

Characterization of Potentially Mycotoxigenic Fungi Associated with Cowpea (*Vigna unguiculata* L. Walp) Seeds from Ogun West Markets

Hamzat- Akinwande, O.T. H¹., Owolade, O. F²., Salau, A. W³ and Abibu, W. A⁴.

¹Crop Production Research Programme, Institute of Food Security, Environmental Resources and Agricultural Research (IFSERAR), Federal University of Agriculture, P.M.B 2240, Abeokuta, Ogun State, Nigeria.

²Institute of Agricultural Research and Training, Obafemi Awolowo University, Ibadan, Oyo State, Nigeria.

³Department of Horticulture, Federal University of Agriculture, P.M.B 2240, Abeokuta, Ogun State, Nigeria.

⁴Department of Microbiology, Federal University of Agriculture, P.M.B 2240, Abeokuta, Ogun State, Nigeria.

Abstract

Cowpea grains stored under sub-optimal conditions are highly susceptible to fungal infection, resulting in significant post-harvest losses, grain quality degradation, and consumer health risks due to mycotoxin contamination. This study performed a morphological identification of seed-borne fungi associated with cowpea grains from major markets in Ogun West Senatorial District, Nigeria, and assessed the occurrence of mycotoxigenic and biocontrol fungal species. Thirty-six cowpea seed samples were randomly collected from retailers across three major Local Government Areas. Fungi were isolated on Czapek Dox Agar and identified by examining morphological characteristics, with emphasis on the known toxigenic nature of the isolates. Eleven fungal species belonging to seven fungal genera were identified. Significantly, the potentially mycotoxigenic species *Fusarium verticillioides*, *F. oxysporum*, *Aspergillus flavus*, and *A. niger* showed the highest occurrence (100% incidence across locations). Other high-incidence species included *Aspergillus tubingensis* and *Rhizopus stolonifera* (91.67%), while the known hyper-parasite and potential biocontrol agent, *Trichoderma* sp., was also isolated (41.67%). The high prevalence of well-known potential mycotoxin producers (e.g., *Aspergillus* spp. and *Fusarium* spp.) in marketed cowpea seeds highlights a critical food safety concern in the region. This characterization establishes a baseline for further genomic and metabolomic analyses of these Nigerian strains, which is essential for developing targeted post-harvest biocontrol strategies utilizing indigenous *Trichoderma* isolates or engineering crops with enhanced resistance. The specific fungal profiles identified, particularly the high frequency of *A. flavus* and *F. verticillioides*, are instrumental for developing cost-effective, high-throughput qPCR assays for early detection. This would enable market regulators in Ogun West to rapidly screen cowpea batches for high-risk contamination before visual symptoms appear.

Key words: Seed borne, fungal genera, *V. unguiculata*, mycological medium, Occurrence.

*Corresponding Author: hamzatoth@funaab.edu.ng; +234-8062326387

Introduction

Cowpea production and storage are hampered by a variety of disease and pests that impact viability, yield, quality, and nutritional value. This unpleasant occurrence, which resulted from more favorable conditions for the growth of fungus while in storage and/or transit, are caused by inadequate storage and transportation facilities (Osipitan *et al.*, 2021). As a result, poor nations have suffered significant post-harvest losses, and consumers face serious health risks if they ingest it in any form. In addition, many these fungi create toxic compounds, such as mycotoxins, which are chronic health issues for plants, animals, and people (Viljoen *et al.*, 2025). Nigeria is reportedly the country that produces and consumes the most cowpea grain. (Horn *et al.*, 2022). The greater percentage of people in the tropics can easily obtain and afford it because it is the main source of plant protein. Cowpeas are also a valuable multifunctional crop. In 2021, Nigeria produced 3.6 million tons of cowpeas, making it the world's top producer. However, the country's high population and significant post-harvest losses mean that demand for cowpeas in Nigeria exceeds supply (Horn *et al.*, 2022). Researchers have identified several major obstacles to Nigeria's cowpea production, including inadequate soil fertility, dryness, heat stress, limited adoption of better cowpea varieties, and high sensitivity to pests and diseases from planting to storage phases (Emechebe and Shoyinka, 1985). Their research indicates that low yield cultivars, low input consumption, and poor

productivity are now the hallmarks of cowpea agriculture. Nigeria has a substantial market for cowpea grains and feed, which has raised demand for the crop over the past few decades. Nigerian cowpea farmers have been seen to regularly plant tainted preserved seeds, which frequently result in seed rot, mycotoxin contamination, and loss of viability (Taylor and Nyaujah, 2016).

