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Antifungal activity of cashew nut shell liquid against fungal pathogens associated with cashew diseases

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Abstract

Cashew production in northern Cameroon is constrained by fungal diseases. This study identified fungi associated with symptomatic cashew fruits and assessed the antifungal activity of ethanolic cashew nut shell liquid (CNSL) obtained by Soxhlet extraction (hot) and cold maceration. Symptomatic fruits were sampled in 14 localities of the North and Adamawa regions. Fungi were characterized by macro- and microscopic traits; pathogenicity was confirmed by inoculation and reisolation. CNSL yield, qualitative phytochemical screening, and antifungal activity were evaluated *in vitro* and *in vivo*. Eleven fungal taxa were recovered, with *Colletotrichum* sp. the most frequent in both regions (30.81% in the North; 22.47% in Adamawa). *Colletotrichum* sp., *Lasiodiplodia* sp., *Fusarium* sp., and *Aspergillus niger* caused lesions and were reisolated. The hot extract gave a higher yield than the cold extract (38.83% vs 33.60%); both contained phenols, tannins, flavonoids, terpenoids, steroids, saponins, and alkaloids. Complete *in vitro* growth inhibition occurred at 40 µg/mL for *A. niger*, 80 for *Fusarium* sp., 150 for *Lasiodiplodia* sp., and 300 for *C. gloeosporioides*. On inoculated fruits, the hot extract completely inhibited lesion development at 90, 100, 180, and 330 µg/mL, respectively; the cold extract required 330 µg/mL for *A. niger* and 360 µg/mL for the other fungi. CNSL, especially the hot ethanolic extract, is a promising plant-derived antifungal candidate; formulation, phytotoxicity, and orchard validation require further study.

Keywords: *Anacardium occidentale*; biocontrol; cashew nutshell liquid; detached-fruit assay; fungal pathogens; Soxhlet extraction.

Introduction

Cashew (Anacardium occidentale L.) is a tropical fruit tree of growing economic importance in many producing countries. The marketing of cashew nuts constitutes its main source of value, but cashew apples, nut shells, and other processing by-products also represent resources that can be valorized through local value chains. This versatility gives cashew an important role in agricultural-income diversification, the development of local processing, and the creation of economic opportunities in rural areas [1].

In Africa, particularly in West and Central African countries, cashew cultivation is gradually expanding. In Cameroon, it is mainly developing in the northern regions, where it represents a diversification opportunity for smallholder farms. However, increasing planted areas does not always lead to a proportional improvement in orchard productivity. Several technical,

climatic, and biotic constraints may compromise tree growth, flowering, fruiting, yield, and the sanitary quality of harvested products.

Among these constraints, fungal diseases occupy a prominent place. They affect different cashew organs, including leaves, inflorescences, fruits, buds, and twigs, and may cause yield losses as well as a deterioration in the commercial quality of nuts and apples. The main reported diseases include anthracnose, leaf spots and blights, fruit rots, bud dieback, twig dieback, pestalotiosis, powdery mildew, and black rust [2–4]. Anthracnose, frequently associated with species of the genus *Colletotrichum*, is particularly concerning because of its impact on the vegetative and reproductive organs of the tree . [5].

In Cameroon, Ngoh Dooh et al. [5] documented several fungal diseases of cashew and the involvement of genera such as Colletotrichum, Lasiodiplodia, Pestalotia, and Curvularia. However, the observation of symptoms or the isolation of a fungus from diseased tissue is not sufficient to establish a causal relationship. Confirmation of pathogenic status requires pure-culture isolation, isolate characterization, inoculation of healthy tissues, observation of induced symptoms, and re-isolation of the microorganism. This step is essential to distinguish true pathogens from microorganisms merely associated with lesions.

The management of fungal diseases still relies largely on the use of synthetic fungicides. Although their effectiveness has been demonstrated against certain agents, particularly Colletotrichum gloeosporioides associated with cashew anthracnose [5], their use may be limited by product cost, availability to smallholder farmers, and concerns related to repeated applications. Risks of residues, potential effects on agroecosystems, and the selection of pathogen populations less sensitive to fungicides justify the search for more accessible alternatives compatible with sustainable disease management [7]

Within this perspective, cashew nut shells represent an interesting resource. They contain cashew nut shell liquid (CNSL), a natural liquid rich in bioactive compounds, particularly phenolic derivatives. The valorization of CNSL has a dual benefit: reducing residues generated by nut processing and developing a natural substance with potential agronomic applications. However, CNSL properties may vary according to shell origin, solvent, and extraction conditions; its physicochemical and phytochemical characterization is therefore necessary before any biological evaluation [7, 8].

Several studies have already demonstrated the antifungal activity of CNSL against different phytopathogenic fungi. Garcia et al. [9] notably showed its potential against Colletotrichum gloeosporioides and Lasiodiplodia theobromae. CNSL extracts have also been reported to

be active against species of Fusarium, Lasiodiplodia, and Aspergillus associated with yam dry rot [8]. Nevertheless, data remain limited regarding fungi associated with cashew symptoms in northern Cameroon, their actual pathogenicity, and their sensitivity to hot- and cold-extracted ethanolic CNSL extracts.

Thus, this study primarily aimed to evaluate the antifungal potential of cashew nut shell liquid against the major fungal pathogens associated with cashew diseases in northern Cameroon. It consisted of isolating and characterizing fungi associated with the observed symptoms, confirming the pathogenicity of the main isolates on healthy fruits through inoculation and re-isolation, and characterizing CNSL extracts obtained by hot and cold ethanolic extraction. The antifungal activity of these extracts was then evaluated *in vitro* against mycelial growth and *in vivo* on inoculated fruits.

2. Materials and methods

2.1. Study sites and agroecological context

The study was conducted in two main cashew-producing areas in Cameroon, namely the Adamawa and North regions. These areas have contrasting agroecological characteristics. The North Region belongs to the Sudano-Sahelian agroecological zone (AEZ1). It is characterized by relatively high temperatures and lower annual rainfall. In contrast, the Adamawa Region belongs to the Guinean high savanna or Sudano-Guinean agroecological zone (AEZ2), characterized by relatively higher rainfall and generally more moderate temperatures. A total of 14 localities were selected for field observations. In the North Region, investigations were carried out in Djola-Bame, Mafakilda, Sanguéré Paul, Sanguéré Ngal, Ngoura II, and Ngong. In the Adamawa Region, the study localities were Mbé, Tchabal, Dang, Gada-Mabanga, Marza, Yoko, Wakwa, and Nyambaka. The main bioclimatic and geographical characteristics of the two study areas are presented in Table 5 *[10], while their spatial distribution is illustrated in Figure 1.*

Table 1. Main bioclimatic and geographical characteristics of the study areas [10].

