyl Sulfoxide (DMSO). The effect of DMSO was previously tested to ensure that it does not influence the development of the selected fungi [26]. The plant extracts were tested at concentrations of 4, 8 and 16 mg/ml for ethanolic extracts and 7, 14 and 28 mg/ml for aqueous extracts. These concentrations were obtained by adding 1ml of each of the previously prepared dilutions of each extract to 19 ml of PDA medium. This culture medium was poured into 90 mm diameter Petri dishes. Petri dishes without plant extracts, and having received 1 ml of distilled water or Monchamp at the manufacturer's dose (0.3 mg/ml), served respectively as negative and positive controls. After solidification of the medium, an explant of mycelium was taken from the growth front of the pure culture of the different fungi aged 10 days using a 5 mm diameter punch and then aseptically placed in the centre of each Petri dish. These dishes were incubated at a temperature of 24 ± 2°C for 10 days in darkness, at the end of which the following parameters were evaluated: the percentage of inhibition of the growth of the different fungi and the percentage of inhibition of sporulation. This experiment was carried out in a completely randomised design with three repetitions

The radial growth of these fungi was assessed by measuring every day across the two orthogonal diameters drawn on the reverse side of the Petri dishes. The radial growth data were transformed into percentage of inhibition (PI) by the following formula:

$$PI\ (\%) = \frac{D - DT}{D} \times 100$$

Where DT and D, are the radial growth diameter of the supplemented and negative control Petri dishes respectively.

From the aged colonies of the control (negative control) Petri dishes of each fungal species, four diameter discs of 5 mm of each test fungus were taken from all the other plates of different concentrations and introduced into 1 ml of sterilized distilled water. The fungal suspension was then vortexed to release the spores from the conidiospores. These experiments were repeated three times. After counting the total number of spores using a Thoma cell [27,28] the percentage of sporulation inhibition (SI) was determined by the following formula:

$$SI\ (\%) = \frac{No - Nc}{No} \times 100$$

Where: No, the average number of spores estimated in the negative control and Nc, the average number of spores estimated in the presence of the extracts.

2.4 Statistical Analyses of the Data

Data collected on isolation frequency of fungi, percentage of inhibition of radial growth and sporulation inhibition were subjected to analysis of variance (ANOVA) using SPSS version 22.0. Means were separated using Duncan's Multiple Range Test (DMRT) and LDS Fisher test at the 5% probability level.

3. RESULTS

3.1 Isolation Frequency of Fungi from *Anacarduim occidentale* L. Seeds

Cashew seeds are reported to harbour a diverse range of fungi. Overall, twelve fungi species were isolated and identified from cashew seeds from different localities in Cameroon and Chad (Table 1). *Cercospora* sp. was the most frequent species (with a frequency ranging from 19.05 to 35.04%) on seeds from all zones except Moundou, where *Aspergillus niger* (27.59%) was the most frequent species. On cashew seeds from Cameroon, *A. niger* was the second most frequent species with frequencies of 15.38%, 17.8% and 18.37% for seeds from Yagoua, Garoua and Ngaroundéré respectively. With seeds from Chad, *P. heterocornis* was the second most frequent species (20.74% in Koumra and 17.24% in Sarh). Except in Moundou, where *A. niger* was the second most frequent species. *E. nigrum* was the least frequent species in Yagoua, Garoua and Koumra with frequencies of 0.85%, 1.69% and 2.22 % respectively. With seeds from Ngaroundéré, Moundou and Sarh, the least frequent fungi species was *Mucor* sp. (0.70%), *A. flavus* (1.56%) and *A. atra* (0.86%) respectively. In generally, the most frequent fungi species were *Cercospora* sp. (26%), followed by *A. niger* (17.78%) and *P. heterocornis* (15.6%). The least frequent were *A. atra* (2.46%), *E. nigrum* (2.35%) and *Mucor* sp. (1.26%).