Both harmful and saprophytic fungi can be found in plant tainted seeds. Saprophytic seed-borne fungi induce discolouration, decreased weight, and germination of seeds while they are being stored. On the other hand, pathogenic seed-borne fungi infect seeds in the field, impair the plant during its early growth phases, destroy seed vigor, and spread disease outbreaks (Shahnaz *et al.*, 2015).

Cowpea seeds have been connected to a few seed-borne fungal species in different parts of the world. *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum*, *Alternaria alternata*, and *Penicillium sp.* have all been connected to cowpea seeds in India (Etaware *et al.*, 2019). Okigbo (2004) found that *Phoma sp.*, *Macrophomina phaseolina*, *Fusarium verticillioides*, and *Fusarium solani* are prevalent on cowpea seeds in Sierra Leone.

In Nigeria, cowpea seeds are predominantly marketed in open markets where storage conditions are often sub-optimal. Seeds are frequently stored in woven sacks or open iron containers and exposed to fluctuating environmental conditions, mechanical damage, insect infestation and microbial contamination.

These dynamics collectively promote fungal growth and subsequent mycotoxin production. Although, the works of authors like Aleruchi *et al.* (2025), Atanda *et al.* (2012) and Ayeni *et al.* (2021) have documented fungal contamination of stored grains and legumes in different parts of Nigeria, information on characterization and diversity of mycotoxigenic fungi associated with cowpea seeds in Yewa markets of Ogun West Local government Area remains scanty. Using microbial tools, knowledge of the prevalent mycotoxigenic fungi can inform public health interventions, improve storage practices and support regulatory frameworks aimed at ensuring food safety. Therefore, this study aims to investigate the potential food safety risks associated with the presence of mycotoxigenic fungi in cowpea seeds sold in selected Yewa markets within Ogun West Senatorial District, Southwestern Nigeria. The study aims to investigate the possibility of identifying pathogenic biotic agents linked to cowpea seeds and grains that are stored and sold in the main markets of some selected local government areas in Ogun West Senatorial District.

Materials and Methods

Collection of samples and sampling procedure

Cowpea seeds from 36 retailers were randomly selected from three local government areas in the Ogun West Senatorial District, Ogun State, Nigeria. The seed samples were kept in cellophane bags and transported to the Plant Pathology laboratory, Institute of Agricultural Research and Training, Obafemi Awolowo University, Ibadan campus, Ibadan, Nigeria. The major markets where the samples were collected included Ayetoro, Ojaodan, Ijoun, Imasai, Sayedero, Sabo, Eredo, Owode, Idiroko, Ijofin, Ipokia and Tube. These markets were randomly selected from three Local Government Areas in Ogun West. These included, Yewa North, Yewa South and Ipokia Local Government Areas (Table 1). The cowpea seed samples were collected from three different retailers from each of the 12 markets and these were labeled as sample A, B and C accordingly. Twelve (12) markets and three (3) retailers sampled gave a total of thirty-six (36) samples taken to the laboratory for further processing.