Région	AEZ	Altitude (m)	Precipitation[a] (mm)	Température[b] (°C)
North	AEZ1	300–1400	400–1200	28–45
Adamawa	AEZ 2	400–2000	600–1500	20–32

AEZ: agroecological zone [10, 11].

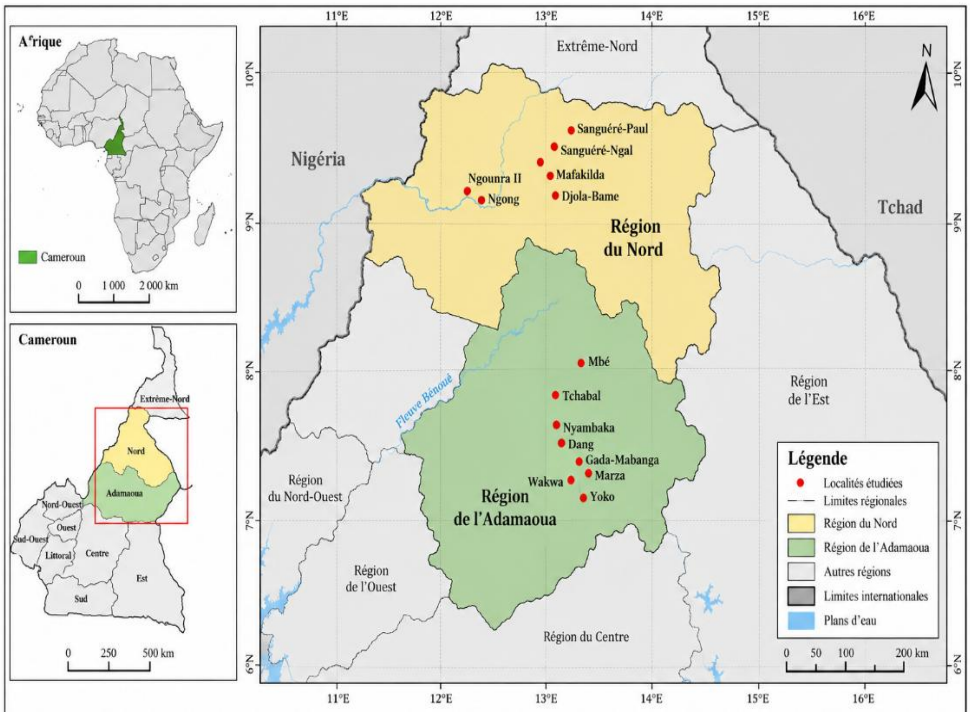


Figure 1. Geographical location of the study sites in the North and Adamawa regions of Cameroon.

2.2. Isolation and characterisation of fungi associated with symptoms on cashew fruits

2.2.1. Sampling of symptomatic fruits

Cashew fruits displaying characteristic symptoms of fungal diseases were collected from the surveyed orchards. The sampled fruits exhibited necrotic lesions, brown or black spots, rots, superficial depressions, or desiccated areas. Sampling was conducted to cover the diversity of symptoms observed across the different localities. Each symptomatic fruit was individually wrapped in clean paper, labelled according to its collection site and symptom type, and transported to the Microbiology Laboratory of the Wakwa Agricultural Research Centre for mycological analyses. This approach was adapted from studies on the identification of fungal diseases of cashew in Cameroon [5].

2.2.2. Isolation and purification of fungi

Fungi were isolated from symptomatic cashew fruits. After washing under running water, small fragments of approximately 2 mm² were excised from the interface between apparently healthy and necrotic fruit tissues. The fragments were surface-disinfected in a 1% sodium hypochlorite solution, rinsed three times with sterile distilled water, and dried on sterile absorbent paper.The fragments were then placed on Potato Dextrose Agar (PDA) supplemented with an antibiotic to limit bacterial growth. Petri dishes were incubated at 25 ± 2 °C for 5–7 days. The resulting fungal colonies were successively subcultured on fresh

PDA, using hyphal tips, until pure cultures were obtained. This method was adapted from protocols for the isolation of phytopathogenic fungi from symptomatic plant tissues [5, 8].

2.2.3. Morphological characterisation and isolation frequency

Pure isolates were characterised based on their cultural and microscopic features. Cultural characteristics included colony colour, texture, appearance, reverse pigmentation, and growth rate on PDA. Microscopic observations were performed using an Olympus CX23 light microscope at ×400 magnification to examine hyphae, conidiophores, conidia, and other reproductive structures.

Identification was considered morphological and presumptive; in the absence of molecular analyses, isolates were assigned to genera or morphotypes. This approach is commonly used in preliminary studies of fungi associated with diseases of cashew and other tropical crops [5, 8].

The isolation frequency of each fungal morphotype in each region was calculated using the following formula:

$$F(\%) = \frac{N_i}{N_{ti}} \times 100$$

where (Ni) is the number of isolates belonging to morphotype (i), and (Nti) is the total number of isolates obtained in the region considered.

2.3. Assessment of the pathogenicity of fungal isolates on cashew fruits

2.3.1. Inoculation and re-isolation

The pathogenicity of the main isolates was assessed on healthy cashew fruits that were physiologically mature but not fully ripe and showed no visible symptoms or mechanical injuries. The fruits were washed under running water, surface-disinfected in 1% sodium hypochlorite for 2 min, and rinsed three times with sterile distilled water.

A superficial wound, 3–4 mm in diameter and 2–3 mm deep, was made on each fruit using a sterile instrument. A 5-mm mycelial plug taken from the actively growing margin of a 7-day-old pure culture was placed on the wounded area. Five fruits were inoculated per isolate. Negative controls received a sterile PDA plug without mycelium.

The inoculated fruits were maintained in a humid chamber at 25 ± 2 °C for 5 days. The occurrence and progression of lesions were monitored daily. Fragments were collected from the margins of induced lesions and subjected to the same isolation procedure to re-isolate

the fungus. An isolate was considered pathogenic when symptoms similar to those initially observed were reproduced and the same fungal morphotype was re-isolated from lesions, but not from control fruits. The protocol was adapted from pathogenicity studies on fruits and tissues of tropical hosts [5, 8, 12].

2.3.2. Lesion measurement and severity assessment

Lesion diameter was measured along two perpendicular axes: length ((L)) and width ((l)). The mean lesion diameter was calculated as follows:

Dm = (L + l) / 2.The severity of pathogenicity was assessed based on the mean lesion diameter:

- very low: Dm < 5 mm;
- moderate: Dm : 5-10 mm;
- significant: Dm 10-20 mm;
- severe: (Dm> 20 mm [12].