In general, the most frequent fungal species in both countries were *Cercospora* sp. followed by *A. niger* and *P. heterocornis*. The frequency of isolation of Cercospora sp. from cashew nuts (26.79%) was significantly the same as in Chad (25.19%) according to LSD Fisher's at 5%. Similarly, no significant differences were observed with species of the genus *Aspergillus*; as well as *Cladosporium* sp. and *E. nigrum*. The isolation frequencies of *C. gloeosporioides* (12.01%) and *F. oxysporium* from Cameroon (8.64%) were higher than those from Chad which had 7.8% and 5.56% respectively.

A. flavus showed yellow mycelial colonies that turned green with age. *A. fumugatus* and *A. niger* showed green and black mycelial colonies respectively. All three fungi species, under ordinary microscopy, showed erect conidiophores, swollen at the end into spherical or ovoid sporangia. It was from the sterigma that the spores were formed in very long chains. *Cercospora* sp. had a greyish cottony mycelium. Under ordinary microscopy, the mycelium of this fungus produced relatively short, compact conidiophores, extended in fascicles. The conidia were long, septate and straight or slightly curved. The pure culture of *C. gloeosporioides* showed a yellow mycelium, spotted with small black granules evolving with mycelial growth. Under the ordinary microscope, the mycelium was septate and the acervuli usually developed with bristles. These conidiophores were fusiform, unpartitioned, simple, straight, light brown, with a bulge of conidia. *P. heterocornis* had a white mycelium which was haloed from place to place by blackish honeydew (acervuli). Under the ordinary microscope, the conidiophores were simple and short. The fusiform conidia were tricellular, golden-yellow in colour; their upper end was extended by three simple filaments or appendages while the opposite, more tapered side was colourless and terminated by a single filament (Fig. 1).

3.2 Efficacy of Extracts from Three Plant Species on the Inhibition of Radial Growth of Major Cashew Seed Fungi

Aqueous and ethanolic extracts of the different plants showed a depressive effect on the radial growth of the *Cercospora* sp., *C. gloeosporioides* and *P. heterocornis*. In general, this depressive effect varied and depended on the type of extracts, the plant and the concentration applied. An increase in the percentage of radial growth inhibition was observed with increasing concentration of the applied extracts (Tables 2 and 3). Like the positive control Petri dishes, the aqueous extracts of *Cupressus lusitanica* and *Lantana camara* at the concentration of 28 mg/ml, completely inhibited the radial growth of *Cercospora* sp. and *C. gloeosporioides*. At the same concentration, the aqueous extract of *Callistemon viminalis* showed a higher inhibition of *Cercospora* sp. (90.59%) than those obtained with the other concentrations and the negative control (0%). This percentage of inhibition was significantly identical to that of the positive control according to the Duncan's test at 5% probability threshold. With *P. heterocornis*, the aqueous extracts of *C. lusitanica* and *C. viminalis* did not completely inhibit the radial growth of the fungus, with the exception of the aqueous extracts of *L. camara*, which inhibited 100% of the development of the fungal species at the concentration of 28 mg/ml.

Table 1. Frequency of isolation (%) of fungi species associated with cashew seeds

	Cameroon			Chad			
Fungi species	**Garoua**	**Ngaoundéré**	**Yagoua**	**Koumra**	**Moundou**	**Sarh**	**Means**
Acremonellia atra	3.39 (4)	1.36 (2)	/	3.7 (5)	5.47 (7)	0.86 (1)	2.46
Aspergillus fumugatus	9.32 (11)	10.20 (15)	6.84 (8)	10.37 (14)	3.91 (5)	10.34 (12)	8.5
Aspergillus niger	17.80 (21)	18.37 (27)	15.38 (18)	11.11 (15)	16.41 (21)	27.59 (32)	17.78
Aspergillus flavus	/	4.76 (7)	8.55 (10)	2.22 (3)	1.56 (2)	6.90 (8)	4
Cercospora sp.	26.27 (31)	19.05 (28)	35.04 (41)	31.85 (43)	27.34 (35)	16.38 (19)	26
Cladosporium sp.	4.24 (5)	4.76 (7)	/	/	3.13 (4)	4.31 (5)	2.74
Colletotrichum gloeosporioides	15.25 (18)	12.24 (18)	8.55 (10)	12.59 (17)	3.91 (5)	6.90 (8)	9.91
Epicocum nigrum	1.69 (2)	5.44 (8)	0.85 (1)	2.22 (3)	3.91 (5)	/	2.35
Fusarium oxysporium	8.47 (10)	5.44 (8)	12.00 (14)	/	14.10 (18)	2.59 (3)	7.09
Mucor sp.	/	0.70 (1)	/	5.19 (7)	/	1.72 (2)	1.26
Pestalotia heterocornis	13.56 (16)	17.69 (26)	11.11 (13)	20.74 (28)	13.28 (17)	17.24 (20)	15.6
Trichoderma harzianum	/	/	1.71 (2)	/	7.03 (9)	5.17 (6)	2.32

