

Research Article

Management of Major Seed-Borne Fungi of Cowpea (*Vigna unguiculata* (L.) Walp) with Four Selected Botanical Extracts

Eric Gyasi , Daniel A. Kotey , Barnabas A. Adongo , Fuleratu K. Adams ,
Edmund O. Owusu , and Abdul Mohammed 

Plant Protection Division, CSIR-Plant Genetic Resources Research Institute, Bunso-Eastern Region, Ghana

Correspondence should be addressed to Eric Gyasi; ericgyasi2010@yahoo.com

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Seed-borne fungal pathogens present significant constrain to the production and conservation of many seed crops including cowpea. Infection of mature seeds by such pathogens could result in mycotoxin contamination, loss of viability, and decay of seeds. This study aimed to identify seed-borne fungi on 200 accessions of cowpea under cold storage at CSIR-Plant Genetic Resources Research Institute (PGRRI), Ghana. Also, the antifungal effect of seeds of *Piper nigrum*, *Xylopi aethiopica*, *Aframomum melegueta*, and fresh leaves of *Cymbopogon citratus* aqueous extracts (100% w/v) on the major seed-borne fungi identified on the cowpea seeds was determined. Seven fungal species belonging to five genera were identified from the seeds of the cowpea accessions evaluated. However, the diversity and infection levels of the pathogenic fungi recorded on the seeds were lower than that of the saprophytic fungi indicating minimal capacity of the seeds to spread pathogenic fungi on the field. Aqueous extract of *Aframomum melegueta* inhibited the growth of *Fusarium verticillioides* by 98.40%, *Colletotrichum* sp. by 97.83%, *Aspergillus niger* by 94.70%, and *Aspergillus flavus* by 63.38%. The only other aqueous extract that inhibited the colony growth above 60% was that of *Piper nigrum* which inhibited colony growth of *Fusarium verticillioides* by 71.7% and *Colletotrichum* sp. by 63.47%. Due to the benign effect of *Aframomum melegueta* extract on the environment and non-target organisms, its use as a seed protectant is highly recommended. Further studies to establish the spectrum of activity and dose levels of *Aframomum melegueta* extract are recommended.

1. Introduction

Cowpea (*Vigna unguiculata* (L.) Walp) is a leguminous crop which due to its high protein content, is of nutritional benefit in most parts of the world [1, 2]. In Sub-Saharan Africa, the production of the crop serves as a source of income for millions of smallholder farmers and traders [3]. In Ghana, cowpea is an important staple crop and is cultivated extensively in the savanna and transitional agroecological zones of the country [4]. Whilst 26 cowpea varieties have been released and registered in Ghana, the majority of the seeds used for cultivation are recycled seeds of landraces. As a way of preventing the diversity of cowpea in Ghana from being irreversibly lost, the CSIR-PGRRI, the national genebank of Ghana, maintains a collection of cowpea germplasm at its seedbank at Bunso in the Eastern Region of

Ghana. At the CSIR-PGRRI, the genetic resources of cowpea are conserved as seeds in cold storage.

Amongst biotic factors that constrain production, seed-borne fungi have been identified as significant in many production areas. According to [5], cowpea seeds in the field or in storage are susceptible to infection by seed-borne pathogens which usually result in mycotoxin contamination, loss of viability, and seed rot. The planting of infected seeds may also result in disease outbreaks and loss of yield. Due to these adverse impacts, seed-borne pathogens are a serious threat to cowpea seed conservation and cultivation. Seed-borne fungi are either saprophytic or pathogenic. Saprophytic seed-borne fungi affect seeds during storage and cause seed discoloration, reduced seed weight, and seed germination. Pathogenic seed-borne fungi, on the other hand, infect seeds in the field and reduce seed vigour,

weaken the plant at its initial growth stages and cause disease epidemics in the field [6, 7]. Various seed-borne fungal species have been reported to be associated with cowpea seeds in different parts of the world. *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum*, *Alternaria alternata*, and *Penicillium* sp. were found to be associated with cowpea seeds in India [8]. The authors in [9] identified *Fusarium verticillioides*, *Fusarium solani*, *Macrophomina phaseolina*, and *Phoma* sp. on cowpea seeds in Sierra Leone. *Aspergillus niger*, *Aspergillus flavus*, *Botryodiplodia theobromae*, *Fusarium oxysporum*, *Penicillium* sp., and *Rhizopus stolonifer* have also been reported to be associated with cowpea seeds [5, 10, 11]. The control of seed-borne fungi using synthetic fungicides is widely practiced in Ghana. Although the use of synthetic fungicides is effective, the approach has the tendency to result in the unintended accumulation of toxic fungicide residues in the ecosystem which may also induce resistance in pathogens [12–14]. Due to the potential adverse effects resulting from the use of synthetic fungicides, there is a shift in focus to the identification of alternative approaches, including the use of botanical extracts, to effectively manage seed-borne pathogens without any harm to the ecosystem and applicators. The use of botanical extracts in the control of seed-borne fungi has been reported as effective, low-cost, readily available, and environmentally safe [15–18]. Several studies have been carried out to evaluate the antifungal effects of aqueous extracts of black pepper (*Piper nigrum*), Ethiopian pepper (*Xylopiya aethiopyca*), lemon grass (*Cymbopogon citratus*), and grains of paradise (*Aframomum melegueta*) on fungal pathogens [19–25]. [21] studied the antifungal activity of extracts of scent leaf (*Ocimum gratissimum*) and alligator pepper (*Aframomum melegueta*) at 100% concentration on the postharvest decay of carrots and reported that *Aframomum melegueta* aqueous extract possessed a stronger antifungal property against the mycelial growth of *Fusarium* species and other fungi. The authors in [23] and [26] also found that *Aframomum melegueta* extract showed high toxicity against some plant fungal pathogens. The authors in [19] used seeds of black pepper (*Piper nigrum*), rhizomes of ginger (*Zingiber officinale*), leaves of neem (*Azadirachta indica*), leaves of pawpaw (*Carica papaya*), and leaves of tobacco (*Nicotiana tabacum*) extracts to control *Penicillium expansum* and concluded that *Piper nigrum* showed high toxicity against the test fungus at 90% concentration. [27] also reported that *Piper nigrum* showed high antimicrobial activity against plant pathogens. [20] and [22]