2.4. Hot ethanol extraction of cashew nut shell liquid

Cashew nut shells were air-dried, ground, and reduced to powder. A mass of 350 g of shell powder was placed in a Soxhlet apparatus and extracted with 2 L of ethanol. Extraction was continued until apparent exhaustion of the plant material.

After extraction, the solvent was removed using a rotary evaporator at 40 °C. The resulting extract, referred to as the hot ethanol extract of cashew nut shell liquid, was weighed and stored in a tightly closed amber bottle until analysis. This method was adapted from studies using Soxhlet extraction for CNSL recovery and characterisation [7, 8].


A


B


CA

Figure 2. Cashew nut shells (A), powdered cashew nut shells (B), and cashew nut shell liquid (CNSL) (C).

2.5. Cold ethanol extraction of cashew nut shell liquid

For cold extraction, 350 g of cashew nut shell powder were macerated in 2 L of ethanol, corresponding to a solid-to-solvent ratio of 1:5.7 w/v. The mixture was maintained at 25 ± 2 °C for 72 h with periodic agitation. After maceration, the mixture was filtered through Whatman No. 1 filter paper. The filtrate was concentrated under reduced pressure using a rotary evaporator at 40 °C. The resulting extract was weighed and stored in an amber bottle until biological assays. Extraction yield was calculated using the following formula:

$$Extraction\ yield\ (\%) = \frac{Mass\ of\ extract\ obtained}{Dry\ mass\ of\ cashew\ nut\ shells} \times 100$$

where (Me) is the Mass of the extract obtained and (Md) is the initial mass of shell powder. This method was adapted from published procedures on solvent extraction and characterisation of CNSL [7, 13].

2.6. Physicochemical and phytochemical characterisation of the extracts

2.6.1. Density

The density of the extracts was determined using a calibrated 25-mL pycnometer. The empty pycnometer, the pycnometer filled with distilled water, and the pycnometer filled with CNSL extract were weighed. The use of a pycnometer was adapted from CNSL characterisation methods reported in the literature (Sekunowo & Uduh, 2022).

$$Density = \frac{Mass\ of\ CNSL}{Volume\ of\ CNSL}$$

[13]

2.6.2. Moisture content

Moisture content was determined by oven drying. A 0.5-g aliquot of extract was placed in a previously weighed crucible and dried at 100 °C for 2 h. After cooling in a desiccator, the crucible was weighed again. Moisture content was calculated using the following formula:

$$Moisture\ content\ (\%) = \frac{M_1 - M_2}{M_1 - M_0} \times 100$$

where (M0) is the mass of the empty crucible, (M1) is the mass of the crucible containing the extract before drying, and (M2) is the mass of the crucible containing the extract after

drying. This approach was adapted from methods used to determine CNSL moisture content [13].

2.6.3. Apparent pH

The apparent pH of the extracts was measured using a Hanna HI98107 portable pH meter, previously calibrated with buffer solutions at pH 4.01 and 7.01. A 20-mL aqueous dispersion of the extract was prepared, and pH was measured in triplicate. This measurement should be reported as the apparent pH of the aqueous dispersion, as CNSL is poorly soluble in water [9, 13].

2.6.4. Qualitative phytochemical screening

Qualitative phytochemical screening of the hot- and cold-extracted CNSL was carried out to detect selected groups of secondary metabolites:

- phenolic compounds, using 2% ferric chloride, indicated by a violet colour;
- alkaloids, using Dragendorff's reagent, indicated by an orange precipitate;
- saponins, using the persistent foam test;
- flavonoids, indicated by a red colour;
- steroids and triterpenes, using the Salkowski test, indicated by a reddish colour [8].

2.7. Evaluation of *in vitro* antifungal activity

2.7.1. Poisoned food assay

The antifungal activity of hot- and cold-extracted CNSL was assessed using the poisoned food technique. The extracts were first solubilised in a small amount of dimethyl sulfoxide (DMSO) and then incorporated into cooled sterile PDA to obtain final concentrations of 10, 20, 40, 80, 150, and 300 µg/mL. Approximately 15 mL of treated PDA medium was poured into each Petri dish. After solidification, a 5-mm mycelial plug taken from the actively growing margin of a 7-day-old pure culture was placed at the centre of each plate. The assays were conducted using the selected pathogenic isolates, including Colletotrichum sp., Lasiodiplodia sp., Fusarium sp., and Aspergillus niger. Each treatment was replicated five times in a completely randomised design. Controls included a negative control without CNSL, a solvent control containing the same final concentration of DMSO as the treatments, and a positive control consisting of mancozeb 80 WP at 500 µg/mL. Plates were incubated at 25 ± 2 °C. The method was adapted from CNSL antifungal activity assays using the poisoned food technique [8, 9].

2.7.2. Assessment of mycelial inhibition, IC_{50}, and MIC

Mycelial growth diameter was measured along two perpendicular axes. The percentage inhibition of mycelial growth was calculated relative to the negative control using the following formula:

$$PI = [(C - T) / C] \times 100$$

where (C) is the mean colony diameter in the negative control and (T) is the mean colony diameter in the corresponding treatment.

The 50% inhibitory concentration (IC_{50}) was estimated by regression between the logarithm of concentration and the percentage inhibition. The minimum inhibitory concentration (MIC) was defined as the lowest concentration showing no visible mycelial growth. The percentage inhibition was calculated according to the method of Dohou et al. [14].

2.7.3. Determination of fungistatic or fungicidal effects

To distinguish fungistatic from fungicidal effects, mycelial plugs from treatments showing no visible fungal growth were transferred to fresh PDA without CNSL. Resumption of growth was interpreted as a fungistatic effect, whereas the absence of growth resumption was considered a fungicidal effect [15].

2.8. Evaluation of *in vivo* antifungal activity on cashew fruits

The *in vivo* antifungal activity of CNSL extracts was evaluated on healthy cashew fruits that were disinfected and artificially inoculated with the selected pathogenic isolates. After disinfection, a superficial wound was made on each fruit, and a 5-mm mycelial plug from a 7-day-old pure culture was placed on the wounded area. Forty-eight hours after inoculation, the fruits were treated by immersion in CNSL solutions at concentrations of 25, 40, 60, 90, 100, 180, and 330 µg/mL. For the cold extract, an additional concentration of 360 µg/mL was tested when complete inhibition was not obtained at lower concentrations. Mancozeb 80 WP at 500 µg/mL was used as the positive control. Negative controls consisted of inoculated but untreated fruits, whereas solvent controls were treated with the DMSO solution without CNSL. Each treatment included five replicates per isolate and per concentration. After treatment, fruits were maintained in a humid chamber at 25 ± 2 °C for 13 days. Mean lesion diameter was calculated from two perpendicular measurements. The percentage inhibition of lesion development was determined relative to the inoculated untreated control

$$I(\%) = \frac{D_c - D_t}{D_c} \times 100$$

where (Dc) is the mean lesion diameter in the negative control and (Dt) is the mean lesion diameter in the treatment [9].

