Valorization of cashew balm in the treatment of cashew tree diseases

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ABSTRACT

Cashew trees have experienced remarkable expansion in northern Cameroon over the past decade. They have recently become the second most lucrative cash crop after cotton. This geographic progression has been largely driven by public policies. However, despite the involvement of multiple stakeholders in its promotion, scientific data on this crop in Cameroon remain scarce. Moreover, recent studies have identified several constraints limiting cashew nut production, notably losses due to fungal diseases that can reach 100% under field conditions. The present study aims to evaluate the efficacy of cashew nut shell liquid (CNSL) in controlling fungal pathogens. The investigation was carried out primarily in the North and Adamawa regions, covering 14 localities and surveying a total of 160 orchards. In each orchard, 30 infected fruits and 30 infected leaves were collected under aseptic conditions and transported to the Veterinary Laboratory of the Institute of Agricultural Research for Development (IRAD) in Wakwa for analysis. Fungal isolates were obtained from lesions observed on leaves and fruits, followed by pathogenicity testing. n total, 11 fungal species were identified: Colletotrichum gloeosporioides, Curvularia sp., Alternaria sp., Pestalotia sp., Lasiodiplodia sp., Verticillium sp., Didymella sp., Penicillium sp., Fusarium sp., Aspergillus niger, and Cercospora sp. Among these, four proved pathogenic on healthy cashew fruits. Colletotrichum gloeosporioides exhibited the highest virulence with a lesion diameter of 24.40 mm, compared to 13.54 mm for A. niger. *In vitro* assays demonstrated that cashew nut shell liquid (CNSL) completely inhibits both mycelial growth and sporulation of C. gloeosporioides, Lasiodiplodia sp., Fusarium sp., and A. niger at respective concentrations of 300 μg/mL, 150 μg/mL, 80 μg/mL, and 40 μg/mL. These findings highlight the strong potential of CNSL as an eco-friendly and sustainable alternative for managing cashew fungal diseases. However, further trials under real agricultural conditions are needed to confirm its efficacy and safety.

Keywords: Anacardium occidentale; cashew nut shell liquid; productivity; mycelium; pathogenicity.

INTRODUCTION

The cashew tree (Anacardium occidentale L.) is a perennial woody species native to the tropical regions of Brazil (Sauer, 1993). It is cultivated primarily for its commercially valuable nut and its false fruit, the cashew apple. The cashew nut represents a strategic commodity on the global market, characterized by growing demand (Ngoh Dooh et al., 2021). According to FAO data, it is the most widely produced tree nut worldwide - surpassing almonds and common walnuts in volume - with an estimated output of 4 087 563 tonnes (FAOSTAT, 2020). West Africa holds a dominant position in global production, accounting for approximately 80 % of supply in 2019 (FAOSTAT, 2022). Côte d'Ivoire stands out as the leading global producer and exporter, with annual yields exceeding one million tonnes (FAOSTAT, 2022). In Cameroon, the cashew sector is progressively establishing itself as a pillar of the local and national economy alongside cotton, supported by the commitment of the State and various private and parastatal actors (Essien et al., 2022). Despite this growth dynamic, scientific data on cashew cultivation in Cameroon remain limited (Bourou et al., 2021). Moreover, several biotic constraints affect its productivity (Olukunle et al., 2023). Beyond insect attacks, more than a dozen diseases have been described on this species (Viana, 2017). The most common include anthracnose (C. gloeosporioides), fruit rot (Lasiodiplodia sp.), red rust (Cryptosporiopsis sp.), powdery mildew (Oidium anacardium), branch and trunk gummosis, bacterial blight (Xanthomonas axonopodis pv. anacardii), and pestalotiosis (Pestalotia heterocornis) (Soro et al., 2020). These diseases lead to considerable economic losses (Tonon et al., 2018). For instance, in Burkina Faso, powdery mildew can cause yield losses ranging from 70 to 100 % (Banito et al., 2021), while in Côte d'Ivoire, C. gloeosporioides is responsible for losses of up to 72 % (Dénis et al., 2018). In that same country, four major diseases prevail in plantations: anthracnose, pestalotiosis, bacterial blight, and gummosis (Lasiodiplodia theobromae) (Won et al., 2017). To combat these diseases, the use of chemical fungicides based on carbendazim or Mancozeb 80 WP has been recommended (Kuete et al., 2022; Ngoh Dooh et al., 2021). However, their application raises concerns regarding toxicity, the development of pathogen resistance, and environmental impact (Danner et al., 2019). In this context, the search for natural, sustainable alternative solutions is essential (Orou et al., 2024). Although cashew shells are generally considered waste and often discarded after kernel extraction, they hold significant valorization potential. Cashew shells are biodegradable, low-cost, and readily available in production areas (Hassana, 2016). CNSL contained in cashew shells is primarily recognized for its insecticidal properties, but recent studies have shown that it may also help control certain phytopathogenic fungi. Indeed, Amoussa et al. (2022) demonstrated that low-dose application of CNSL significantly reduces populations of Plutella xylostella, Hellula undalis, and Lipaphis erysimi, resulting in a marked increase in marketable cabbage yield from 2.20 t/ha (control) to 13.74 t/ha. Similarly, Bajpai et al. (2012) showed that extracts rich in anacardic acid significantly inhibit the mycelial growth of phytopathogenic fungi such as Aspergillus niger, Fusarium oxysporum, and Colletotrichum gloeosporioides.