**Numbers in brackets represent the number of fungi isolates*

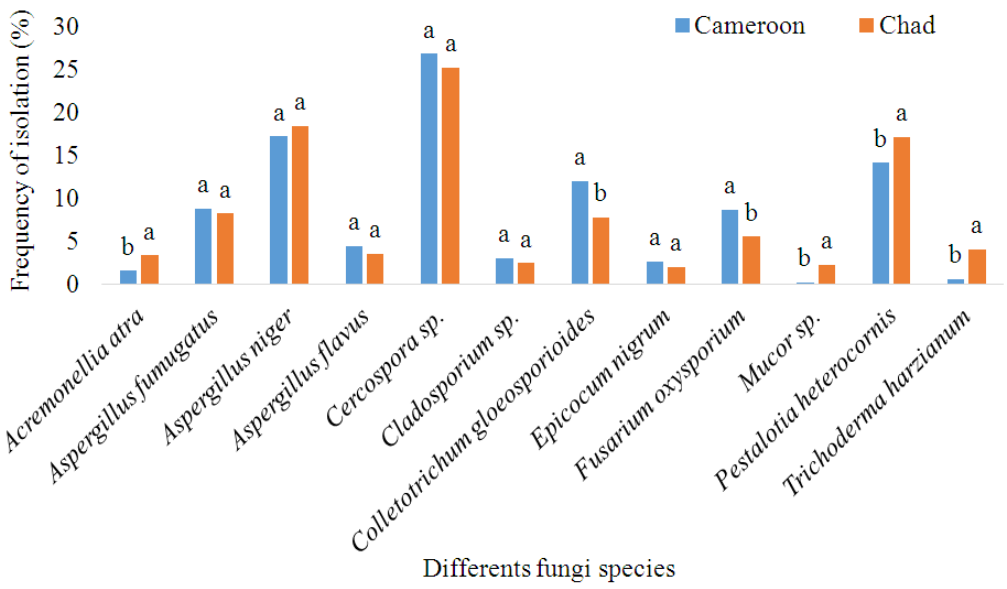

Fig. 1. Frequency of isolation (%) of different fungi species

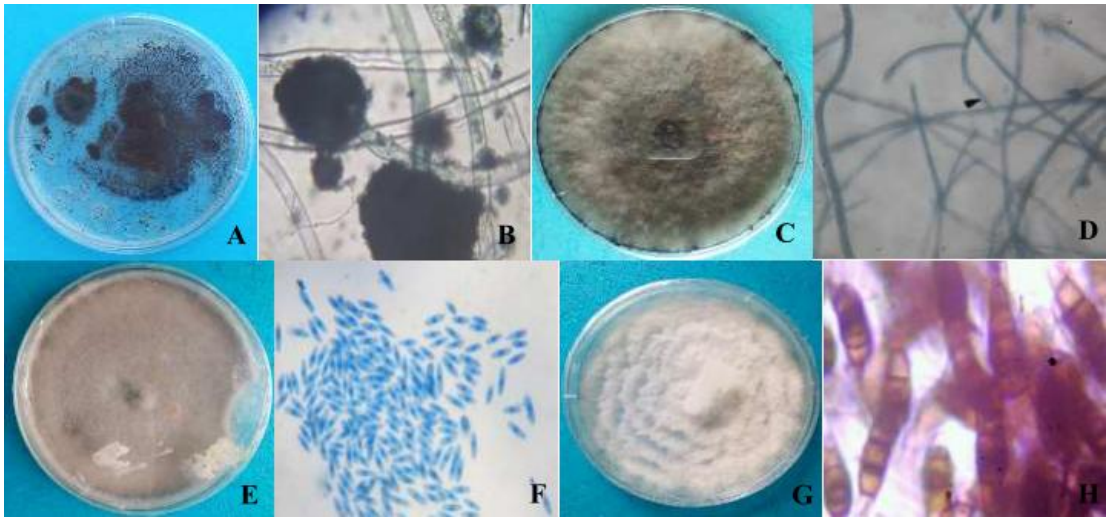

Fig. 2. 10 days old of pure culture and microscopic morphological characters (mycelia and conidia) of some fungi associated with *Anacardium occidentale* L. seeds. A and B: *Aspergillus niger*, C and D: *Cercospora* sp., E and F: *Colletotrichum gloeosporioides*, G and H: *Pestalotia heterocornis*

Ethanolic extracts of *C. lusitanica, C. viminalis* and *L. camara* inhibited 100% of the radial growth of *C. gloeosporioides* at the concentration of 16 mg/ml. At the same concentration (16 mg/ml) the radial growth of *P. heterocornis* was completely inhibited with ethanolic extracts of *C. lusitanica* and *L. camara*; that of *Cercospora* sp. with ethanolic extracts of *C. viminalis* and *L. camara*. The ethanolic extract of *C. lusitanica* at the concentration of 16 mg/ml showed an inhibition of the radial growth of *Cercospora* sp. of 98.82%. This inhibition was significantly identical to that of the positive control and significantly higher than that of the negative control (0%) and the other Petri dishes supplemented with this plant extract at a concentration of 4 mg/ml (47.45%) and 8 mg/ml (80.39%) according to Duncan's test at the 5% probability threshold.