reported that *Xylopiya aethiopyca* showed some level of toxicity against some seed-borne fungi on cowpea seeds. The authors [28] evaluated the antifungal efficacy of 21 plant extracts against the seed-borne fungus *Colletotrichum lindemuthianum* and reported that lemon grass was less effective compared to the other plant extracts. This study aimed (i) to identify seed-borne fungi of 200 cowpea accessions in cold storage at the CSIR-PGRRI, Bunso and (ii) to assess the antifungal effects of black pepper (*Piper nigrum*), Ethiopian pepper (*Xylopiya aethiopyca*), lemon grass (*Cymbopogon citratus*), and grains of paradise (*Aframomum melegueta*) extracts on the major seed-borne fungi identified.

2. Materials and Methods

2.1. Collection of Cowpea Seed Samples and Seed Health Testing. Samples of seeds of 200 randomly selected cowpea accessions were obtained from the stock conserved under cold storage conditions (-20°C) at the CSIR-PGRRI, Bunso in February, 2019. The selected seed samples were examined for the presence of seed-borne fungi using the blotter method [29]. One hundred (100) seeds, randomly selected from each of the cowpea accession, were surface sterilised in 5% sodium hypochlorite solution for 2–3 minutes and rinsed three times with sterilised distilled water. Ten (10) seeds were then plated on sterilized, moistened three-ply blotter paper in Petri dishes and replicated ten times (Figure 1). The plates were placed on a laboratory bench under a 12 h light and a 12 h darkness photoperiod for seven (7) days. On the eighth day, each incubated seed was examined under a stereobinocular microscope. All the seed-borne fungi on plated seeds were identified based on the colony morphology, colour of mycelium, and the shape and other characteristics of conidia produced with the aid of the laboratory manuals developed by [30] and [31]. The diversity of fungi, the incidence of each fungus, and the infection range were recorded. The incidence of each type of fungus identified was calculated using the following formula:

$$\text{fungal incidence} = \frac{\text{number of infected samples}}{\text{total number of samples tested}} \times 100\%. \quad (1)$$

The infection level of each fungus for each accession was also calculated using the formula:

$$\text{infection level} = \frac{\text{number of seeds infected by each fungus in an accession}}{\text{total number of seeds tested per accession}} \times 100. \quad (2)$$

2.2. Culturing of Identified Seed-Borne Fungi on PDA. *Fusarium verticillioides*, *Colletotrichum* sp., *Aspergillus flavus*, and *Aspergillus niger* identified on the cowpea seeds

were the fungal isolates used in the efficacy studies of the botanical extracts. Small portions of mycelia of the four fungal isolates were placed on potato dextrose agar (PDA) in

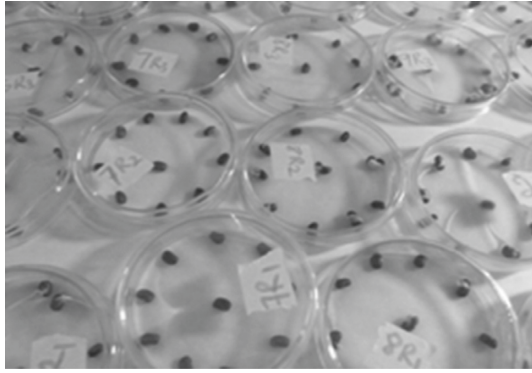


FIGURE 1: Cowpea seeds plated on moistened filter papers in Petri dishes.

Petri dishes and incubated at $28 \pm 2^\circ\text{C}$ for seven (7) days. Single conidia culturing was carried out to obtain pure cultures of the four fungal isolates for further studies.

2.3. Sources of Plant Products (Botanical Materials). The seeds of the black pepper (*Piper nigrum*), Ethiopian pepper (*Xylopia aethiopica*), and grains of paradise (*Aframomum melegueta*) used in this study were obtained from the conservation fields of the CSIR-PGRRI, Bunso. Fresh leaves of lemon grass (*Cymbopogon citratus*) were also obtained from households within the Bunso community (Figure 2).