OBJECTIVES

Main objective: This study aims to evaluate, under *in vitro* conditions, the efficacy of cashew nut shell liquid (CNSL) in controlling fungal pathogens affecting the cashew tree (*Anacardium occidentale* L.).

Specific objectives:

- Identify and characterize the pathogenic fungi associated with cashew tree diseases.
- Evaluate the antifungal activity of CNSL on the growth of pathogens responsible for cashew fruit deterioration.

METHODOLOGY

Study area.

This research was conducted in two main regions: the North region, encompassing the localities of Djola-Bame, Mafakilda, Sanguéré-Paul, Sanguéré-Ngal, Ngounra II, and Ngong; and the Adamawa region, covering Mbé, Tchabal, Dang Gada-Mabanga, Marza, Yoko, Wakwa, and Nyambaka.

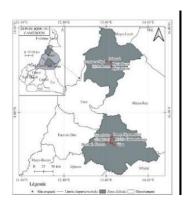


Figure 1: Location of the study area

Collection of Plant Material: As part of this study, fruit and leaf samples were collected from a total of 160 orchards, with 30 fruits and 30 leaves taken from each orchard. The selection focused on fruits and leaves showing lesions. After collection, the samples were carefully labeled and individually wrapped in newspaper, then stored in a cooler containing cold packs. Finally, the samples were transported to the IRAD Veterinary Laboratory in Wakwa for further analysis.

Preparation of Potato Dextrose Agar (PDA) Culture Medium: The pre-prepared Potato Dextrose Agar (PDA) medium was used for the isolation of fungal species. Its preparation involved dissolving 39 grams of PDA powder in 100 milliliters of distilled water. The resulting mixture was brought to a boil and then sterilized at 121°C for 15 minutes. After cooling, one gram of ampicillin was added to the homogenized medium. The medium was then distributed into Petri dishes at a rate of 15 milliliters per dish, under sterile conditions near a flame produced by a Bunsen burner (Yaouba *et al.*, 2019).

Inoculation, Purification, and Identification of Isolated Fungi: Leaf and fruit samples were thoroughly washed with tap water using a strong water jet. Fragments were then taken from the edges of necrotic areas and disinfected with 1% sodium hypochlorite for three minutes. This step was followed by three successive rinses in sterile distilled water, lasting 5, 10, and 15 minutes respectively. After drying on sterile paper, epicarp fragments of approximately 2 mm² were aseptically transferred into Petri dishes containing PDA medium. The dishes were carefully labeled and incubated at a constant temperature of 25°C for seven days

(Djeugap et al., 2015). Fungal strain identification was carried out using a standard microscope, based on the identification key by Botton et al. (1990).