Table 2. Effect of aqueous extracts on the percentage inhibition (%) of the development of major fungi associated with cashew seeds

Concentration (mg/ml)	*Cupressus lusitanica*	*Callistemon viminalis*	*Lantana camara*
	***Cercospora* sp.**		
0 (C-)	0.00 ± 0.00d*	0.00 ± 0.00d	0.00 ± 0.00c
7	6.18 ± 2.35c	39.61 ± 9.51c	75.29 ± 5.13b
14	87.84 ± 2.96b	70.98 ± 7.83b	95.69 ± 4.80a
28	100.00 ± 0.00a	90.59 ± 4.24a	100.00 ± 0.00a
1 (C+)	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
	Colletotrichum gloeosporioides		
0 (C-)	0.00 ± 0.00d	0.00 ± 0.00e	0.00 ± 0.00d
7	47.45 ± 4.90c	44.31 ± 5.30d	55.29 ± 4.24c
14	84.71 ± 3.53b	68.24 ± 5.13c	86.27 ± 1.80b
28	100.00 ± 0.00a	82.35 ± 1.18b	100.00 ± 0.00a
1 (C+)	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
	Pestalotia heterocornis		
0 (C-)	0.00 ± 0.00e	0.00 ± 0.00e	0.00 ± 0.00d
7	41.57 ± 2.96d	32.94 ± 4.24d	58.04 ± 5.56c
14	77.65 ± 1.18c	62.75 ± 2.45c	74.90 ± 1.36b
28	89.02 ± 2.96b	75.69 ± 1.80b	100.00 ± 0.00a
1 (C+)	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a

**Means with the same letter in the same column are not significantly different according to Duncan's test at 5% probability threshold. C- = negative control (without any supplement) and C+ = positive control (addition of Monchamp)*

Table 3. Effect of ethanolic extracts on the percentage inhibition (%) of the development of major fungi associated with cashew seeds