2.4. Preparation of the Botanical Aqueous Extracts

2.4.1. Preparation of Botanical Seed Extracts. Seeds of black pepper (*Piper nigrum*), Ethiopian pepper (*Xylopia aethiopica*), and grains of paradise (*Aframomum melegueta*) were washed thoroughly with distilled water and soaked in a 5% sodium hypochlorite solution for 10 minutes. The seeds were then rinsed three times using sterilised distilled water in the laboratory and air dried in a laminar flow hood for 30 minutes. Fifty grams (50 g) of the seeds of each of the botanicals were weighed and ground using an electric blender (ELBEE, 2 in 1, LB-47) until a fine powder was obtained. The powder obtained from each of the plant species was separately poured into different beakers, after which 50 ml of sterilised distilled water was added to each and stirred thoroughly with a sterilised glass rod. After stirring, the contents of each beaker were left to stand for one hour. Each of the extract was then filtered through folds of sterilised cheese cloth to obtain an aqueous extract with a concentration of 100% (w/v) (Figures 3(a), 3(c), and 3(d)). The procedure described above was carried out in the laminar flow hood.

2.4.2. Preparation of the Botanical Leaf Extract. Fresh leaves of lemon grass (*Cymbopogon citratus*) were cut into pieces with a knife and washed thoroughly with distilled water. The cut leaves were soaked in a 5% sodium hypochlorite solution for 10 minutes and rinsed in sterilised distilled water three times in the laboratory. The leaves were air dried in the

laminar flow hood for 30 minutes. After air drying, 50 g of the leaves were weighed and blended using an electric blender (ELBEE, 2 in 1, LB-47) in the laboratory. The paste obtained from blended leaves was put into a beaker and 50 ml of sterilised distilled water was added to it, stirred thoroughly with a sterilised glass rod and left to stand for one hour. The resulting solution was then filtered through folds of sterilised cheese cloth to obtain an aqueous extract with a concentration of 100% (w/v) (Figure 3(b)).

2.5. Management of Fungal Isolates with Prepared Aqueous Botanical Extracts. A modified poisoned food technique method by [32] was used to determine the effect of the aqueous botanical extracts on fungal growth. Sixty pre-sterilised Petri dishes were divided into four equal sections by drawing two perpendicular lines at the bottom of each Petri dish before dispensing potato dextrose agar (PDA) into each. About 5 ml of the aqueous extract of each plant species was separately introduced into each Petri dish, and 20 ml of PDA was added and shaken gently until uniform mixtures were obtained in each dish placed in a laminar flow hood. The contents of each dish were allowed to solidify, after which a seven-day old disc (4 mm in diameter) of the pure culture of fungal isolates (*Fusarium verticillioides*, *Colletotrichum* species, *Aspergillus flavus*, and *Aspergillus niger*) was placed on the solidified botanical extract and PDA mixture just at the point of intersection of the two lines drawn at the bottom of each Petri dish. Control experiments were set up without the addition of any botanical aqueous extract. The experimental design used was factorial arranged in a completely randomised design with three replications. The effectiveness of the botanical aqueous extracts was recorded in terms of percentage colony inhibition of the fungal isolates and calculated according to the formula [33]:

$$\text{growth inhibition (\%)} = \frac{DC - DT}{DC} \times 100\%, \quad (3)$$

where DC = average diameter of control; DT = average diameter of fungal colony with treatment.

2.6. Data Analysis. The data obtained from the evaluation of the botanical materials was subjected to an analysis of variance (ANOVA) using the Statistix statistical package, edition 10.0. Differences in treatment means were compared for significance using the least significance difference (LSD) at a probability level of 5%.

3. Results

3.1. Identified Seed-Borne Fungi on the Cowpea Seeds. Seven fungal species belonging to five genera were identified from the 200 cowpea accessions evaluated. These included five saprophytic fungi; *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus tamari*, *Penicillium* sp., and *Rhizopus stolonifer*, and two pathogenic fungi such as *Fusarium verticillioides* and *Colletotrichum* sp. The saprophytic fungus, *Aspergillus flavus*, recorded the highest fungal incidence of 54.5%, with an infection level ranging from 3.0 to 57.0%. The lowest