Fungal Isolation Frequency: It was determined using Walder's formula (1996):

FI (%) = $\frac{Ni}{NTI}$ × 100. Where, FI= Isolation frequency (in percentage), Ni= Number of isolations of a fungal species across all samples and NTI= Total number of isolations of all fungal genera (Kuete *et al.*, 2022)

• Pathogenicity test

The pathogenicity test was conducted on physiologically mature fruits that had not yet reached full ripeness and showed no visible symptoms or mechanical injuries. The wound inoculation method described by Rivera *et al.* (2006) was used. Each fruit was thoroughly washed with running tap water, then surface-disinfected with a 1% sodium hypochlorite solution for 2 minutes, followed by three rinses with sterile distilled water. A wound measuring 2–3 mm in depth and 3–4 mm in diameter was made using a sterile punch, and inoculated with a 5 mm diameter mycelial disc taken from a 7-day-old pure culture. For each fungal strain, five fruits were inoculated (n = 5), in accordance with Rivera *et al.* (2006). Control fruits received a sterile PDA medium disc without mycelium. The inoculated fruits were placed in airtight containers lined with moistened filter paper and incubated at 25 ± 2 °C in darkness (Snowdon, 1990; Bautista-Baños *et al.*, 2002). After five days of incubation, lesion diameters were measured in two perpendicular directions, and the average was calculated for each fruit. The isolates were then classified into virulence categories according to the Sanders and Korten scale, adapted to the observed lesion sizes. Measuring lesion diameter five days post-inoculation allowed for quantification of each strain's virulence.

Classification of Isolates Based on Pathogenicity Scale The values obtained were used to classify the isolates according to the pathogenicity scale of Sanders and Korten (ranging from 0 to 4), where: Class 0: No symptoms; Class 1: Very mild lesions (< 5 mm); Class 2: Moderate lesions (5–10 mm); Class 3: Significant lesions (10–20 mm); Class 4: Severe lesions (> 20 mm).

The average lesion diameter was calculated using the following formula:

$$Dm = \frac{\sum (L+I)}{2N}$$

Where, **Dm**= Average lesion diameter; **L**= Lesion length; **l**= Lesion width; **N**= Total number of inoculated fruits (N'Guettia *et al.*, 2006)

Inhibition Tests of Fungal Strains at Different CNSL Concentrations

CNSL Extraction Method: A total of 100 kg of cashew nuts (*Anacardium occidentale* L.) were harvested from seven-year-old trees at the Agricultural Research Center (CRA) in Wakwa. The fruits were carefully sorted to retain only healthy nuts free from any disease. CNSL (Cashew Nut Shell Liquid) extraction was carried out at the Chemistry Laboratory of the National Advanced School of Agro-Industrial Sciences (ENSAI) at the University of Ngaoundéré.

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Extraction Yield Calculation: The extraction yield was determined using the following formula:

R (%) =
$$\frac{\text{Mass of extracted oil (g)}}{\text{Initial mass of dry shells (g)}} \times 100$$
. (Rivera *et al.*, 2006)

Evaluation of CNSL Antifungal Activity.

Preparation of Solutions for In Vitro Tests: To assess the effect of CNSL on fungal growth, Petri dishes containing 15 mL of PDA medium were prepared with increasing concentrations of CNSL (10 μg/mL, 20 μg/mL, 40 μg/mL, 80 μg/mL, 150 μg/mL, and 300 μg/mL). Each dish was inoculated with a 5 mm disc of a 7-day-old culture of one of the following fungi: *C. gloeosporioides, Lasiodiplodia* sp., *Fusarium* sp., and *A. niger*. The negative control consisted of PDA medium without CNSL, while the positive control was PDA medium supplemented with mancozeb 80 WP (Tonon *et al.*, 2018). Each dish was incubated at 25°C for six days. Radial colony growth was measured daily along two perpendicular axes.

Growth Rate (GR):

$$GR = \frac{(Df - Di)}{n}$$

Where, Df = Final diameter; Di = Initial diameter (5 mm) and n = Number of incubation days (Kossonou *et al.*, 2019).

Growth Inhibition:

Growth inhibition was determined by the average radial growth of the mycelium for each fungal strain.

$$I = \frac{C - T}{T} \times 100$$

Where, C = Control growth and T = Treated growth.

Procedure when no mycelial growth is observed: When, for a given concentration, no mycelial growth is observed, the inoculum is subcultured onto an agar medium free of CNSL. Resumption of growth indicates a fungistatic effect, while absence of growth indicates a fungicidal effect (Kossonou *et al.*, 2019).

Target antifungal parameters

- **Minimum inhibitory concentration (MIC)** = Lowest concentration preventing visible growth.
- **IC50** = Concentration that induces 50% inhibition.

Statistical analyses: Data were entered into Excel and subjected to analysis of variance (ANOVA). Means were separated using Student's t-test at the 5% significance level. Analyses were performed with R software, version 3.5.1.