Concentration (mg/ml)	*Cupressus lusitanica*	*Callistemon viminalis*	*Lantana camara*
	***Cercospora* sp.**		
0 (C-)	0.00 ± 0.00d*	0.00 ± 0.00d	0.00 ± 0.00 e
4	47.45 ± 4.90c	25.49 ± 6.79c	67.45 ± 12.47c
8	80.39 ± 8.01b	76.86 ± 0.68b	86.27 ± 1.80b
16	98.82 ± 2.04a	100.00 ± 0.00a	100.00 ± 0.00a
1 (C+)	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
	Colletotrichum gloeosporioides		
0 (C-)	0.00 ± 0.00d	0.00 ± 0.00d	0.00 ± 0.00d
4	71.37 ± 1.80c	55.29 ± 1.18c	74.90 ± 1.80c
8	80.39 ± 8.01b	80.00 ± 5.13b	92.16 ± 3.59b
16	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
1 (C+)	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
	Pestalotia heterocornis		
0 (C-)	0.00 ± 0.00d	0.00 ± 0.00 e	0.00 ± 0.00d
4	63.92 ± 2.72c	55.29 ± 1.18c	62.75 ± 2.45c
8	87.06 ± 3.11b	67.45 ± 3.78c	78.82 ± 1.18b
16	100.00 ± 0.00a	82.35 ± 1.18b	100.00 ± 0.00a
1 (C+)	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a

**Means with the same letter in the same column are not significantly different according to Duncan's test at 5% probability threshold. C- = negative control (without any supplement) and C+ = positive control (addition of Monchamp)*

Table 4. Effect of aqueous extracts on the percentage inhibition (%) of sporulation of major fungi associated with cashew seeds

Concentration (mg/ml)	*Cupressus lusitanica*	*Callistemon viminalis*	*Lantana camara*
	***Cercospora* sp.**		
0 (C-)	0.00 ± 0.00d*	0.00 ± 0.00e	0.00 ± 0.00c
7	56.69 ± 3.40c	46.10 ± 3.22d	78.00 ± 5.24b
14	87.57 ± 2.00b	67.10 ± 2.10c	96.61 ± 1.62a
28	100.00 ± 0.00a	94.38 ± 4.10b	100.00 ± 0.00a
1 (C+)	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
	Colletotrichum gloeosporioides		
0 (C-)	0.00 ± 0.00d	0.00 ± 0.00e	0.00 ± 0.00d
7	50.42 ± 1.34c	43.46 ± 2,66d	57.00 ± 1.37c
14	94.04 ± 0.49b	64.96 ± 1.34c	97.06 ± 0.74b
28	100.00 ± 0.00a	85.27 ± 1.30b	100.00 ± 0.00a
1 (C+)	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
	Pestalotia heterocornis		
0 (C-)	0.00 ± 0.00d	0.00 ± 0.00e	0.00 ± 0.00d
7	44.33 ± 2.47c	33.02 ± 2.56d	54.94 ± 2.95c
14	79.61 ± 5.25c	64.52 ± 4.21c	71.62 ± 2.26b
28	94.55 ± 2.30b	82.00 ± 1.92b	100.00 ± 0.00a
1 (C+)	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a

**Means with the same letter in the same column are not significantly different according to Duncan test at 5% probability threshold. C- = negative control (without any supplement) and C+ = positive control (addition of Monchamp)*

Table 5. Effect of ethanolic extracts on the percentage inhibition (%) of sporulation of major fungi associated with cashew seeds