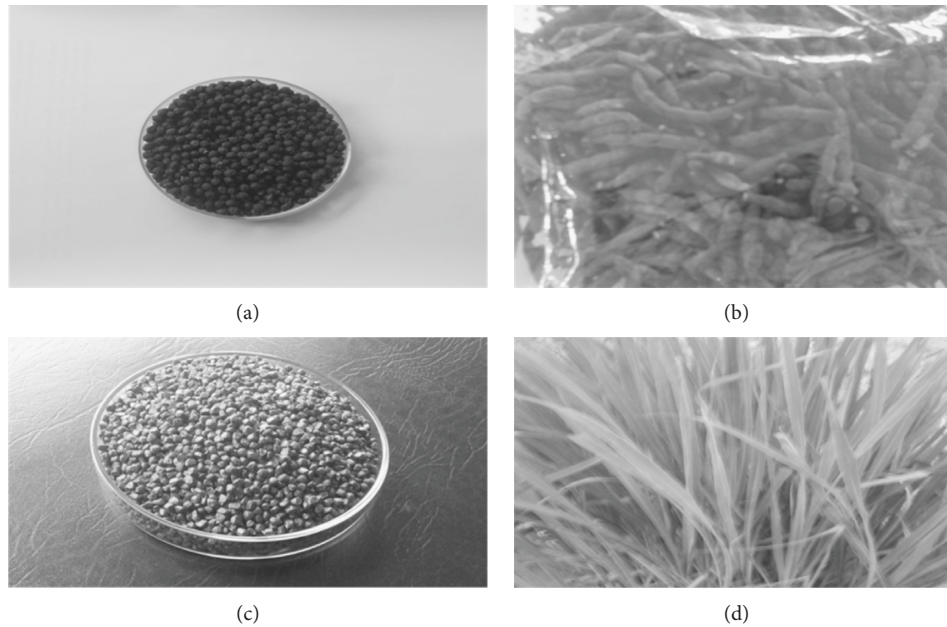


FIGURE 2: Botanical materials used: (a) black pepper (*Piper nigrum*), (b) Ethiopian pepper (*Xylopiya aethiopiya*), (c) grains of paradise (*Aframomum melegueta*), and (d) lemon grass (*Cymbopogon citratus*).

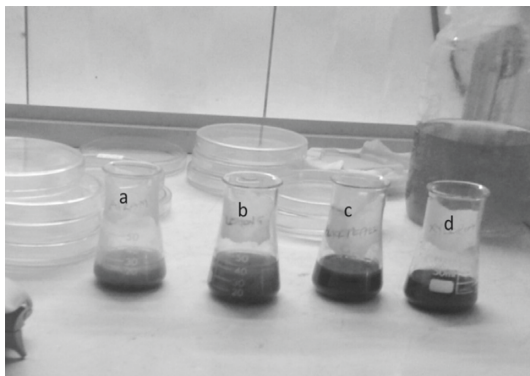


FIGURE 3: Botanical aqueous extracts: (a) *Aframomum melegueta*, (b) *Cymbopogon citratus*, (c) *Piper nigrum*, and (d) *Xylopiya aethiopiya*.

fungal incidence was recorded by *Aspergillus tamari* (1.0%), with an infection range of 6.0–10.0%. Lower diversity and infection levels of the pathogenic fungi were recorded on the cowpea seeds as compared to the saprophytic fungi (Table 1).

3.2. Antifungal Effect of the Aqueous Botanical Extracts on the Major Seed-Borne Fungi. Significant differences were observed between the botanical aqueous extracts and the control. *Aframomum melegueta* aqueous extract inhibited the colony growth of *Fusarium verticillioides* by 98.4%. This was followed by black pepper (*Piper nigrum*) aqueous extract (71.7% inhibition), Ethiopian pepper (*Xylopiya aethiopiya*) aqueous extract (25.6% inhibition), and lemon grass (*Cymbopogon citratus*) aqueous extract (17.23%). However, there was no significant difference between the colony inhibition of *Fusarium verticillioides* by *Xylopiya aethiopiya* and *Cymbopogon citratus* aqueous extracts.

For *Colletotrichum* species inhibition by the aqueous botanical extracts, there were significant differences between the botanical extracts and the control. *Aframomum melegueta* aqueous extract recorded the highest inhibition of 97.83% of *Colletotrichum* species colony growth. This was followed by black pepper (*Piper nigrum*) aqueous extract with a 63.47% inhibition and then *Xylopiya aethiopiya* extract with a 32.17% inhibition. The lowest inhibition (10%) of colony growth of *Colletotrichum* species was recorded by aqueous lemon grass (*Cymbopogon citratus*) extract.

Significant differences were also observed between the aqueous botanical extracts and the control in their inhibition of *Aspergillus niger*. Grains of paradise (*Aframomum melegueta*) aqueous extract recorded the highest inhibition of *Aspergillus niger* colony growth of 94.7%. This was followed by Ethiopian pepper (*Xylopiya aethiopiya*) aqueous extract (35% inhibition) then lemon grass (*Cymbopogon citratus*) aqueous extract (21.83%). The lowest inhibition (3.0%) was recorded by black pepper (*Piper nigrum*) aqueous extract.

With regards to the inhibition of *Aspergillus flavus* by the aqueous botanical extracts, significant differences were also observed between the botanical aqueous extracts and the control. The highest inhibition of colony growth of *Aspergillus flavus* (63.38%) was recorded by *Aframomum melegueta* extract. This was followed by lemon grass (*Cymbopogon citratus*) aqueous extract with 14.37% inhibition then *Xylopiya aethiopiya* aqueous extract with 8% inhibition. The lowest colony growth inhibition (1.7%) of *Aspergillus flavus* was recorded by black pepper aqueous extract (Figure 4; Table 2).

4. Discussion

4.1. Identified Seed-Borne Fungi on the Cowpea Seeds. The seed-borne fungi identified from cowpea seeds have also been reported to be associated with cowpea seeds in

TABLE 1: Incidence of infection (%) and infection level (%) by fungal species from cowpea seed samples.