3 Results

3.1. Types of diseases and symptoms observed

Field surveys revealed five diseases, namely anthracnose, gummosis, leaf stunting, fruit rot, and *die-back*. Anthracnose was characterized by brown to black necrotic spots on leaves and fruits, which could develop into sunken lesions and fruit rot. Gummosis was manifested by lesions on the trunk or branches accompanied by gum exudation, whereas leaf stunting was associated with reduced leaf size and deformation of the leaf blade. Fruit rot was characterized by tissue browning followed by softening, whereas *die-back* was characterized by progressive drying of twigs and leaves, which could lead to branch death. The main symptoms observed are illustrated in Figure 3.

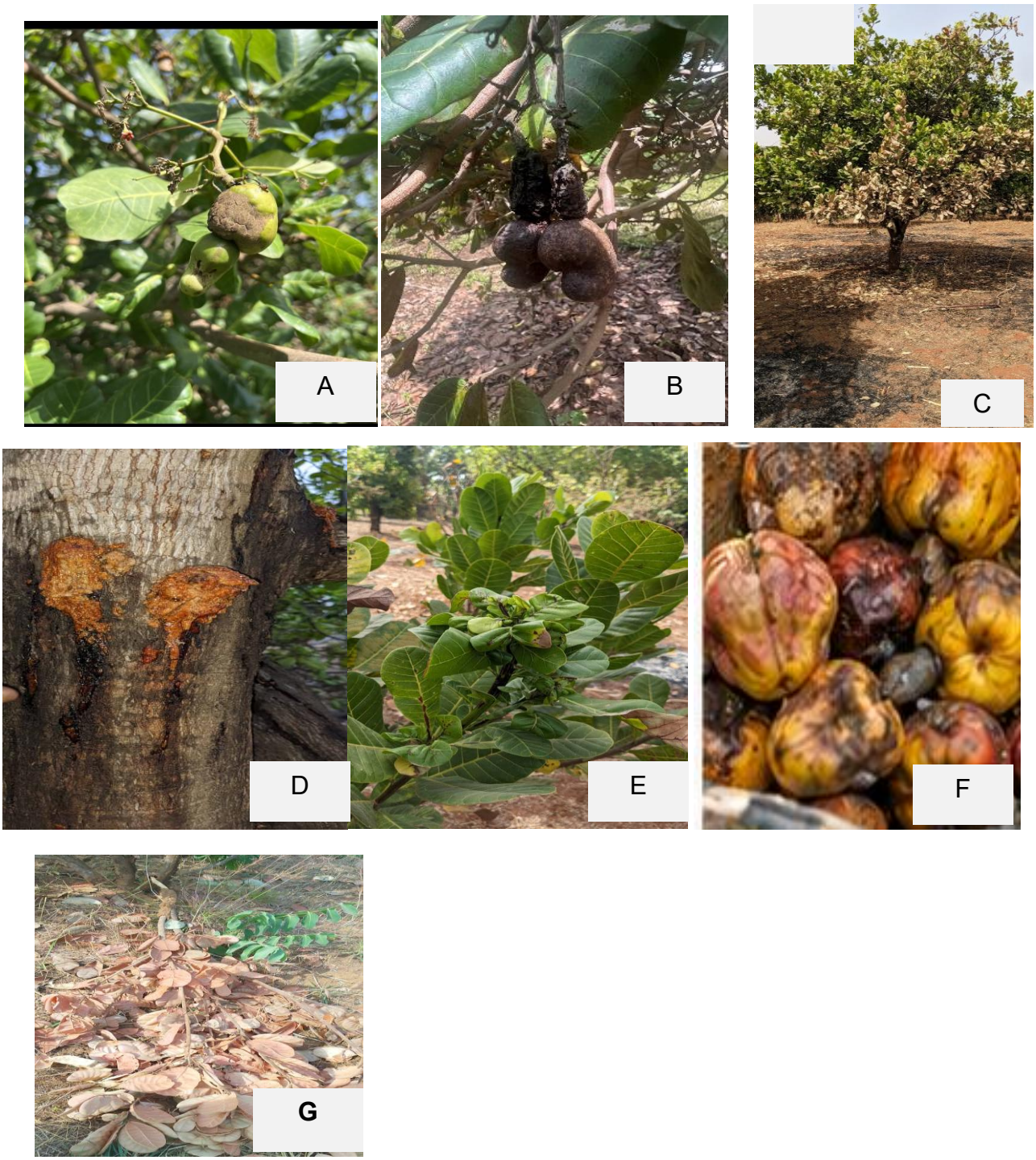


Figure 3. Anthracnose on fruits (A, B), anthracnose on leaves (C), gummosis (D), leaf stunting (E), fruit rot (F), and *die-back* (G).

3.2. Diversity of fungi isolated from symptomatic organs

Eleven fungal taxa were recognised based on their macroscopic and microscopic characteristics: *Colletotrichum* sp., *Lasiodiplodia* sp., *Fusarium* sp., *Aspergillus niger*, *Curvularia* sp., *Alternaria* sp., *Pestalotia* sp., *Verticillium* sp., *Didymella* sp., *Penicillium* sp., and *Cercospora* sp.

3.3. Frequency of fungal isolation

Colletotrichum sp. showed the highest isolation frequency in both regions, with 30.81% in the North and 22.47% in Adamawa. *Lasiodiplodia* sp. was particularly frequent in the North (19.75%), whereas *Fusarium* sp., *Alternaria* sp., *Verticillium* sp., and *Cercospora* sp. were proportionally more represented in Adamawa.

Table 2. Frequency of morphologically assigned fungal taxa recovered from symptomatic cashew tissues in the North and Adamawa regions of Cameroon.

Fungi	North	Adamawa
Colletotrichum sp.	30,81	22,47
Lasiodiplodia sp.	19,75	2,10
Pestalotia sp.	12,03	10,40
Didymella sp.	12,05	4,23
Curvularia sp.	9,31	3,91
Fusarium sp.	5,19	14,71
Aspergillus niger	5,25	8,21
Cercospora sp.	2,01	10,23
Alternaria sp.	1,58	11,66
Verticillium sp.	1,45	10,34
Penicillium sp.	0,13	0,00

Frequency was calculated independently within each region as the proportion of recovered isolates assigned to the taxon. Taxonomic assignments are morphology based.

