

## Antifungal potential of three plant extracts in the control of some major fungi associated with cashew seeds (*Anacardium occidentale* L.)

### Abstract

Cashew (*Anacardium occidentale* L.), is an economically export oriented crop that could play an important role in the ~~cameroonian~~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 ~~ongoingon-~~going under *in vivo* field conditions to practically evaluate on their potential in cashew seeds disease management.

**Key words:** Antifungal potential, plant extracts, seed-borne fungi, *Anacardium occidentale*.

### 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].

Comment [I1]:

Comment [I2]: Not italic

Comment [I3]:

Comment [I4]:

Comment [I5]: Not italic

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 (*Oidium anacardii*) [13, 14].

Comment [16]: Italic

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.

## Materials and methods

### 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 mm<sup>2</sup>, 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:

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

### Preparation of plant extracts

Aerial parts of *Callistemon viminalis* 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].

### 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 19ml 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  $24 \pm 2^{\circ} \text{C}$  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:

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

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:

$$\text{SI (\%)} = \frac{N_o - N_c}{N_o} \times 100$$

Where:  $N_o$ , the average number of spores estimated in the negative control and  $N_c$ , the average number of spores estimated in the presence of the extracts.

#### **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.

#### **Results**

##### **Isolation Frequency of fungi from *Anacardium 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, *Pestalotia 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. *Epicoccum 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 %), *Aspergillus flavus* (1.56 %) and *Acremonellia 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 *Acremonellia atra* (2.46 %), *Epicocum nigrum* (2.35 %) and *Mucor* sp. (1.26 %).

Comment [17]: P.

Comment [18]: A.

Comment [19]: B.

Comment [110]: A.

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

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

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

In general, the most frequent fungal species in both countries were *Cercospora* sp. followed by *Aspergillus niger* and *Pestalotia 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 *Epicocum nigrum*. The isolation frequencies of *Colletotrichum gloeosporioides* (12.01 %) and *Fusarium oxysporium* from Cameroon (8.64%) were higher than those from Chad which had 7.8 % and 5.56 % respectively.

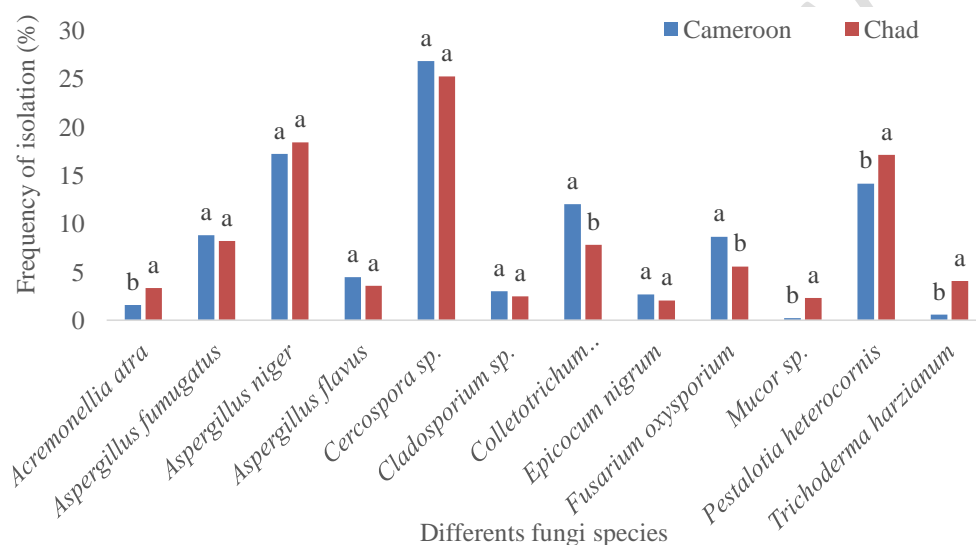


Figure 1: Frequency of isolation (%) of different fungi species

*Aspergillus flavus* showed yellow mycelial colonies that turned green with age. *A. fumigatus* 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 *Colletotrichum 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. *Pestalotia 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 (figure 1).