Fungal species	Total no. of accessions evaluated	Number of infested accessions	Incidence of fungi (%)	Infection level (%)
<i>Aspergillus flavus</i>	200	109	54.5	3.0–57.0
<i>Aspergillus niger</i>	200	52	26.0	3.0–100.0
<i>Penicillium</i> sp.	200	37	18.5	3.0–14.0
<i>Aspergillus tamari</i>	200	2	1.0	6.0–10.0
<i>Rhizopus stolonifer</i>	200	4	2.0	3.0–10.0
<i>Fusarium verticillioides</i>	200	97	48.5	3.0–34.0
<i>Colletotrichum</i> sp.	200	4	2.0	6.0–10.0

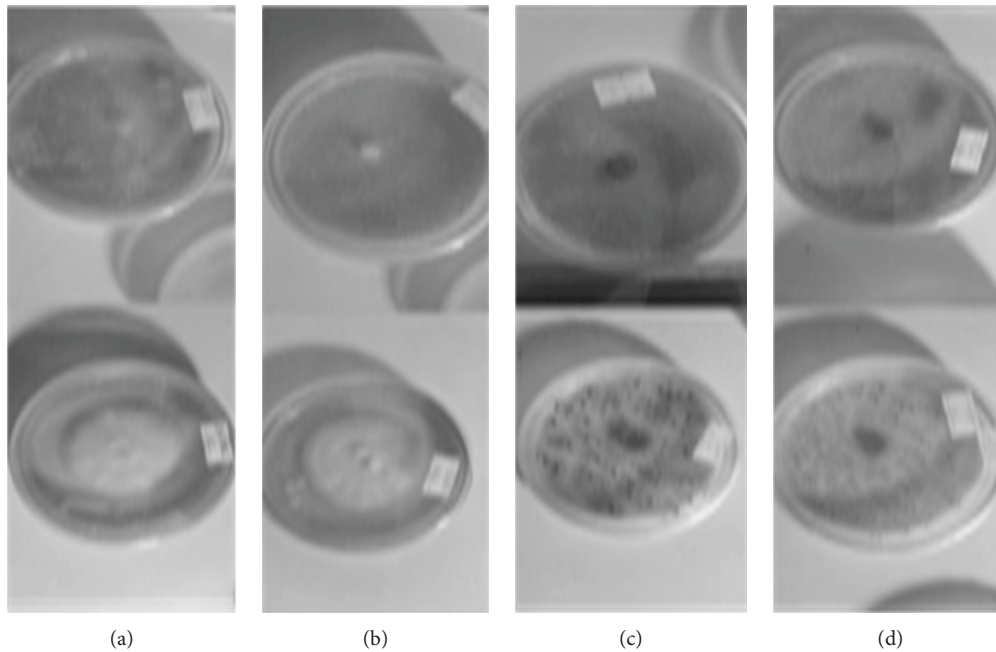
FIGURE 4: Growth inhibition by *Aframomum melegueta* extract and their corresponding controls: (a) *Fusarium verticillioides*, (b) *Colletotrichum* species, (c) *Aspergillus niger*, and (d) *Aspergillus flavus*.

TABLE 2: Inhibition of fungal growth by the aqueous botanical extracts.

Treatment	Percentage inhibition of fungal growth (%)			
	<i>F. verticillioides</i>	<i>Colletotrichum</i> species	<i>A. niger</i>	<i>A. flavus</i>
Aframomum	98.40 ^a	97.83 ^a	94.70 ^a	63.38 ^a
Black pepper	71.70 ^b	63.47 ^b	3.00 ^d	1.70 ^d
Xylopia	25.60 ^c	32.17 ^c	35.0 ^b	8.00 ^c
Lemon grass	17.23 ^c	10.00 ^d	21.83 ^c	14.37 ^b
Control (PDA)	0.00 ^d	0.00 ^e	0.00 ^e	0.00 ^e
Lsd (5%)	15.51	2.41	1.19	1.58
CV (%)	19.34	3.15	2.04	4.80

Each value is the mean of three replicates. Values with different letters are significantly different at $p < 0.05$.

different studies. *Aspergillus niger*, *Aspergillus flavus*, and *Penicillium* sp. were found to be associated with cowpea seeds in a study by [8] in India. [9] identified *Fusarium verticillioides* together with some other fungal species on cowpea seeds in their studies on seed-borne fungi of cowpea in Sierra Leone. The authors [20] in their studies on seed-borne mycoflora of stored cowpea in Nigeria identified *Colletotrichum* species, *Fusarium* species, and *Aspergillus* species. *Aspergillus niger*, *Aspergillus flavus*, *Penicillium* species, *Colletotrichum* species, and *Rhizopus*

stolonifer have also been reported to be associated with cowpea seeds [5] in Botswana, [10] in Pakistan, and [11] in Nigeria.

4.2. Antifungal Effect of the Aqueous Botanical Extracts on *Fusarium verticillioides*, *Colletotrichum* sp., *Aspergillus flavus*, and *Aspergillus niger*. *Aframomum melegueta* aqueous extract performed best at inhibiting both the pathogenic and saprophytic fungi identified on the cowpea seeds compared