3.4. Pathogenicity and re-isolation

All four selected isolates induced lesions on wounded fruits, whereas the sterile-PDA control remained symptom free. *Colletotrichum* sp. produced the largest mean lesion diameter (24.40 ± 1.31 mm), followed by *Lasiodiplodia* sp. (18.32 ± 0.76 mm), *Fusarium* sp. (15.68 ± 0.33 mm), and *Aspergillus niger* (13.54 ± 0.21 mm). The re-isolated cultures were morphologically concordant with their respective inoculated isolates. These four isolates were consequently retained for CNSL assays.

Table 3. Pathogenicity and morphotype-level re-isolation of selected fungi on detached cashew fruit.

Inoculum	Lesion diameter (mm)	Re-isolation result
Colletotrichum sp.	24.40 ± 1.31 [a]	Concordant morphotype recovered
Lasiodiplodia sp.	18.32 ± 0.76 [b]	Concordant morphotype recovered
Fusarium sp.	15.68 ± 0.33 [c]	Concordant morphotype recovered
Aspergillus niger	13.54 ± 0.21 [d]	Concordant morphotype recovered
Sterile PDA plug	0.00 ± 0.00[e]	No fungus recovered

Different letters indicate Tukey-separated means at α = 0.05. Re-isolation confirms only morphological concordance with the inoculated culture.

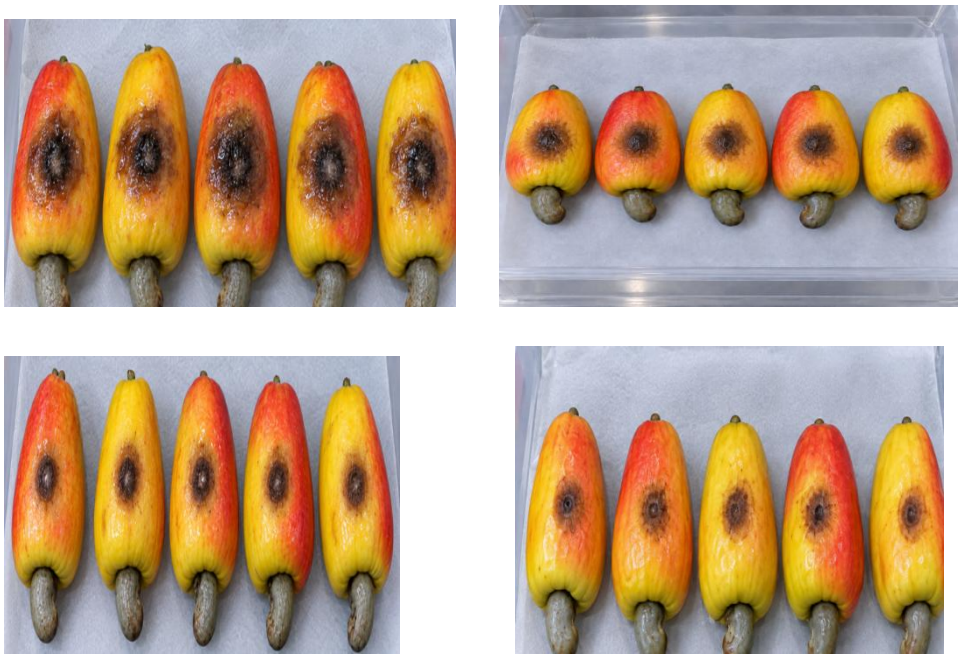


Figure 4. Representative lesions on detached cashew fruit following inoculation with (A) *Colleto-trichum* sp., (B) *Lasiodiplodia* sp., (C) *Fusarium* sp., and (D) *Aspergillus niger*. Original embedded species-level labels were removed because identifications in this study were morphology based.

3.5. Extraction yield and basic extract characteristics

Soxhlet extraction yielded more crude ethanolic extract than cold maceration. The hot system produced 135.91 ± 2.43 g of extract (38.83 ± 1.34%), compared with 117.60 ± 4.56 g (33.60 ± 0.32%) after maceration. The cold extract had a slightly higher density and

moisture content, whereas the hot extract showed a higher apparent pH in the standardized dispersion. All qualitative reactions were positive for both extracts.

Table 4. Yield and basic physicochemical characteristics of ethanolic CNSL prepared by hot Soxhlet extraction and cold maceration.

Parameter	Hot extract	Cold extract
Extraction route	Soxhlet (hot)	Maceration (cold)
Shell mass per batch (g)	350	350
Ethanol volume per batch (L)	2.0	2.0
Extract mass (g)	135.91 ± 2.43 ^{a}	117.60 ± 4.56 ^{b}
Yield (%)	38.83 ± 1.34 ^{a}	33.60 ± 0.32 ^{b}
Density at 30 °C	0.933 ± 0.002 ^{b}	0.953 ± 0.003 ^{a}
Moisture content (%)	2.78 ± 0.09 ^{b}	3.31 ± 0.11 ^{a}
Apparent pH of aqueous dispersion	4.75 ± 0.04 ^{a}	4.26 ± 0.05 ^{b}

Values are mean ± SD. For a given parameter, values bearing different letters differ at α = 0.05 by Tukey's test. Yield is based on three independent extraction batches; physicochemical determinations were performed in triplicate.

Table 5. Qualitative phytochemical reactions of the hot and cold ethanolic CNSL extracts.

Class screened	Observed reaction	Hot extract	Cold extract
Phenolics	Violet coloration with $FeCl_3$	Positive	Positive
Flavonoids	Red coloration	Positive	Positive
Alkaloids	Orange precipitate with Dragendorff reagent	Positive	Positive
Steroids	Red Salkowski-type reaction	Positive	Positive
Saponins	Persistent stable foam	Positive	Positive

3.6. *In vitro* activity and fungitoxicity

Mycelial-growth inhibition increased with CNSL concentration for all four fungi (Figure 2). With hot-extracted CNSL, complete visible inhibition was reached at 40 μg mL^{-1} for *Aspergillus niger*, 80 μg mL^{-1} for *Fusarium* sp., 150 μg mL^{-1} for *Lasiodiplodia* sp., and 300 μg mL^{-1} for *Colletotrichum* sp. Cold-extracted CNSL required higher thresholds for the first three isolates (80, 150, and 300 μg mL^{-1}, respectively); *Colletotrichum* sp. remained below complete inhibition at 300 μg mL^{-1}. At 300 μg mL^{-1}, its inhibition was 98.73 ± 0.50%.