Table 1. Location and Affiliated Local Government Area where cowpea seeds were sampled

S/N	Location	Affiliated local government area	Market Accession code
1	Ayetoro	Yewa north	YN1
2	Ojaodan	Yewa north	YN2
3	Ijoun	Yewa north	YN3
4	Imasai	Yewa north	YN4
5	Sayedero	Yewa south	YS1
6	Sabo	Yewa south	YS2
7	Eredo	Yewa south	YS3
8	Owode	Yewa south	YS4
9	Idiroko	Ipokia	IP1
10	Ijofin	Ipokia	IP2
11	Ipokia	Ipokia	IP3
12	Tube	Ipokia	IP4

Sterilization of Glassware

After collection of all the samples, glassware such as beakers, measuring cylinders, spatula, conical flask, were washed thoroughly with liquid detergent, rinsed severally with water and then with sterile distilled water and allowed to air-dry. Petri dishes and other glassware were arranged in canisters before sterilization in the oven (Gallenkamp Hotbox oven, Gallenkamp, UK) at 160 °C for 90 min. Distilled water was sterilized using Schott Duran bottles in the autoclave at temperature of 121 °C and 15 psi for 15 min. The sterilized glasswares were used within 24 h. Forceps and microscopic glass slides were sterilized by dipping them into methylated spirit and passing them over the flame three times at an angle of 45 °C at the upper part of the flame. The inoculating needles were sterilized by heating to "red hot" over a Bunsen burner flame. Plastic materials were sterilized by soaking them in 10% sodium hypochlorite (NaOCl) for 1 hour and were later rinsed with sterile distilled water. The working benches, lamina hood and environment

where sample preparation took place were sterilized by cleaning with methylated spirit.

Czapek Dox Agar (CZA) incorporated with streptomycin was used for the isolation and identification of the organisms. A semi-synthetic medium processing sodium nitrate as the only source of nitrogen. It supports the growth of most fungi in abundance. It also has good buffering action due to the presence of many salts. Streptomycin injection is generally used to avoid bacterial contamination; it has a bactericidal effect on both gram - positive and gram-negative bacterial strains. It is generally binding 30s ribosomal subunits of bacteria and block the initiation and elongation steps in protein synthesis.

The workbench was surface sterilized with 70% ethanol. Ten (10) seeds of cowpea counted thrice for each retailer's sampled from the different markets, and wrapped with the aluminum foil were labeled properly. The process was repeated for all the 36 samples of cowpea. The forceps

used were aseptically sterilized at interval to prevent transfer of inoculum between samples

Isolation of fungi from cowpea seeds

For the detection of seed- borne mycoflora, ISTA (International Seed Testing Association) technique was employed using standard agar plates (Anon, 1993). Seeds were surface-sterilized by dipping in 1% Na(OCl)₂ (Sodium hypochlorite) for 2 min followed by rinsing in several changes of sterile distilled water. Ten (10) surface sterilized seeds were plated on the cooled pre-poured Czapek Dox Agar and duly labeled. Plates sealed with paper tapes and were incubated at 25±2 °C for 5-7 d under 12h alternating cycles of natural day light and darkness (Anon, 1993). The isolated fungi were identified using both macroscopical and microscopical methods. The colors and general appearance of the fungal growth on plates were examined. Little samples of the pure fungal cultures were agitated, preserved in lactophenol in cotton blue dye, placed on sterile slides with a fresh cover slip, and examined under a microscope (Fawole and Osho, 1995). The fungal identities were verified by matching them with verified representatives found using a key, as well as by utilizing cultural traits and microscopic analyses.

After 72 hr of incubation, the plates were observed daily for growth. The number of colonies observed on the individual plate containing the sampled seeds was counted in the growth area. The colony was observed and where necessary documentation of the organism suspected, then the individual colony that appeared in different

plates was selected. Using a sterile inoculating needle, a small portion of each sample was cut out and transferred to a new prepared culture medium in petri dish containing CZA's (streptomycin incorporated). This was done to obtain pure fungal cultures. These new plates were labeled accordingly and wrapped using paper tapes/aluminum foil and stored in the incubator at room temperature of 27 °C for another 72 hr to obtain a pure culture of the suspected organism.

Isolation of pure cultures

Ten milliliters of molten (10 ml) Czapek Dox was poured into sterilized McCartney bottles and allowed to solidify in a staining rack in slanted position. After solidification, pure cultures of the sample were obtained from the sub cultured sample petri dishes using a sterile inoculating needle and placed in each of the already prepared McCartney bottles containing solidified agar slant. These were stored for further microbial examination as cultures in agar slant survive longer than those kept in petri dishes. Although it was not kept for too long because the fungi could consume the entire agar in the bottles if kept for a while. The stock culture was monitored to avoid excessive growth.