RESULTS

Fungal pathogens isolated from cashew plant tissues

At the end of the study, 11 fungal species were isolated and identified from fruits and leaves. These were: *Colletotrichum* sp., *Curvularia* sp., *Alternaria* sp., *Pestalotia* sp., *Lasiodiplodia* sp., *Verticillium* sp., *Didymella* sp., *Penicillium* sp., *Fusarium* sp., *Aspergillus niger*, and *Cercospora* sp. (Annex 1).

Isolation frequency of fungi

The results showed a significant difference in isolation frequencies between fungi and collection localities. The fungus *Colletotrichum* sp. was the most frequent in both production basins, with values of 30.81% and 22.47%, respectively. Lasiodiplodia sp. was the second most frequent fungus (19.75%) and was absent in the Vina. In the Vina, fungi such as *Fusarium* sp. (15.71%), *Cercospora* sp. (11.23%), *Verticillium* sp. (10.34%), and *Alternaria* sp. (11.66%) showed higher isolation frequencies than those observed in the Bénoué basin by 5.19%, 2.01%, 1.45%, and 1.58%, respectively (Annex 2).

Pathogenicity test

Pathogenicity tests were performed with all isolated fungi. Of these, 4 caused lesions on the fruits: C. gloeosporioides, *Lasiodiplodia* sp., *Fusarium* sp., and A. niger. However, lesion diameters varied significantly depending on the fungus. The mean lesion diameter ranged from 24.40 mm to 13.54 mm. C. *gloeosporioides* showed the largest lesion diameter (24.40 mm), followed by *Lasiodiplodia* sp., *Fusarium* sp., and *A. niger*, with mean lesion diameters of 18.32 mm, 15.68 mm, and 13.54 mm, respectively ((Annex 3).

Antifungal activity of CNSL

The results show fungal growth inhibition strongly correlated with CNSL concentration. As the concentration increased, the inhibition percentage rose progressively, reaching 100% for all tested species. *A. niger* was the most sensitive, with 71.8% inhibition at 10 μg/mL and complete inhibition at 40 μg/mL. *Fusarium* sp. showed moderate sensitivity, reaching 100% inhibition only at 80 μg/mL. *Lasiodiplodia* sp. exhibited intermediate sensitivity, with total inhibition at 150 μg/mL. Finally, *C. gloeosporioides* was the most resistant, with an MIC of 300 μg/mL. Overall, minimum inhibitory concentrations (MICs) ranged from 40 μg/mL for *A. niger* to 300 μg/mL for *C. gloeosporioides*, while all IC50 values were below 10 μg/mL except for *C. gloeosporioides* (22.2 μg/mL).

DISCUSSION

The results obtained during this study made it possible to identify eleven fungal species from lesions observed on leaves and fruits in orchards of the North and Adamaoua regions. These species include *Colletotrichum* sp., *Curvularia* sp., *Alternaria* sp., *Pestalotia* sp., *Lasiodiplodia* sp., *Verticillium* sp., *Didymella* sp., *Penicillium* sp., *Fusarium* sp., *Aspergillus niger*, and *Cercospora* sp. This fungal diversity confirms the observations of Sali Bourou (2021) in the Benue Basin, who had already reported *C. gloeosporioides*, *Fusarium* solani, *A. niger*, and *Phytophthora* sp. on cashew fruits. *C. gloeosporioides* was the most frequent fungus in the Vina division (24.41 %) and in the Benue division (30.81 %). Similarly, the severity of the lesions (24 mm) caused by *C. gloeosporioides* confirms the high aggressiveness of certain isolates already described in Côte d'Ivoire (Soro *et al.*, 2025). Furthermore, these results align with observations by Muntala *et al.* (2020) in Ghana, who reported an average anthracnose incidence of 22.9 % accompanied by pronounced leaf and fruit lesions. In addition, the FAO/Plantwise guide notes that such lesions not only compromise the market quality of fruits but can also accelerate wet rot and promote pathogen spread in orchards with high relative humidity and elevated temperatures.

The average lesion size of 19.32 mm induced by *Lasiodiplodia* sp. on cashew fruits confirms the significant aggressiveness of this fungus, consistent with findings by Ngnankam *et al.* (2023) in Cameroon. These authors reported that *Lasiodiplodia* sp. emerges as a major pathogen of cashew fruits in Cameroon, capable of undermining the marketability of the harvest. Similarly, Afouda *et al.* (2013) in Benin highlighted its role in cashew fruit rots.