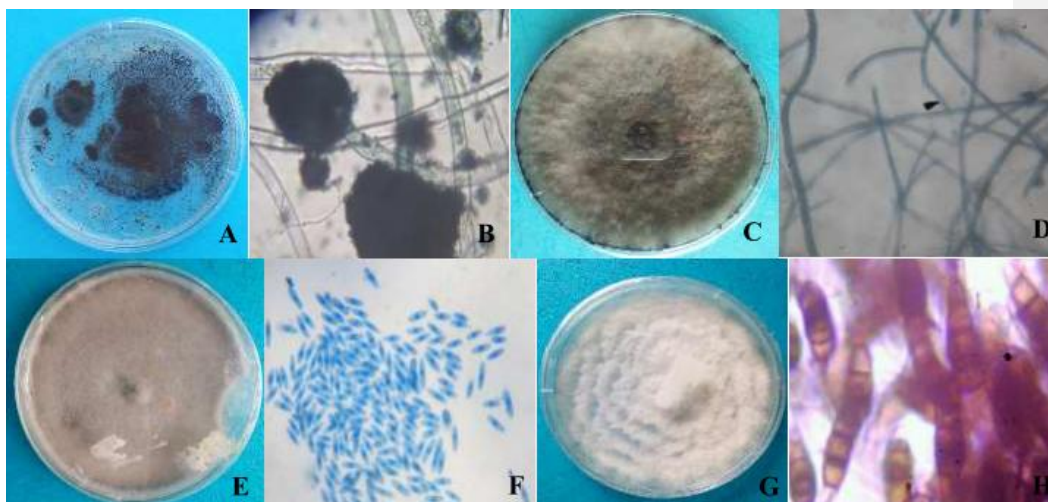


Figure 2: 10 days old of pure culture and microscopic morphological characters (mycelia and conidia) of some fungi associated with *Anacardium occidentale* L. seeds. A and B: *Aspergillus niger*, C and D: *Cercospora* sp., E and F: *Colletotrichum gloeosporioides*, G and H: *Pestalotia heterocornis*.

#### 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., *Colletotrichum gloeosporioides* and *Pestalotia 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

Comment [I20]: C.

Comment [I21]: P.



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

Concentration (mg/ml)	<i>Cupressus lusitanica</i>	<i>Callistemon viminalis</i>	<i>Lantana camara</i>
<i>Cercospora</i> 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
<i>Colletotrichum gloeosporioides</i>			
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
<i>Pestalotia heterocornis</i>			
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).

Ethanollic extracts of *Cupressus lusitanica*, *Callistemon C. viminalis* and *Lantana camara* inhibited 100 % of the radial growth of *Colletotrichum CC. gloeosporioides* at the concentration of 16 mg/ml. At the same concentration (16 mg/ml) the radial growth of *Pestalotia 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.

Comment [I22]: C.

Comment [I23]: C.

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

Concentration (mg/ml)	<i>Cupressus lusitanica</i>	<i>Callistemon viminalis</i>	<i>Lantana camara</i>
<i>Cercospora</i> 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
<i>Colletotrichum gloeosporioides</i>			
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
<i>Pestalotia heterocornis</i>			
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).

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., *Colletotrichum*-*C. gloeosporioides* and *Pestalotia* *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.

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

Concentration (mg/ml)	<i>Cupressus lusitanica</i>	<i>Callistemon viminalis</i>	<i>Lantana camara</i>
<i>Cercospora</i> 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
<i>Colletotrichum gloeosporioides</i>			
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
<i>Pestalotia heterocornis</i>			
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).

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 *Callistemon C. viminalis* and *Lantana-L. camara* on sporulation of the three test fungi. The ethanolic extracts of *Cupressus-CC. lusitanica* at 16 mg/ml showed inhibitions of sporulation of 98.14 and 81.89 % respectively on *Cercospora* sp. and *P. heterocornis*.

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

Concentration (mg/ml)	<i>Cupressus lusitanica</i>	<i>Callistemon viminalis</i>	<i>Lantana camara</i>
<i>Cercospora</i> 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
<i>Colletotrichum gloeosporioides</i>			
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
<i>Pestalotia heterocornis</i>			
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).

## Discussion

### Fungi associated with cashew seeds

Twelve fungi species are associated with *Anacardium occidentale* L. seeds. These fungal species included *Acremonella atra*, *Aspergillus niger*, *Cercospora* sp., *Colletotrichum gloeosporioides*, *Epicoccum nigrum*, *Fusarium oxysporium*, *Mucor* sp. and *Pestalotia heterocornis*. Our results are inline 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

Comment [124]: Space

leaf spot caused by *Pestalotia-P.heterocornis* is one of the major fungal diseases of cashew in Burkina Faso.