The identification of isolates was done culturally and microscopically for the classification and identification of various fungi. The pure cultures were viewed under the microscope using microscopic slides. A sterile glass slide was prepared with a drop of lactophenol cotton blue stain (LPCS). Using a sterile inoculating needle, a

small portion of the fungal culture was gently removed from the agar plate. The fungal material was placed in the drop of stain and gently teased apart with the needle to ensure even distribution. It was covered using a cover slip before viewing under the microscope, (x10) and (x40). This work was replicated thrice, starting from the initial isolation process.

Determination of the frequency of occurrence of the fungal isolates

The frequency of occurrence was determined by how often an organism occurred on a sample from the individual retailer. This was carried out using occurrence count on the agar plates that had the cowpea seeds. Total isolates count for each sample compared with the total number of samples collected within a location using the technique outlined by Giridher and Ready (1997).

Determination of percentage (%) occurrence of the fungal isolates

This was done in order to ascertain the frequency of occurrence of the various fungal isolates that were obtained from cowpea seeds in the local government area that was chosen for each site. The number of times each isolate occurred in the sampled regions was calculated and compared to the total number of isolates in all screened sampling locations. Thus, the approach outlined by (Giridher and Ready, 1997) was used to calculate the frequency of occurrence.

Percentage of
frequency

$$= \frac{\text{Number of observations in which species appear}}{\text{Total Number of observations}} \times 100$$

Data analysis: Analysis of variance was performed on the gathered data using GenStat 12th edition (2009) to identify significant differences between treatments and their interactions. Duncan's Multiple Range Test (DMRT) was used to differentiate treatment means at a 5% probability level. Values in percentages were analyzed statistically after angular transformation (Peterson, 1985).

Results

Characteristics of identified fungi isolated from the cowpea seeds from the selected areas

This research was carried out to examine the fungi associated with seeds of cowpea collected from twelve markets (12) in three local government (Yewa north, Yewa south and Ipokia) areas, Ogun State, Nigeria. Results from Table (2) showed the cultural and microscopic characters of the isolated fungi from different market locations in the three local government areas. Identification was based solely on morphological characteristics and should be considered presumptive. It was observed that eleven fungi were isolated from cowpea seeds collected.

The occurrence of fungi, *Fusarium verticillioides*, *Fusarium oxysporium*, *Aspergillus flavus*, *Aspergillus niger* and *Penicillium citrinum* were

highest across the locations (Table 3). These were closely followed by *Aspergillus tubingensis* and *Rhizopus stolonifera* while *Trichoderma* sp. least occurred amongst the fungi isolated from the

cowpea seeds. However, the number of fungi isolated from these samples collected at different locations ranged between eight (08) and eleven (11) organisms (Table 3).

Table 2: Characteristics and Identification of fungi Isolated from cowpea seeds sampled

S/N	Fungal Species	Cultural Appearance	Microscopic characteristics
1	<i>Fusarium verticillioides</i>	Initially pale salmon coloured with cottony aerial mycelium	Macroconidia were moderately falcate, septated, hyaline with a curved and tapering apical cell. It grows very well at room temperature.
2	<i>Fusarium oxysporum</i>	Superficial mycelium observed, Pinkish to off-white and Cottony colonies were observed, Light orange pigmentation often observed and presence of radial growth	Septated hyphae; sickle-shaped macroconidia with presence of chlamydospores
3	<i>Colletotrichum</i> sp.	Grayish to black in colour, with formation of concentric ring pattern	Hyaline with septated conidia; some conidia curved while some were straightened
4	<i>Macrophomina phaseolina</i>	Gray to black colour were observed with the presence of microsclerotia	Dark brown, round or spherical microsclerotia; presence of septated hyphae
5	<i>Aspergillus flavus</i>	the culture appeared Yellowish green on the surface with the procession of powdery texture	conidia are yellowish green pyriform or globose, smooth
6	<i>Aspergillus niger</i>	Observation showed black surface with off- white to yellow edges and in addition, powdery appearances were observed	Large globose vesicle with dark brown conidia presence in long chains
7	<i>Aspergillus tubingensis</i>	Black colonies were observed and were similar to that of <i>A. niger</i> but with less compact conidial heads	It was similar to observation made in <i>A. niger</i> , but this with smoother and slightly larger conidia
8	<i>Aspergillus fumigatus</i>	Observation on colony colour with smoky gray-green surface, texture was observed as powdery at first later turning into smoky green	Observation of white to tan conidia with possession of smooth conidiophore, has uniseriate phialides (single) usually covering the upper half vesicle located parallel to the axis of the stalk.
		Reverse Pigmentation	