Concentration (mg/ml)	*Cupressus lusitanica*	*Callistemon viminalis*	*Lantana camara*
	***Cercospora* sp**		
0 (C-)	0.00 ± 0.00d*	0.00 ± 0.00d	0.00 ± 0.00c
4	33.40 ± 3.78c	43.45 ± 2.82c	61.65 ± 11.02b
8	77.03 ± 7.69b	84.91 ± 7.21b	91.43 ± 4.26a
16	98.14 ± 3.05a	100.00 ± 0.00a	100.00 ± 0.00a
1 (C+)	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
	Colletotrichum gloeosporioides		
0 (C-)	0.00 ± 0.00d	0.00 ± 0.00d	0.00 ± 0.00c
4	52.45 ± 1.06c	71.73 ± 3.31c	75.97 ± 2.04b
8	77.07 ± 2.20b	96.88 ± 0.84b	98.22 ± 0.61a
16	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
1 (T+)	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
	Pestalotia heterocornis		
0 (T-)	0,00 ± 0,00e	0.00 ± 0.00d	0.00 ± 0.00d
4	33.30 ± 7,10d	50.21 ± 3.12c	54.76 ± 2.33c
8	67.37 ± 1.50c	93.77 ± 2.91b	91.10 ± 1.20b
16	81.89 ± 2.92b	100.00 ± 0.00a	100.00 ± 0.00a
1 (T+)	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a

**Means with the same letter in the same column are not significantly different according to Duncan's test at 5% probability threshold. C- = negative control (without any supplement) and C+ = positive control (addition of Monchamp)*

The percentages of sporulation inhibition of the different fungi depended on the concentration applied; the plant and the type of extracts (Tables 4 and 5). Aqueous extracts of *L. camara* at the concentration of 28 mg/ml, inhibited 100% the sporulation of the different fungi tested. Similarly, *C. lusitanica* extracts at the same concentration (28 mg/ml) completely inhibited the sporulation of *Cercospora* sp. and *C. gloeosporioides*. The same observation was

made with the positive control Petri dishes on the sporulation of the different fungi species. No concentration of the aqueous extracts of *C. viminalis* inhibited at 100% of the sporulation of the different fungi. The highest percentages of sporulation inhibition were obtained at the concentration of 28 mg/ml. These sporulation inhibition percentages were 94.38, 85.27 and 82% for *Cercospora* sp., *C. gloeosporioides* and *P. heterocornis* respectively. They were significantly higher than those obtained with the other concentrations (7 and 14 mg/ml) and the negative control experiments according to Duncan test at 5% probability threshold.

The ethanolic extracts of all plants at the concentration of 16 mg/ml and the positive control experiments completely sporulated *C. gloeosporioides*. This was same for ethanolic extracts of *C. viminalis* and *L. camara* on sporulation of the three test fungi. The ethanolic extracts of *C. lusitanica* at 16 mg/ml showed inhibitions of sporulation of 98.14 and 81.89% respectively on *Cercospora* sp. and *P. heterocornis*.

4. DISCUSSION

4.1 Fungi Associated with Cashew Seeds

Twelve fungi species are associated with *Anacardium occidentale* L. seeds. These fungal species included *Acremonellia atra*, *Aspergillus niger*, *Cercospora* sp., *Colletotrichum gloeosporioides, Epicoccum nigrum, Fusarium oxysporium, Mucor* sp. and *Pestalotia heterocornis*. Our results are similar with those of [12,13,21], who showed that cashew fruits harbour a diversity of fungi that could be transmitted to the seeds. This fungal diversity is thought to be due to the fact that cashew seeds are an important source of carbohydrate for fungi species or they appear due to poor seed storage conditions. Indeed, the work of [29] have shown that high humidity conditions during storage favour the proliferation of mould on foodstuffs. Fungi species such as *Cercospora* sp., *Aspergillus* spp., *P. heterocornis* and *C. gloeosporioides* were the most frequent in all locations compared to other fungi species isolated. This high frequency of these fungi is thought to be due to the fact that they are pathogens and cause significant damage to cashew seeds. The works of [30], showed that *C. gloeosporioides*, the causal agent of anthracnose in *A. occidentale* L. is the main constraint to cashew nuts production in Ivory Coast. [31] reported that anthracnose contributes to a drop of more than 50% in production by affecting apples and cashew nuts. [12] showed that cashew leaf spot caused by *P. heterocornis* is one of the major fungal diseases of cashew in Burkina Faso.