Plugs taken from the hot-extract plates with complete inhibition did not resume growth on CNSL-free PDA, consistent with fungicidal activity under the test conditions. For the cold extract, a fungistatic effect was observed at 80 μg mL^{-1} against *Fusarium* sp.; absence of regrowth was observed at 150 μg mL^{-1}. The cold route achieved no complete inhibition against *Colletotrichum* sp. within the 10–300 μg mL^{-1} range, so a fungitoxicity classification was not assigned for this isolate at the tested doses.

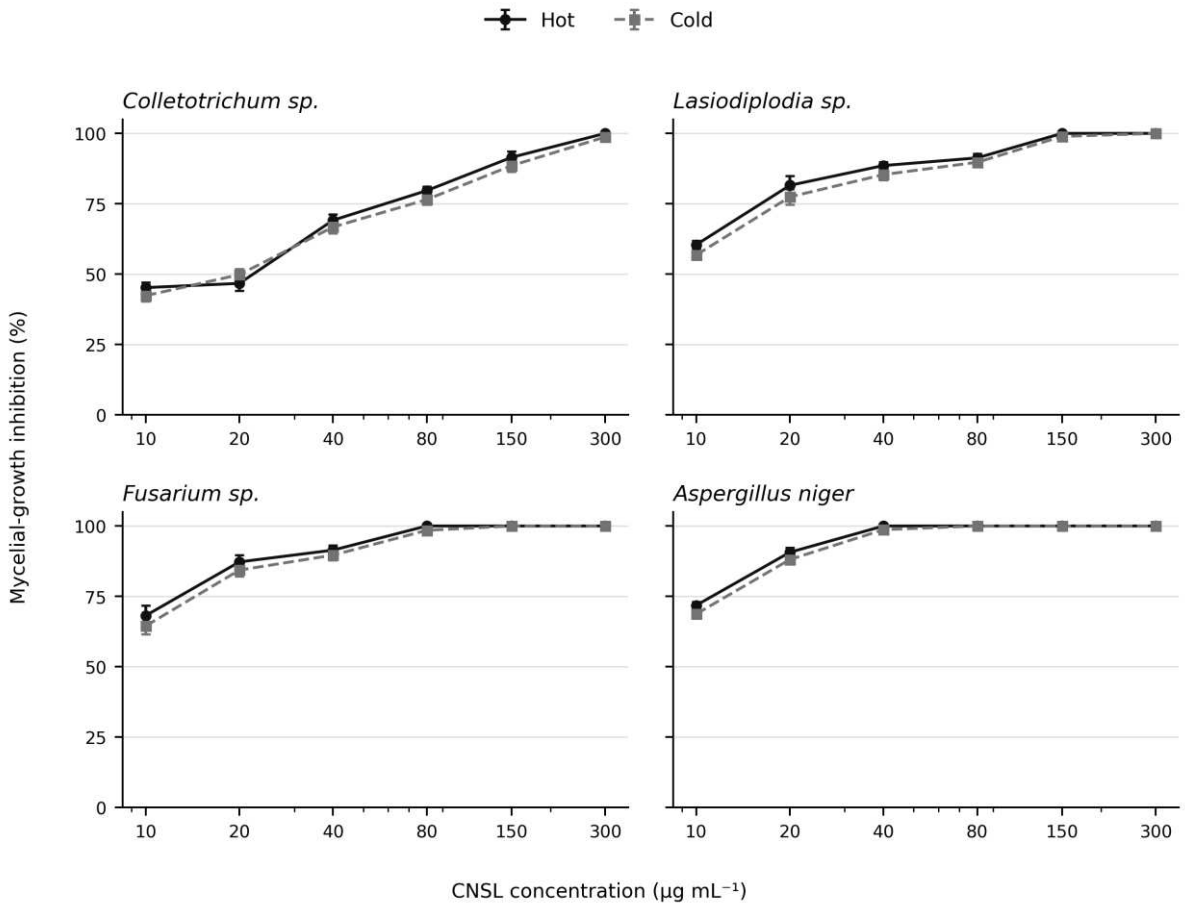


Figure 5. Concentration-response curves for inhibition of fungal mycelial growth by hot- and cold-extracted ethanolic CNSL on PDA.

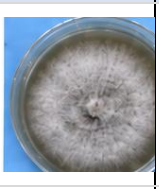
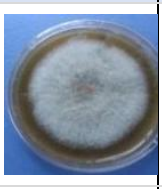
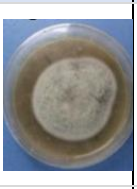
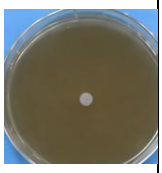
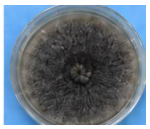
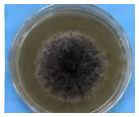
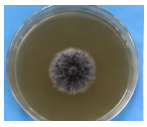
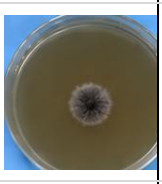
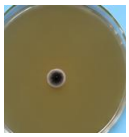
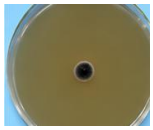
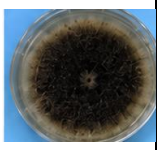
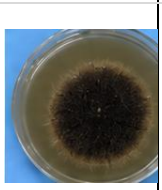
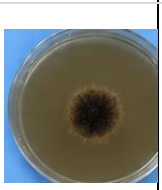
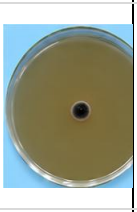
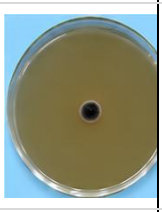
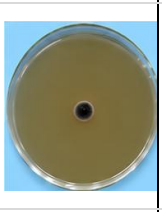
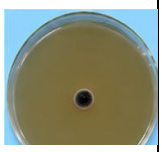
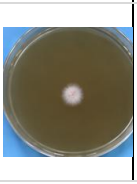
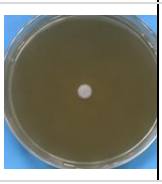
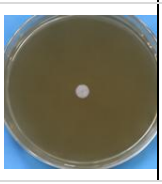
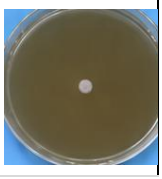
Fungi	0 µg/mL	10 µg/mL	20 µg/mL	40 µg/mL	80 µg/mL	150 µg/mL	300 µg/mL
Colletothicum sp.							
Lasiodiplodia sp.							
A. niger							
Fusarium sp.							

Table 6. Summary of the *in vitro* inhibitory thresholds and viability outcomes for ethanolic CNSL.