with the other aqueous extracts. This finding is in line with previous reports by other authors on the antifungal effects of *Aframomum melegueta* aqueous extract on plant fungal pathogens. The author [34] who evaluated the antifungal effects of 12 different plant species on the growth of *Fusarium verticillioides* causing rot disease of maize, concluded that, *Aframomum melegueta* and *Piper guineense* aqueous extracts were the best botanicals found to inhibit the growth of *Fusarium verticillioides* in his studies. The authors [21], also found that *Aframomum melegueta* aqueous extract possessed a stronger antifungal property against the colony growth of *Fusarium* species tested at 100% concentration. The authors [35] who evaluated the antifungal effects of different spices in controlling fungal pathogens isolated from rotten okra reported that *Aframomum melegueta* aqueous extract was effective in controlling *Fusarium* species, *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus stolonifer*, *Penicillium* species, and *Lasiodiplodia theobromae*. Bamidele [36] in his study on the antifungal potency of *Aframomum melegueta* seed extracts on postharvest rot fungi of two citrus species also found that *Aframomum melegueta* aqueous extract was effective in inhibiting the colony growth of *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus stolonifer*, and *Rhizoctonia* species. In their studies in Nigeria, [23], the authors found that *Aframomum melegueta* extract showed high toxicity against *Aspergillus niger*, *Penicillium digitatum*, *Mucor piriformis*, and *Helminthosporium solani*. Okwu and Njoku [26] similarly found that *Aframomum melegueta* extract showed high toxicity against some plant pathogens in their studies. Aside from its antifungal effect, *Aframomum melegueta* has been reported by several studies [37–40] to possess a broad spectrum of biological activities against arthropods. These effects, which may be initiated through contact, ingestion, or fumigation [41], include feeding and oviposition deterrence, repellence, growth disruption, reduced fitness, and sterility. [42] reported that essential oils of *Aframomum melegueta* have a repellent effect on storage insect pests. This, coupled with the findings of the current study, makes *Aframomum melegueta* an ideal botanical for the integrated management of storage insect pests and diseases of cowpea in smallholder settings.

Although black pepper (*Piper nigrum*) was less effective in inhibiting the colony growth of the saprophytic fungi *Aspergillus niger* and *Aspergillus flavus*, it was more effective against the pathogenic fungi *Fusarium verticillioides* and *Colletotrichum* species. It inhibited the colony growth of *Fusarium verticillioides* by 71.70% and *Colletotrichum* species by 63.47%. These findings concur with those of [19] and [27], who found that *Piper nigrum* has high antimicrobial activity against plant pathogens.

Xylopi aethiopica and *Cymbopogon citratus* also showed some level of effectiveness in the inhibition of the colony growth of the tested cowpea seed-borne fungi. It was, however, not as effective as *Aframomum melegueta* aqueous extract. The authors in [20] and [23] reported that *Xylopi aethiopica* showed some level of toxicity against some seed-borne fungi on cowpea seeds. In the studies by [28], it was found that lemon grass was less effective in inhibiting the colony growth of *Colletotrichum lindemuthianum*. The

authors in [43] studied the antifungal activity of lemon grass against *Aspergillus niger* and *Penicillium digitatum* identified from maize seeds. They also reported that lemon grass was less effective in inhibiting the colony growth of *Aspergillus niger*.

5. Conclusion

Seven fungal species belonging to five genera were identified from the 200 cowpea accessions in this study. However, the diversity and infection levels of the pathogenic fungi recorded on the cowpea seeds were low as compared to the saprophytic fungi, an indication of good phytosanitary practice on the regeneration fields. Evaluation of the antifungal effects of the four different botanical aqueous extracts against the major seed-borne fungi identified from the cowpea seeds showed that grains of paradise (*Aframomum melegueta*) aqueous extract (100% w/v) was effective in inhibiting the colony growth of the two pathogenic fungi, *Fusarium verticillioides* and *Colletotrichum* species, and the two saprophytic fungi, *Aspergillus niger* and *Aspergillus flavus*. Black pepper (*Piper nigrum*) aqueous extract was moderately effective, whilst Ethiopian pepper (*Xylopi aethiopica*) and fresh leaves of lemon grass (*Cymbopogon citratus*) aqueous extracts were less effective in controlling the major seed-borne fungi on cowpea accessions at the CSIR-PGRRI, Bunso.

5.1. Recommendations.

- (i) Due to the benign effect of *Aframomum melegueta* on the environment and non-target organisms, the use of its extract as a seed protectant is highly recommended
- (ii) Further studies to establish the spectrum of activity and dose levels of *Aframomum melegueta* extract are recommended

Data Availability

The data used to support the findings of this study are available upon request from the corresponding author.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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References

- [1] C. H. A. Matos Filho, R. L. F. Gomes, M. M. Rocha, F. R. Freire Filho, and Á. C. d. A. Lopes, "Productive potential of cowpea progenies with erect plant architecture," *Ciência Rural*, vol. 39, no. 2, pp. 348–354, 2009.
- [2] S. R. Akande, "Genotype by environment interaction for cowpea seed yield and disease reactions in the forest and derived savanna agro-ecologies of south-west Nigeria,"