On the other hand, species such as *Mucor* sp., *Epicoccum-E.nigrum*, *Acremonellia-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<sub>2</sub>) solution which would have had a detrimental effect on the frequency of these opportunistic fungi.

#### **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., *Colletotrichum-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 *Colletotrichum-C. kahawae* was more inhibited by *Carica papaya* and *Eucalyptus saligna* extracts when the concentrations applied were high. Aqueous and ethanolic extracts of *Cupressus-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*, *Cereospora-C. 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-P.colocasiae*, the causal agent of taro blight. Extracts of *Callistemon-C. 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 *Callistemon viminalis* on the development of seed-associated fungi of *Moringa oleifera*. In contrast, those of [37], reported that *Callistemon*

*viminalis* extracts inhibited the development of *Phytophthora P. colocasiae*, the causal agent of taro blight. This difference in results would be due to the fact that the test fungi (*Cercospora* sp., *Colletotrichum C. gloeosporioides* and *Pestalotia 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 *Ascochyta rabiei* and *Fusarium F. oxysporium* were more inhibited by ethanolic extracts of *Punica granatum* barks with the high concentrations.

### 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 *Eupressus E. lusitanica* and *Lantana 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.

### References

1. Coutinho IBL, Freire FCO, Lima JS, Gonçalves FJT, Machado AR, Silva AMS, Cardoso JE. Diversity of genus *Lasiodiplodia* associated with perennial tropical fruit plant in northeastern Brazil. Plant Pathology. 2016 ; 66 (1) : 90-104.
2. Dedehou ESCA, Dossou J, Soumanou MM. Etude diagnostique des technologies de transformation de la pomme de cajou en jus au Bénin. International Journal of Biological and Chemical Sciences. 2015 ; 9 (1) : 371-387.
3. Dieng F, Ngom D, Dia D, Sy R. Efficience technique de la production d'anacarde (*Anacardium occidentale* L.) dans les grandes régions de production du Sénégal. International Journal of Biological and Chemical Science. 2019 ; 13 (6) : 2627-2645.

4. Jules PND, Christine BDA, Bouba D, Dany BTT, Alain H, Serge BM, William NTK, Zachée A. Major fungi diseases of cashew trees (*Anacardium occidentale* L.) in Cameroon. Journal of Agricultural Science. 2020 ; 13 (3) : 124-134.
5. Martinez AR, Penarredona MA, Pheng B, Hoyos DE, Ting JCH, Alvarez NFP. Global Enterprise Experience ; 2011.
6. Dénis E, Tonon H, Afio Z, Rachidatou S, Aristide A, Valérien Z, Sharif B, Kouami N'D. Les pertes économiques dues à l'anthracnose de l'anacardier au Bénin. European Scientific Journal. 2018 ; 14 (15) : 127138.
7. ACFAM (Association des Communes Forestières du Cameroun). Etude de faisabilité technique et socio-économique de l'introduction de l'arboriculture d'anacarde par les collectivités territoriales décentralisées en zone de savane sèche du Cameroun. Centre Technique de la Forêt Communale ; 2020.
8. Sali B, Madou C, Nome A, Kuate J. Caractérisation socio-économique des grands bassins de productions d'anacardiers (*Anacardium occidentale*) et étude comportementale de leur peuplement dans le Cameroun septentrional. International Journal of Biological and Chemical Science. 2020 ; 14 (6) : 2094-2111.
9. Bezerra MA, Lacerda CF, Filho EG, Abreu CEB, Prisco JT. Physiology of cashew plants grown under adverse conditions. Brazilian Journal of Plant Physiology. 2007 ; 19 (4): 449-461.
10. Mekonnen G. Review on: Impact of Seed-Borne Pathogens on Seed Quality. American Journal of Plant Biology. 2020 ; 5 (4) : 77-81.
11. Sultana N, Ali Y, Jahan S, Yasmin S. Effect of Storage Duration and Storage Devices on Seed Quality of Boro Rice Variety BRR Idhan 47. Journal of Plant Pathology and Microbiology. 2016 ; 8 (2) : 386-392.
12. Wonni I, Drissa S, Ouédraogo I, Kassakogno AI. Diseases of cashew nut plants (*Anacardium occidentale* L.) in Burkina Faso. Advances in Plants and Agriculture Research. 2017 ; 6 (3) : 207-216.
13. Afouda LCA, Zinsou V, Balogoun RK, Onzo A, Ahohuendo BC. Inventaire des agents pathogènes de l'anacardier (*Anacardium occidentale* L.) au Bénin. Bulletin de la Recherche Agronomique du Bénin. 2013 ; 73 (1) : 13-19.
14. Chagas JFR, Sajio SA, Leao EU, Chagas AFJ, Giongo MV, Raimoundo WSA, Fidelis RR, Gil RDS. Sanitary analysis, transmissibility and pathogenicity of fungi associated with cashew nuts. African Journal of Agricultural Research. 2017 ; 12 (4) : 229-236.