3.7. Curative activity on detached fruits

Inhibition of lesion development on inoculated detached fruits increased with CNSL concentration (Figure 3). Higher concentrations were generally required on fruit than on PDA. The hot extract achieved complete lesion inhibition at 90 µg mL^{-1} for *Aspergillus niger*, 100 µg mL^{-1} for *Fusarium* sp., 180 µg mL^{-1} for *Lasiodiplodia* sp., and 330 µg mL^{-1} for *Colletotrichum* sp. In contrast, the cold extract required 330 µg mL^{-1} for *Aspergillus niger* and 360 µg mL^{-1} for the remaining three isolates. *Colletotrichum* sp. was the least responsive isolate in both assay systems.

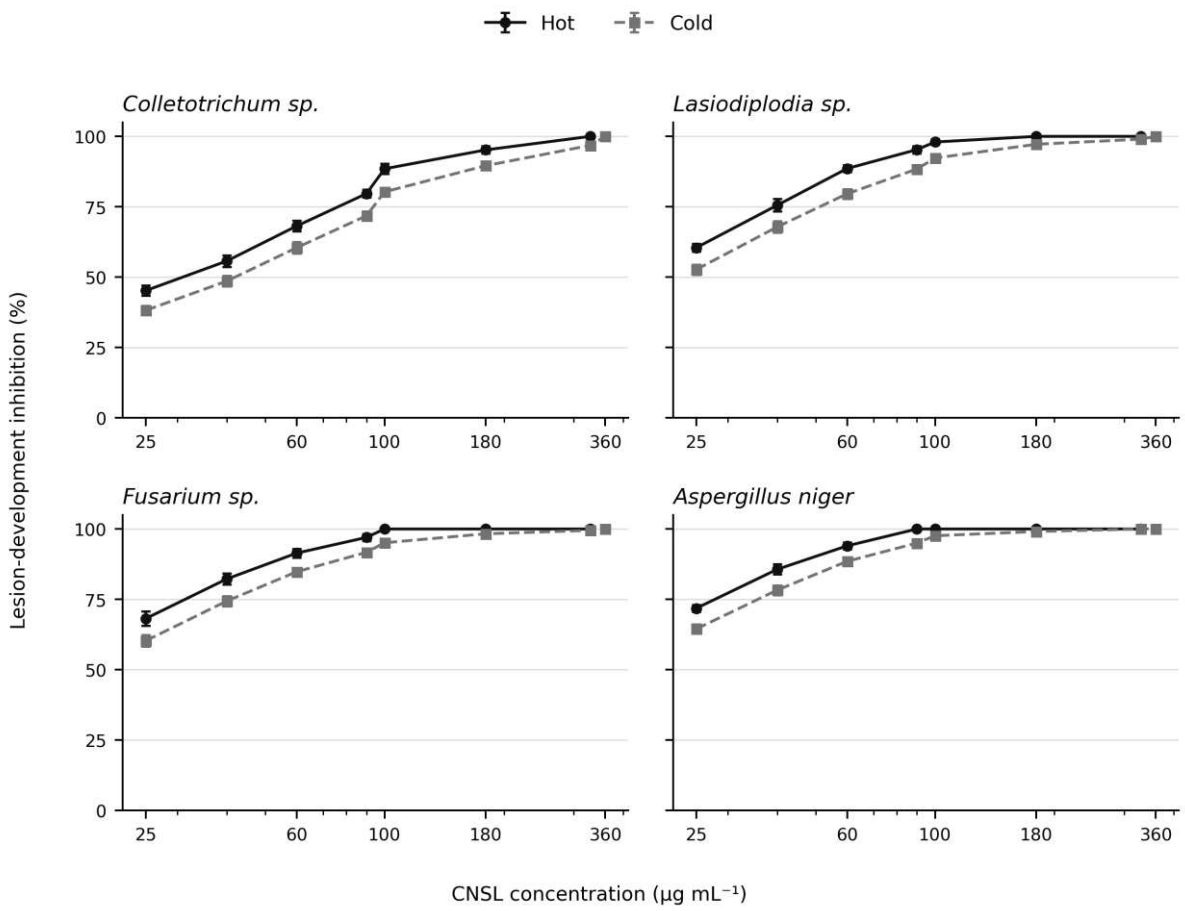


Figure 6. Curative concentration-response curves for hot- and cold-extracted ethanolic CNSL on detached cashew fruit inoculated with each fungal isolate. CNSL was applied 48 h after inoculation. Points are means and error bars are SD (n = 5 fruits per treatment).

Table 7. Concentrations associated with complete lesion inhibition and estimated EC50 values in the detached-fruit assay.

Fungus	Hot: complete lesion inhibition	Cold: complete lesion inhibition	Hot EC50	Cold EC50
Colletotrichum sp.	330	360	31.8	42.4
Lasiodiplodia sp.	180	360	20.7	23.7
Fusarium sp.	100	360	18.3	20.7
Aspergillus niger	90	330	17.4	19.4

All concentrations are µg mL⁻¹. Complete lesion inhibition refers to 100% inhibition of lesion development in this controlled detached-fruit experiment. EC50 values are log-probit estimates.

4 Discussion

The predominance of Colletotrichum sp. in both regions, with isolation frequencies of 30.81% in the North and 22.47% in Adamawa, confirms that this genus is a major fungal group associated with cashew diseases. From an agronomic perspective, this high occurrence is of concern because Colletotrichum species are generally involved in anthracnose, a disease capable of simultaneously affecting leaves, inflorescences, young shoots, and fruits. This trend is consistent with several studies. In Côte d'Ivoire, Silué et al. [16] identified Colletotrichum gloeosporioides as the most frequently isolated fungus in cashew orchards, with a mean frequency of 63.45%. In Cameroon, Ngoh Dooh et al. [5] also associated Colletotrichum with anthracnose, bud dieback, and certain foliar necroses of cashew. Similarly, Soro et al. [17], in Côte d'Ivoire, obtained 85 isolates of C. gloeosporioides during their study of anthracnose and reported a national mean severity index of 28.48%. These findings confirm the agronomic importance of this fungal group in cashew production systems in West and Central Africa.

The relatively high frequency of *Lasiodiplodia* sp. in the North region (19.75%) also deserves particular attention. From an agronomic perspective, this result may indicate increased orchard vulnerability to fungi associated with twig dieback, gummosis, and fruit rots. *Lasiodiplodia* species are often favoured by wounds, tissue ageing, and stress affecting trees. In the hot and drier conditions of the North region, this frequency could therefore be linked to interactions among water stress, high temperatures, tree physiological status, and inadequate sanitation practices. However, this interpretation remains an agronomic hypothesis, as the direct effects of climatic factors on the isolates were not assessed in the present study.