Cashew balm markedly inhibited the growth of *C. gloeosporioides*, *Lasiodiplodia* sp., *Fusarium* sp., and *A. niger*, confirming its antifungal potential. This activity is attributed to the balm's richness in anacardic acids, which disrupt membrane permeability and induce leakage of cytoplasmic contents. Santos et al. demonstrated that anacardic acid isolated from cashew pericarp liquid exhibited MIC values between 50 and 200 μg/mL against *C. gloeosporioides* and *L. theobromae*, consistent with our MICs of 300 μg/mL and 150 μg/mL, respectively. Likewise, Silva et al. reported IC₅₀ values below 10 μg/mL for *Fusarium* sp. and *A. niger*, indicating a high sensitivity of these species to the phenolic compounds of cashew balm. These converging findings validate the use of cashew balm as a bioproduct in integrated disease management against anthracnose, fruit rot, and other fungal diseases in orchards, either alongside or in place of conventional fungicides.

Moreover, the fact that *C. gloeosporioides* requires an MIC of 300 μ g/mL to achieve complete inhibition illustrates the robustness of its cell wall and its capacity to withstand fungicidal treatments. This high level of resistance corresponds to its aggressiveness observed under natural conditions where it causes severe fruit lesions and in laboratory settings, where only high doses can effectively curb its growth. Indeed, Wang *et al.* (2020) showed that fungicide concentrations as high as 400–20 500 μ g/mL are required to achieve 50 % inhibition.

CONCLUSION

The study revealed a high level of fungal diversity in the orchards of the North and Adamaoua regions, with eleven species isolated from cashew leaves and fruits. *C. gloeosporioides* proved to be the dominant pathogen, causing average lesions of 24 mm and confirming its high aggressiveness as previously reported in Côte d'Ivoire (Soro *et al.*, 2025). *Lasiodiplodia* sp. also exhibited significant virulence (lesions of 19.32 mm), in agreement with

findings by Ngnankam *et al.* (2023) in Cameroon and Afouda *et al.* (2013) in Benin. Cashew balm demonstrated strong *in vitro* antifungal activity. Assays showed that it completely inhibited mycelial growth and sporulation of *C. gloeosporioides* at 300 μg/ml, *Lasiodiplodia* sp. at 150 μg/ml, *Fusarium* sp. at 80 μg/ml, and *A. nige*r at 40 μg/ml. This efficacy attributable to the balm's high anacardic acid content disrupting membrane permeability positions it as a promising bioproduct for integrated management of cashew fungal diseases in orchards. However, field trials are needed to evaluate its real-world effectiveness across diverse agroecosystems and to characterize the active compounds and their modes of action in order to optimize formulations.

RECOMMENDATIONS

- Train and raise awareness among producers in the extraction, safe handling, and application of cashew balm as an alternative or complement to conventional treatments
- Develop application protocols (dosage, frequency, and method of administration) tailored to local farming practices

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ANNEX 1: Fungi isolated from fruits and leaves of Anacardium occidentale L.



Figure: *C. gloeosporioides* on 7-day-old PDA culture medium (A); Microscopic view (400×) (B)

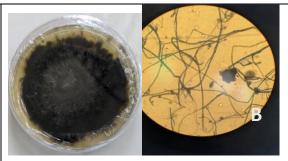


Figure: Curvularia sp. on 7-day-old PDA culture medium (A); Microscopic view $(400\times)$ (B)





Figure: *Didymella* sp. on 7-day-old PDA culture medium (A); Microscopic view (400×) (B)

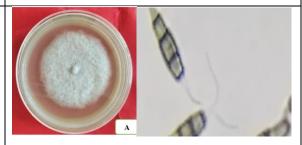
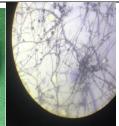
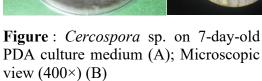


Figure: *Pestalotia* sp. on 7-day-old PDA culture medium (A); Microscopic view (400×) (B)







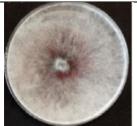
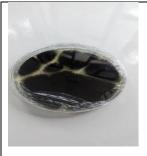




Figure: Lasiodiplodia sp on 7-day-old PDA culture medium (A); Microscopic view (400×) (B)



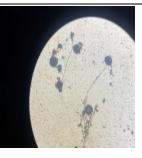


Figure: on 7-day-old PDA culture medium (A); Microscopic view (400×) (B)