Comment [I25]: Put the, No, pages, volume

Comment [I26]: ?

Comment [I27]: Put pages, vol, No

Comment [I28]:

15. Jemal T, Yalew M. Cashew production benefits and opportunities in Ethiopia: A Review. *Journal of Agricultural and Crop Research*. 2019 ; 7 (2) : 18-25.
16. Provost D, Anne C, Pierre L, Anne J, Véronique L, Hugues L, Anne V, Patrick B, Isabelle B. Brain tumours and exposure to pesticides : a case control study in south-western France. *Occupational and Environmental Medicine*. 2007 ; 64 (8) :509-514.
17. Toé AM, Guissou IP, Héma OS. Contribution à la toxicologie Agro-Industrielle au Burkina Faso. Etude des intoxications d'agriculteurs par des pesticides en zone cotonnière du Mouhoun. Résultats, analyse et proposition de prise en charge du problème. *Revue de médecine de travail*, tome XXIX, numéro unique ; 2002.
18. Okigbo RN, Nmeka IN. Control of Yam tuber rot with leaf Extracts of *Xylophia aethiopica* and *Zingiber officinale*. *African Journal of Biotechnology*. 2005 ; 4 (8) : 804-807.
19. Okigbo RN, Omodamiro OD. Antimicrobial effect of leaf extract of pigeon pea (*Cajanus cajan* (L) Mill sp.) on some human pathogen. *Journal of Herbs, Spices and Medicinal Plants*. 2006 ; 12 (1/2) : 117-127.
20. Ngoh-Dooch JP, Mbou TPR, Asta DBC, Deurnaye P, Djongnang G, Boydoul FU, Djile B, Ambang Z. *In vitro* and *in vivo* effect of Mancozeb 80 WP on development of *Pestalotia heterocornis* agent of leaf blight of Cashew in Far North Cameroon. *Journal of Applied Biosciences*. 2021 ; 162 (1) : 16694-16706.
21. Nyaka AICN, Fadimatou SNMZ, Dzokouo CUD, Bourou S, Yaouba A. Effet antifongique de deux extraits de plantes sur les agents pathogènes identifiés sur des fruits de l'anacardier (*Anacardium occidentale* L.) au Nord Cameroun. *International Journal of Biological and Chemical Science*. 2021 ; 15 (3) : 1121-1139.
22. Barnett HL, Hunter BB. Illustrated genera of imperfect fungi. 3rd edition. Burgess Publishing Company ; 1972.
23. Champion R. Identifier les champignons transmis par les semences. Institut Nationale de la Recherche Agronomique. 147, rue de l'Université, 75338, Paris Cedex, France ;1997.
24. Keuete KE, Tsopmbeng NG, Yaouba A, Djeugap FJ, Serferbe S. Antifungal potential of some plant extract against tree post-harvest fungal pathogens of avocado (*Persea americana*) fruits. *International Journal of Multidisciplinary Research and Development*. 2015 ; 2 (1) : 148-152.

Comment [I29]: Put, No, Pages & vol.

Comment [I30]:

Comment [I31]: Put the pages, No, Vol

Comment [I32]: ?