		Special Feature can grow at 55 °C and survive up to 70 °C	
9	<i>Rhizopus stolonifer</i>	Cottony growth were observed with blackish sporangia	sporangiophores emerged from well-developed rhizoids that were dark brown in color.
10	<i>Trichoderma</i> spp.	Observation of green surface with white border that appeared compact and fast-growing on CZA plates	long, hyaline, branched, with individual phialide appearance, or in groups of 2 or 3 phialides
11	<i>Penicillium citrinum</i>	Blue-green surface was observed with yellow back view	Branched conidiophores were observed with chains of spherical conidia from phialides

Distribution of fungi associated with cowpea seed samples collected from different locations.

Results from Table (3) showed that eleven fungal species from seven genera were identified from the cowpea accession evaluated in this study, including *Fusarium verticillioides*, *Fusarium oxysporium*, *Colletotrichum sp*, *Macrophomina phaseolina*, *Aspergillus tubingensis*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Rhizopus stolonifera*, *Trichoderma* spp., and *Penicillium citrinum*. This study was limited to the isolation and characterization of fungi associated with cowpea (*Vigna unguiculata* L. Walp) seeds obtained from selected markets in Ogun West, Nigeria. Although several fungal species identified in this study particularly those belonging to the genera *Aspergillus*, *Fusarium*, and *Penicillium* are widely reported in the literature as mycotoxin producers, direct quantification of mycotoxins was not conducted. Consequently, the mycotoxigenic status of the isolates could not be experimentally confirmed, and their classification should be

regarded as potentially mycotoxigenic rather than definitively toxin-producing. It was observed that among eleven potential mycotoxigenic fungal organisms found associated with the cowpea seed samples collected from the study areas, four were pathogenic fungi such as; *Fusarium verticillioides*, *Fusarium oxysporium*, *Colletotrichum sp*, and *Macrophomina phaseolina*, six were saprophytic fungi; *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus tubingensis*, *Penicillium sp.* and *Rhizopus stolonifera* and one Hyper-pathogenic fungus; *Trichoderma sp.*

The *Fusarium verticillioides*, *Fusarium oxysporium*, belongs to Phylum: *Ascomycota* and Class: *Ascomycetes*, while the *Colletotrichum sp.* belongs to the class: *Sordariomycetes*. The *Macrophomina phaseolina* is in the class of *Dothideomycetes*. The *Aspergillus sp.* is in the class of *Eurotiomycetes* while the *Penicillium citrinum*, belong to the class of *Ascomycetes* (Table 3).

The occurrence of fungi, *Fusarium verticillioides*, *Fusarium oxysporium*, *Aspergillus flavus*, *Aspergillus niger* and *Penicillium citrinum* was highest across the locations (Table 3). These were closely followed by *Aspergillus tubingensis* and *Rhizopus stolonifera* while *Trichoderma* sp. least occurred amongst the fungi isolated from the cowpea seeds collected at these locations. However, the number of fungi isolated from these samples collected at different locations were between eight (08) and eleven (11) organisms (Table 3).