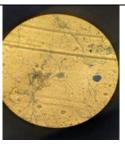
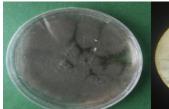


Figure: *Verticillium* sp. on 7-day-old PDA culture medium (A); Microscopic view (4)



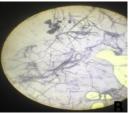


Figure: *Penicillium* sp. on 7-day-old PDA culture medium (A); Microscopic view (400×) (B)



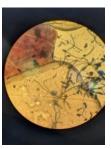


Figure: Alternaria sp. on 7-day-old PDA culture medium (A); Microscopic view (400×) (B)

ANNEX 2: Frequency of fungal isolation by location

Fungi	Bénoué	Vina		
Colletotrichum sp.	30, 81 ±3,84 ^a *	22,47± 1,09°		
Lasiodiplodia sp.	19,75± 2, 15 ^b	$0.00\pm0.00^{\rm g}$		
Pestalotia sp.	$12,03\pm 1,12^{c}$	$10,40\pm 1,15^{d}$		
Didymella sp.	$12,05 \pm 1,3^{cd}$	$4,23\pm 1,04^{\circ}$		
Curvularia sp.	$9,31 \pm 0,05^{d}$	$3,91\pm 2, 14^{\rm f}$		
Fusarium sp.	$5,19 \pm 0,71^{e}$	$15,71\pm 1,57^{\mathrm{b}}$		
Aspergillus niger	$5,25 \pm 2,12^{ef}$	8,21± 2, 41°		
Cercospora sp.	$2,01 \pm 0,16^{\mathrm{f}}$	$11,23\pm 1,54^{cd}$		
Alternaria sp.	$1,58\pm0,\!12^{fg}$	$11,66\pm0,67^{c}$		
Verticillium sp.	$1,45 \pm 0,17^{g}$	$10,34\pm 1,89^{de}$		
Penicillim sp.	$0,13\pm 0,\!06^{h}$	$0.00\pm0,00^{g}$		

^{*} Means followed by the same superscript letter within a column are not significantly different according to Student's test at $P \le 0.05$.

ANNEX 3: Mean lesion diameters caused by *C. gloeosporioides*, *Lasiodiplodia* sp., *Fusarium* sp. and *A. niger* strains on fruits, 5 days after inoculation.

Fungi	Mean lesion diameters (mm)	Categories		
C. gloesporiodes	24,40±1,31 ^a	Severe lésions		
Lasidiplodia sp.	$18,32\pm0,76^{b}$	Significant lesions		
Fusarium sp.	15,68± 0,33°	Significant lesions		
A. niger	13,54±0,21 ^d	Modérate lesions		

^{*} Means in the same column followed by the same superscript letter are not significantly different according to Student's test at $P \le 0.05$.

ANNEXE 4: Effect of different concentrations of cashew balm on the inhibition of *C. gloeosporioides*, *Lasiodiplodia* sp., *Fusarium* sp., and *A. niger*, and the measured MIC and IC₅₀ values.

Fungi	Positive control	10 μg/ml	20 μg/ml	40 μg/ml	80 μg/ml	150 μg/ml	$300~\mu g/ml$	Negative control	СМІ	CI 50
C.gloesporiodes	91,27± 1,52 ^b	45,22±1,88°	46,73±2,6°	69,15±2,05 ^d	79,66±1,43°	91,49±2,17 ^b	100,00±0,00a	0,00±0,00 ^{f*}	300	22,2
Lasiodiplodia sp.	90,30± 2,21 ^b	60,46±1,43°	81,55±3,36 ^d	88,57±1,22°	91,29±1,51 ^b	100,00±0,00°	100,00±0,00a	0,00±0,00f*	150	< 10
Fusarium sp.	91,07± 1,10 ^b	68,19±3,55 ^d	87,25±2,43°	91,42±1,64 ^b	100,00±0,00 ^a	100,00±0,00a	100,00±0,00a	0,00±0,00e*	80	< 10
A. niger	90,60± 2,02 ^b	71,83±1,30°	90,67±1,66 ^b	100,00±0,00°	100,00±0,00°	100,00±0,00°	100,00±0,00°	0,00±0,00 ^{d*}	40	< 10

Means followed by the same superscript letter in the same row are not significantly different according to Student's test at $P \le 0.05$.