25. Amsalu A, Fihre L, Diriba M. The Antifungal Activity of Some Medicinal Plant against Coffee Berry Disease Caused by *Colletotrichum kahawae*. International Journal of Agriculture Research. 2011 ; 6 (3) : 268-279.
26. Yemo NY, Tsopmbeng NGR, Keuete KE, Nchongboh CG. Antifungal Activities of Plant Extracts against Coffee Berry Disease Caused by *Colletotrichum kahawae* L. International Journal of Current Research in Biosciences and Plant Biology. 2017 ; 4 (7) : 60-66.
27. Tsopmbeng GR, Fontem DA, Yamde KF. Evaluation of culture media for growth and sporulation of *Phytophthora colocasiae* Racib., causal agent of taro leaf blight. International Journal of Biological and Chemical Sciences. 2012 ; 6 (4) : 1566-1573.
28. Keuete KE, Tsopmbeng NGR, Kuate JR. Cross-Infectivity of *Colletotrichum* Isolates, Causal Agent of Anthracnose on Fruits in Three Regions of Cameroon. International Journal of Pure and Applied Bioscience. 2018 ; 6 (4) : 1-10.
29. Serferbe S, Tsopmbeng NG, Yaouba A, Djeugap FJ, Keuete KE. Efficacy of tree local plant extract as seed treatment on the germination, infection and vigour index of two cotton seed varieties from Chad. International Journal of Applied Biology and Pharmaceutical Technology. 2015 ; 6 (2) : 39-44.
30. Silué N, Abo K, Johnson F, Camara B, Koné M, Koné D. Evaluation *in vitro* et *in vivo* de trois fongicides de synthèse et d'un fongicide biologique sur la croissance et la sévérité de *Colletotrichum gloeosporioides* et de *Pestalotia heterornis*, champignons responsables de maladies foliaires de l'anacardier (*anacardium occidentale* L.) En Côte d'Ivoire. Agronomie Africaine. 2018 ; 30 (1) : 107-122.
31. Cardoso JE, Carlos AA, Rosseti AG, Vidal JC. Relationship between incidence and severity of cashew gummosis in semiarid north-eastern Brazil. Plant Pathology. 2004 ; 53 (3) : 363-367.
32. Everett KR, Boyd LM, Pak HA, Cutting JGM. Calcium, fungicide sprays and canopy density influence postharvest rots of avocado. Australasian Plant Pathology. 2007 ; 36 (4) : 223-230.
33. Ogbo EM, Oyibo AE. Effect of three plant extracts (*Ocimum gratissimum*, *Acalypha wilkesiana* and *A. macrostachya*) on post-harvest pathogen of *Persea americana*. Journal of medicinal plants research. 2008 ; 2 (11) : 311-314.
34. Keuete KE. Inventaire des champignons post-récoltes des fruits d'avocatier (*Persea americana* Mill) et essai de lute antifongique par l'utilisation des extraits de quelques

Comment [I33]: Not italic

plantes. Thèse de master II. Faculté des sciences, Université de Dschang, Cameroun ; 2014.

Comment [I34]: Put No, Pages, Vol.

35. Mousavi SM, Mirzargar SS, Mousavi HEZ, Baigi RO, Khosravi A, Bahonar A, Ahmadi MR. Evaluation of antifungal activity of new combined essential oils in comparison with malachite green on hatching rate in rainbow trout (*Oncorhynchus mykiss*) eggs. Journal Fisheries Aquatic Sciences. 2009 ; 4 (3) : 103-110.

36. Safary A, Motamedi H, Maleki S, Seyyednejad SM. A preliminary study on the antifungal activity of *Quercus brantii* against bacterial pathogens, particularly enteric pathogens. International Journal of of botany. 2009 ; 5 (1) : 176-180.

Comment [I35]:

Comment [I36]:

Comment [I37]:

37. Tsopmbeng NG, Megatche CJP, Lienou JA, Yaouba A, Djeugap FG, Fontem DA. Evaluation des activités antifongiques des extraits de plantes contre *Phytophthora colocasiae*, Agent causal du mildiou du taro (*Colocasia esculenta* (L.) Schott). Journal of Applied Biosciences. 2014 ; 81 (1) : 7221-7232.

Comment [I38]:

38. Serghat S, Mouria A, Ouazzani Touhami A, Badoc A, Douira A. Effet de quelques fongicides sur le développement *in-vitro* de *Pyricularia grisea* et *Helminthosporium oryzae*. Bulletin Société de Pharmacie Bordeaux. 2004 ; 143 (1) : 7-18.

39. Khedoudja K, Bouziane A, Mohamed LB, Fatima ZB, Bakhta M. Etude de l'Efficacité de l'Extrait Ethanolique d'Ecorces de *Punica granatum* L. sur deux Souches Phytopathogenes: *Ascochyta rabiei* (pass.) Labr. et *Fusarium oxysporum* f.sp.radicis – lycopersici. European Scientific Journal. 2014 ; 10 (12) : 301-315.