Table 3: Distribution of Fungi Isolated from Cowpea Seeds Collected from Selected Markets in each Location

LGA	Location	Market accession code	<i>F. ver.</i>	<i>F. oxy.</i>	<i>Coll. sp</i>	<i>M. pha.</i>	<i>A. fla.</i>	<i>A. nig.</i>	<i>A. tub.</i>	<i>A. fum.</i>	<i>R. stol.</i>	<i>Tric. spp.</i>	<i>P. citr.</i>	Number of Fungi Isolated
Yewa North	Ayetoro	YN1	+	+	-	-	+	+	+	+	+	-	+	08
Yewa North	Ojaodan	YN2	+	+	+	+	+	+	+	+	+	+	+	11
Yewa North	Ijoun	YN3	+	+	+	+	+	+	+	+	+	-	+	10
Yewa North	Imasai	YN4	+	+	-	+	+	+	-	+	-	+	+	08
Yewa South	Sayedero	YS1	+	+	+	+	+	+	+	+	+	-	+	10
Yewa South	Sabo	YS2	+	+	+	+	+	+	+	-	+	+	+	10
Yewa South	Eredo	YS3	+	+	+	+	+	+	+	+	+	-	+	10
Yewa South	Owode	YS4	+	+	+	-	+	+	+	-	+	+	+	09
Ipokia	Idiroko	IP1	+	+	+	-	+	+	+	+	+	-	+	09
Ipokia	Ijofin	IP2	+	+	-	+	+	+	+	+	+	-	+	09
Ipokia	Ipokia	IP3	+	+	-	+	+	+	+	-	+	+	+	09
Ipokia	Tube	1P4	+	+	+	-	+	+	+	+	+	-	+	09
% Occurrence			100	100	66.67	66.67	100	100	91.67	75.0	91.67	41.67	100	Range= 08 & 11

Key: *F. ver.* *Fusarium verticillioides*; *F. oxy.* *Fusarium oxysporum*; *Coll. sp* *Colletotrichum* sp.; *M. pha.* *Macrophomina phaseolina*; *A. fla.* *Aspergillus flavus*; *A. nig.* *Aspergillus niger*; *A. tub.* *Aspergillus tubingensis*; *A. fum.* *Aspergillus fumigatus*; *R. stol.* *Rhizopus stolonifera*; *Tric. spp.* *Trichoderma* spp.; *P. citr.* *Penicillium citrinum*

+ = presence of organism

- = Absence of organism

Frequency of occurrence of fungi associated with cowpea seeds collected from choice Local Government Areas

Penicillium citrinum, the frequency was similar across the three local government area (Table 5).

The Analysis of Variance presented in Table (4) showed that local government area significantly affected *F. verticillioides*, *Colletotrichum* sp, *Macrophomina phaseolina* and *Rhizopus stolonifera* ($p < 0.05$), while location affected only *Penicillium citricum*. Also interaction of local government areas and location significantly affected *Aspergillus niger*, *Aspergillus tubingensis*, *Aspergillus fumigatus* and *Trichoderma spp* (Table 4). Retailers' sampling did not significantly ($p > 0.05$) affect the frequency of isolated fungi.

Among the fungi isolated from selected local government area in Ogun west, the frequency of *F. verticillioides* was higher in Ipokia and Yewa south relative to Yewa north and for *F. oxysporium*, the frequency was similar across the local government areas examined (Table 5). The frequency of *Colletotrichum sp.* in Yewa south did not differ from that obtained from Yewa north and relative to Ipokia, while the frequency of *M. phaseolina* was similar across the three local government areas investigated. Frequency of *A. flavus* was higher in Ipokia, while *A. niger* was higher in both Yewa north and Ipokia; *A. tubingensis* was higher in Ipokia, while *A. fumigatus* was highest in Yewa north but similar compared to the frequency in Ipokia (Table 5). The frequency of *R. stolonifera* was highest in Ipokia, while for *Trichoderma spp.*, it was more prevalent in Yewa north and Yewa south. For

Table 4: ANOVA Showing the Frequency of Occurrence of Various Fungal Organisms Isolated from the Samples across Locations