- American-Eurasian Journal of Agricultural & Environmental Sciences*, vol. 2, pp. 163–168, 2007.
- [3] A. Langyintuo, S. Lowenberg-Deboer, M. Faye et al., “Cowpea supply and demand in west and central Africa,” *Field Crops Research*, vol. 82, no. 2-3, pp. 215–231, 2003.
 - [4] CSIR-Crops Research Institute, *Annual Report, CSIR-CRI, Fumesua, Kumasi, Ghana*, 2006.
 - [5] K. B. Khare, D. Loeto, K. Wale, and M. Salani, “Seed-borne fungi of cowpea (*Vigna unguiculata* (L.) Walp) and their possible control *in-vitro* using locally available fungicides in Botswana,” *International Journal of Bioassays*, vol. 5, no. 11, pp. 5021–5024, 2016.
 - [6] R. B. Maude, *Seed-Borne Diseases and their Control*, p. 280, CAB International Cambridge, Wallingford, UK, 1996.
 - [7] P. Neergaard, *Seed Pathology*, S. Chand & Company Ltd., New Delhi, India, 1986.
 - [8] S. Zanjare, Y. Balgude, S. S. Zanjare, A. Suryawanshi, and V. Shelar, “Detection of seed borne myco-flora associated with cowpea (*Vigna unguiculata* L. Walp),” *International Journal of Chemical Studies*, vol. 8, no. 1, pp. 1585–1587, 2020.
 - [9] D. R. Taylor and A. S. Nyaujah, “Seed-borne fungi of rice, maize, sorghum, fundi, cowpea, groundnut, pigeon pea and pepper cultivated in the Kambia district of Sierra Leone,” *International Journal of Agriculture: Research and Review*, vol. 4, no. 8, pp. 569–578, 2016.
 - [10] D. Shahnaz, K. Maimona, and R. Summiaya, “Seed-borne fungi associated with cowpea (*Vigna unguiculata* L. Walp),” *International Journal of Biotechnology*, vol. 12, no. 4, pp. 565–569, 2015.
 - [11] N. G. Iyanyi and A. E. Ataga, “Fungal species associated with cowpea (*Vigna unguiculata* L. Walp) seeds from parts of Enugu state Nigeria,” *Scientia Africana*, vol. 1, pp. 103–108, 2014.
 - [12] P. M. Etaware, E. U. Etaware, O. O. Olaoluwa, O. Oj, O. O. Aiyelaagbe, and A. C. Odebode, “The impact crude plant extracts: as potential biofertilizers and treatment against tomato plant infection,” *Journal of Plant Pathology & Microbiology*, vol. 10, no. 5, pp. 481–491, 2019.
 - [13] R. N. Okigbo, “A review of biological control methods for post-harvest yams in storage in south eastern Nigeria KMITL,” *Journal of Science*, vol. 4, pp. 207–215, 2004.
 - [14] S. Sighamony, I. Anees, T. Chandrakal, and J. S. Kaiser, “Indigenous plant products as grain protectants against *Sitophilus oryzae* (L) and *Rhizopertha dominica* (F),” *Journal of Stored Products Research*, vol. 22, pp. 21–23, 1990.
 - [15] I. Mehedi, A. Sultana, and M. A. U. Raju, “Control of seed borne fungi on tomato seeds and their management by botanical extracts,” *Research in Agriculture Livestock and Fisheries*, vol. 3, no. 3, pp. 403–410, 2016.
 - [16] S. G. Malkhan, A. Shahid, A. Masood, and S. S. Kangabam, “Efficacy of plant extracts in plant disease management,” *Agricultural Sciences*, vol. 3, no. 3, pp. 425–433, 2012.
 - [17] R. N. Okigbo, “Variation in phytochemical properties of selected fungicidal aqueous extract of some plant leaves in Kogi state, Nigeria,” *American-Eurasian Journal of Sustainable Agriculture*, vol. 3, no. 3, pp. 407–409, 2009.
 - [18] C. O. Akueshi, C. O. Kadir, E. U. Akueshi, S. E. Agina, and C. Ngurukwen, “Antimicrobial potential of hyptis *saueolen* poit (*Lamiaceae*),” *Nigerian Journal of Botany*, vol. 15, pp. 37–41, 2002.
 - [19] V. I. Gwa, A. O. I. Nwankiti, and E. J. Ekefan, “Antifungal effect of five aqueous plant extracts on mycelial growth of *Penicillium expansum* isolated from rotted yam tubers in storage,” *ACTA Scientific Agriculture*, vol. 2, no. 6, pp. 65–70, 2018.
 - [20] A. N. Awurum, D. N. Enyiukwu, and P. O. Uchegwu, “Effectiveness of duration of storage and phytochemicals in the management of seed-borne mycoflora of stored cowpea seeds,” *Greener Journal of Agronomy, Forestry and Horticulture*, vol. 2, no. 2, pp. 22–26, 2014.
 - [21] I. O. Arikpo, S. Effiong-Udo, E. M. Eka, H. E. Kingsley, N. O. Alobi, and O. Magdalene, “Antifungal activity of extracts of scent leaf (*Ocimum gratissimum*) and alligator pepper (*Aframomum melegueta*) on the postharvest decay of carrot in Calabar, Nigeria,” *Journal of Biology, Agriculture and Healthcare*, vol. 3, no. 14, pp. 26–30, 2013.
 - [22] A. N. Awurum and P. O. Uchegwu, “Effect of duration of contact of seed extracts of *Piper guineense*, *Monodora myristica* and *Xylopiya aethiopica* on the germination and incidence of seed-borne fungi of stored cowpea (*Vigna unguiculata* L. Walp) seeds,” *Continental Journal of Biological Sciences*, vol. 6, no. 1, pp. 37–42, 2013.
 - [23] N. V. Chiejina and A. J. Ukeh, “Efficacy of *Aframomum melegueta* and *Zingiber officinale* extracts on fungal pathogens of tomato fruits,” *Journal of Pharmacy and Biological Sciences*, vol. 4, no. 6, pp. 13–16, 2013.
 - [24] R. N. Okigbo and O. U. Ogbonnaya, “Antifungal effects of two tropical plant extracts; *Ocimum gratissimum* and *Aframomum melegueta* on post-harvest yam rot,” *African Journal of Biotechnology*, vol. 5, pp. 727–731, 2006.
 - [25] E. O. Nwachukwu and C. I. Umechuruba, “Antifungal activities of some leaf extract on seed-borne fungi of African yambean seeds, seed germination and seed emergence,” *Journal of Applied Sciences & Environmental Management*, vol. 5, pp. 29–32, 2001.
 - [26] D. E. Okwu and E. E. Njoku, “Chemical composition and *in-vitro* antifungal activity screening of seed and leaf extracts from *Aframomum melegueta* and *Monodora myristica* against *Sclerotium rolfsii* of cowpea,” *Pest Technology*, vol. 3, no. 1, pp. 58–67, 2009.
 - [27] I. Sasidhran and A. N. Menon, “Comparative chemical composition and antimicrobial activity of berry and leaf essential oils of *Piper nigrum* L.” *International Journal of Biological & Medical Research*, vol. 1, no. 4, pp. 215–218, 2010.
 - [28] J. Bhardwaj, M. K. Setha, and R. Gogoi, “Evaluation of antifungal efficacy of plant extracts against seed-borne fungi *Colletotrichum lindemuthianum*,” *International Journal of Current Science*, vol. 18, pp. 49–54, 2015.
 - [29] International Seed Testing Association (ISTA), “Proceedings of the international seed testing association,” *International Rules of Seed Testing, Seed Science and Technology*, vol. 15, pp. 1–9, 2005.
 - [30] S. B. Mathur and O. Kongsdal, *Common Laboratory Seed Health Testing Methods for Detecting Fungi*, International Seed Testing Association (ISTA), Bassersdorf, Switzerland, 2003.
 - [31] H. L. Barnett and B. B. Hunter, *Illustrated Genera of Imperfect Fungi*, APS Press, St. Paul, MN, USA, 4th edition, 1998.
 - [32] A. C. Amadioha and V. I. Obi, “Control of anthracnose disease of cowpea by *Cymbopogon citratus* and *Occimum gratissimum*,” *Acta Phytopathologica et Entomologica Hungarica*, vol. 34, pp. 83–89, 1999.
 - [33] D. K. Pandey, N. N. Tripathi, R. O. Tripathi, and S. N. Dixit, “Fungitoxic and phytotoxic properties of essential oil of *Phyllis sauvolensis*,” *Pfkrankh. Pfschutz*, vol. 89, pp. 344–346, 1982.
 - [34] T. O. Adejumo, “Evaluation of botanicals as biopesticides on the growth of *Fusarium verticillioides* causing rot diseases and