This observation is supported by Dianda et al. [18] in Burkina Faso, who identified several Lasiodiplodia species associated with gummosis and dieback of cashew. The isolates were pathogenic and were able to induce dieback symptoms on cashew.

Hot ethanol extraction produced a higher CNSL yield than cold maceration, with 38.83% and 33.60%, respectively. This difference of 5.23 percentage points, corresponding to an approximately 16% increase with hot extraction, suggests that heating promotes solvent diffusion into the shell matrix and the solubilisation of extractable compounds. Continuous solvent renewal in the Soxhlet apparatus may also contribute to a more complete recovery of CNSL. The resulting extract was dark brown and viscous, which is consistent with the typical appearance of CNSL.

This result is comparable to that of Nyirenda et al. [19] in Zambia, who obtained an ethanol extraction yield of 38.2%, a value close to that recorded with hot extraction in the present study. Similarly, Alaka et al. [20] in Nigeria showed that hot extraction generally produced higher CNSL yields than cold extraction, regardless of the solvent used. However, differences among studies may depend on solvent type, solid-to-solvent ratio, shell particle size, shell pre-treatment, extraction duration, and the origin of the plant material.

Phytochemical screening showed that the hot- and cold-extracted CNSL had a broadly similar qualitative profile, characterised by a strong presence of phenolic compounds, as well as tannins, flavonoids, terpenoids, steroids, saponins, and alkaloids. This similarity indicates that both extraction methods recovered the main groups of CNSL bioactive compounds. However, it does not demonstrate that these compounds were present at identical concentrations in the two extracts, since the tests were qualitative.

These findings agree with those of Sanyaolu et al. [8], who reported phenolic compounds, flavonoids, tannins, alkaloids, saponins, terpenoids, and steroids in CNSL. The strong positive reaction for phenolic compounds in the present study is particularly relevant because CNSL phenolic derivatives, including anacardic acids, cardol, and cardanol, are considered the main compounds likely to contribute to its biological activity. Nevertheless, hot extraction may partially modify CNSL composition. León et al. [21] showed that Soxhlet extraction may increase cardanol content and alter certain physicochemical properties of CNSL. Thus, the main advantage of hot extraction lies in its higher yield, but comparisons between the two extracts should also consider their actual antifungal activity.

The CNSL showed a clear concentration-dependent antifungal activity against the four fungi tested. *Aspergillus niger* was the most sensitive, with complete inhibition of mycelial growth at 40 µg/mL, followed by *Fusarium* sp. at 80 µg/mL, *Lasiodiplodia* sp. at 150 µg/mL, and *Colletotrichum gloeosporioides* at 300 µg/mL. This variation reflects differences in fungal sensitivity to CNSL. From an agronomic perspective, this result is important because CNSL was active against several agents associated with fruit rots and anthracnose. However, the higher concentration required for *C. gloeosporioides* indicates that this fungus was the least sensitive among the isolates evaluated and represents the most demanding target for a future antifungal formulation.

The *in vivo* efficacy observed confirms that CNSL activity is not limited to culture medium. Following curative application to inoculated fruits, the hot extract caused complete lesion inhibition at 90 µg/mL for *A. niger*, 100 µg/mL for *Fusarium* sp., 180 µg/mL for *Lasiodiplodia* sp., and 330 µg/mL for *C. gloeosporioides*. The cold extract required higher concentrations:

330 µg/mL for *A. niger* and 360 µg/mL for the other three fungi. The higher concentrations required on fruits compared with the *in vitro* assays are expected. In PDA medium, CNSL is in direct contact with the mycelium, whereas on the fruit, its action may be limited by the cuticle, host tissues, uneven diffusion of the product, and the progression of an already established infection.

The overall superiority of the hot ethanolic extract is consistent with its higher extraction yield. It may reflect a better recovery of antifungal compounds, particularly phenolic derivatives. However, this relationship should be interpreted cautiously: phytochemical screening revealed the same major families of compounds in both extracts, without allowing comparison of their actual concentrations.

The results obtained are consistent with those of Garcia et al. [9] in Brazil, who showed that CNSL inhibited mycelial growth and sporulation of C. gloeosporioides and Lasiodiplodia theobromae at 320 µg/mL. The concentration required in the present study to completely inhibit C. gloeosporioides in vitro (300 µg/mL) is therefore comparable to that reported by these authors.

Similarly, Sanyaolu et al. [8] in Nigeria reported CNSL activity against Fusarium solani, Lasiodiplodia theobromae, Aspergillus fumigatus, and A. niger associated with yam dry rot. This convergence confirms the broad-spectrum antifungal potential of CNSL. However, direct comparisons of concentrations should be interpreted cautiously, as the fungal species, solvents, formulations, assessment methods, and host plants differ among studies.

5.Conclusion

This study showed that symptomatic cashew fruits in northern Cameroon harbour a diverse fungal community, with *Colletotrichum* sp. being the most frequently isolated taxon. Pathogenicity assays subsequently confirmed that *Colletotrichum gloeosporioides*, *Lasiodiplodia* sp., *Fusarium* sp., and *Aspergillus niger* were able to induce lesions on healthy fruits. The ethanolic CNSL extracts contained several bioactive metabolite groups, and hot extraction provided the higher yield. Both extracts inhibited the four pathogenic fungi *in vitro* and *in vivo*, but the hot extract consistently achieved complete inhibition at lower concentrations. CNSL, particularly the hot ethanolic extract, therefore represents a promising option for valorising cashew shells and developing an alternative antifungal product for cashew disease management. Further formulation, phytotoxicity, and field-validation studies are needed before practical application.

Data availability

The datasets generated during this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they are no confllicts of interests.

Ethics declaration

The plant material used in this study originated from cultivated cashew trees (*Anacardium occidentale* L.). Cashew fruits were collected from orchards in the North and Adamawa regions of Cameroon as part of research activities conducted at the Institute of Agricultural Research for Development (IRAD), with prior permission from the orchard owners. Cashew nut shells used for CNSL extraction were obtained from a cashew nut processing facility. The collection and use of plant material were conducted in accordance with applicable local and national guidelines. No additional administrative collection permits or licences were obtained.

Author Contributions Statement

C.C.B. and Y.A. conceived and designed the study. C.C.B. and K.N.B.C. carried out the experimental work. M.E.W. performed data curation and statistical analysis. C.C.B. wrote the first draft of the manuscript. P.M.O. and Y.A. contributed to the review and editing of the manuscript. Y.A. supervised the study. All authors read and approved the final manuscript.

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