On the other hand, species such as *Mucor* sp., *E. nigrum, A. atra* and *Cladosporium* sp. were the least frequent. The low frequency of these fungi could be due to the fact that they are opportunists. Similarly, those of [32, 33], showed that opportunistic fungi occur at a low frequency. For [34], the low frequency of some fungi could be explained by the fact that the different fragments from which the fungi species should be isolated were disinfected with 95° alcohol and 5% Sodium hypochlorite ($NaOCl_2$) solution which would have had a detrimental effect on the frequency of these opportunistic fungi.

4.2 Efficacy of Extracts from the Three Plant Species on the Radial Growth of Major Fungi Associated with Cashew Seeds

The aqueous and ethanolic extracts of the four plants showed overall a greater depressive effect on the radial growth of the three fungi tested than the negative control. This depressive effect of the plant extracts would be due to the fact that the plants used could contain compounds with antifungal properties that would have influenced the growth of the fungal species tested. Indeed, the work of [35, 36], showed that certain plants would contain in their organs, compounds with antifungal properties (alkaloids, sterols, flavonoids, anthraquinone phenols, saponins or tannins). The percentages of radial growth inhibition of *Cercospora* sp., *C. gloeosporioides* and *P. heterocornis* were influenced by the plant used, the concentration applied and the type of extracts (aqueous and ethanolic). The percentage inhibition of the different fungi tested increased with increasing concentration. This could suggest that higher concentrations of extracts are more fungicidal than lower concentrations. Similarly, [26] showed that, radial growth of *C. kahawae* was more inhibited by *Carica papaya* and *Eucalyptus saligna* extracts when the concentrations applied were high. Aqueous and ethanolic extracts of *C. lusitanica* and *Lantana camara* showed antifungal activity against these major fungi associated with cashew seeds. These results corroborate those of [24] who showed that aqueous and ethanolic extracts of *C. lusitanica* inhibited the

development of *Botryosphaeria dothiorella, Cercospora purpurea* and *C. gloeosporioides*, causal agents of diseases in avocado fruits. Similarly, those of [37], showed the extracts of *C. lusitanica* inhibited the radial growth of *Phytophthora colocasiae*, the causal agent of taro blight. Extracts of *Callistemon viminalis* did not inhibit at 100% the radial growth of *C. gloeosporioides* and *P. heterocornis*. These results are similar to those [38] who reported a weak inhibitory action of *C. viminalis* on the development of seed-associated fungi of *Moringa oleifera*. In contrast, those of [37], reported that *C. viminalis* extracts inhibited the development of *Phytophthora colocasiae*, the causal agent of taro blight. This difference in results would be due to the fact that the test fungi (*Cercospora* sp., *C. gloeosporioides* and *P. heterocornis*) would be less susceptible to C. *viminalis* extracts compared to *P. colocasiae*.

The percentages of sporulation inhibition varied according to the concentration applied, the plant, the type of extracts and the fungi species tested. The higher the concentration applied, the higher the inhibition of sporulation of the test fungi. This would be due to the fact that by inhibiting radial growth, the extracts would also simultaneously inhibit the sporulation of these test fungi. Similar results were reported by [38] where mycelial growth and sporulation of *Pyricularia grisea* were inhibited by tricyclazole. Similarly, those of [39] showed that radial growth and sporulation of *Ascocyhta rabiei* and *F. oxysporium* were more inhibited by ethanolic extracts of *Punica granatum* barks with the high concentrations.

5. CONCLUSION

This study revealed that cashew seeds harbour a variety of fungi amongst which *Cercospora* sp., *P. heterocornis* and *C. gloeosporioides* are major field fungi and that *C. lusitanica* and *L. camara* extracts, showed significant *in vitro* antifungal activity in the control of the three fungi tested. Their antifungal activities were similar to that of the synthetic fungicide Monchamp, this suggests their potential use in the management of seed borne fungi affecting cashew nuts. Further studies in controlled greenhouse and in field conditions are needed to practically confirm their use in the framework of cashew seeds disease management.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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