- fumonisin production of maize,” *Journal of Microbiology and Antimicrobials*, vol. 4, no. 1, pp. 23–31, 2012.
- [35] B. O. Ejechi, A. Ojeata, and S. B. Oyeleke, “The effect of extracts of some Nigerian spices on biodeterioration of okra by fungi,” *Journal of Phytopathology*, vol. 145, pp. 469–472, 2008.
- [36] H. O. Bamidele, “Antifungal potency of *Aframomum melegueta* (alligator pepper) seed extracts on postharvest rot fungi of two citrus species,” *Sustainable Food Production*, vol. 6, pp. 1–11, 2019.
- [37] M. B. Isman, O. Koul, A. Kaminski, and J. Keminski, “Insecticidal and antifeedant bioactivities of neem oils and their relationship to azadirachtin content,” *Journal of Agricultural and Food Chemistry*, vol. 38, no. 6, pp. 1406–1411, 1990.
- [38] O. Koul, M. B. Isman, and C. M. Ketkar, “Properties and uses of neem, *Azadirachta indica*,” *Canadian Journal of Botany*, vol. 68, no. 1, pp. 1–11, 1990.
- [39] H. Schmutterer, “Properties and potential of natural pesticides for integrated pest control in developing and industrialized countries,” *Journal of Insect Physiology*, vol. 34, pp. 713–719, 1990.
- [40] P. C. Saxena, D. Kumar, and K. Navaneetham, “Language as a pull factor for migration to major cities in India,” in *Dynamics of Population and Family Welfare, 1989*, K. Srinivasan and K. B. Pathak, Eds., pp. 174–187, Himalaya Publishing House, Bombay, India, 1989.
- [41] A. Plata-Rueda, G. D. S. Rolim, C. F. Wilcken, J. C. Zanuncio, J. E. Serrão, and L. C. Martínez, “Acute toxicity and sublethal effects of lemongrass essential oil and their components against the granary weevil, *Sitophilus granarius*,” *Insects*, vol. 11, no. 6, p. 379, 2020.
- [42] D. A. Ukeh, “Bioactivities of essential oils of *Aframomum melegueta* and *Zingiber officinale* both (*Zingiberaceae*) against *Rhyzopertha dominica* (fabricius),” *Journal of Entomology*, vol. 5, no. 3, pp. 193–199, 2008.
- [43] M. N. Suleiman and O. M. Omafè, “Activity of three medicinal plants on fungi isolated from stored maize seeds (*Zea mays* (L.)),” *Global Journal of Medicinal Plant Research*, vol. 1, no. 1, pp. 77–81, 2013.