Source of variation	d.f.	<i>F.vert</i>	<i>F.oxy.</i>	<i>C. sp</i>	<i>Mac phas</i>	<i>A. flav</i>	<i>A. nig</i>	<i>A. tub</i>	<i>A. fum</i>	<i>R. stol</i>	<i>Tric. spp</i>	<i>Pen. Citr</i>
REP stratum	2	1.361	5.250	0.194	1.028	5.028	7.843	5.778	2.009	0.954	0.528	4.731
REP.LGA stratum												
LGA	2	16.583*	1.750*	2.583*	0.583*	22.750*	13.898*	3.000*	32.065*	6.509*	5.250*	0.898
Residual	4	2.111	0.333	1.028	0.528	1.403	0.940	0.319	4.426	0.176	0.194	4.231
REP.LGA.LOCATION stratum												
LOCATION	11	4.705*	3.318*	4.705*	7.773*	8.614*	18.371*	6.727*	15.977*	3.530	11.250*	3.462*
Residual	16	1.969	1.844	0.427	0.781	2.729	2.729	0.948	1.823	1.698	0.844	1.323
REP.LGA.LOCATION.*Units* stratum												
SAMPLE	2	1.361	0.250	0.083	0.083	0.250	0.398	0.250	0.231	0.120	0.000	2.065
LGA.SAMPLE	4	0.861	0.500	0.083	0.083	0.875	0.454	0.250	0.273	0.134	0.000	0.731
LOCATION.SAMPLE	22	0.295	0.250	0.068	0.068	0.636	0.326	0.114	0.182	0.258	0.000	0.326
Residual	44	1.288	1.652	0.652	0.712	2.197	2.460	1.318	1.177	0.990	0.788	1.662
Total	107											

*Significant at 5% (p< 0.05) probability

Key: *F. ver.* *Fusarium verticillioides*; *F. oxy.* *Fusarium oxysporum*; *Coll. sp* *Colletotrichum sp.*; *M. pha.* *Macrophomina phaseolina*; *A. fla.* *Aspergillus flavus*; *A. nig.* *Aspergillus niger*; *A. tub.* *Aspergillus tubingensis*; *A. fum.* *Aspergillus fumigatus*; *R. stol.* *Rhizopus stolonifera*; *Tric. spp.* *Trichoderma spp.*; *P. citr.* *Penicillium citrinum*

Table 5: Frequency of Fungi Isolated From Cowpea Seeds Collected From Selected Local Government Areas

LGA	<i>F. ver.</i>	<i>F. oxy.</i>	<i>Coll. Sp</i>	<i>M. pha.</i>	<i>A. fla.</i>	<i>A. nig.</i>	<i>A. tub.</i>	<i>A. fum.</i>	<i>R. stol.</i>	<i>Tric. spp.</i>	<i>P. citr.</i>
YN	2.250 ^b	2.583 ^a	0.917 ^a	1.083 ^a	5.667 ^b	6.556 ^a	1.500 ^b	3.333 ^a	1.528 ^b	1.250 ^a	2.278 ^{ns}
YS	3.333 ^a	2.250 ^{ab}	1.000 ^a	1.250 ^a	6.333 ^b	5.333 ^b	1.500 ^b	1.500 ^b	1.694 ^b	1.000 ^a	2.500 ^{ns}
IP	3.500 ^a	2.167 ^b	0.500 ^a	1.000 ^a	7.250 ^a	6.139 ^a	2.000 ^a	2.028 ^{ab}	2.333 ^a	0.500 ^b	2.583 ^{ns}
Mean Value	3.028	2.333	0.806	1.111	6.417	6.009	1.667	2.287	1.852	0.917	2.454

Means followed by the same letter in any vertical column are not significantly different at $p = 0.05$ according to Duncan's Multiple Range Test.

Key: *F. ver.* *Fusarium verticillioides*; *F. oxy.* *Fusarium oxysporum*; *Coll. sp* *Colletotrichum* sp.; *M. pha.* *Macrophomina phaseolina*; *A. fla.* *Aspergillus flavus*; *A. nig.* *Aspergillus niger*; *A. tub.* *Aspergillus tubingensis*; *A. fum.* *Aspergillus fumigatus*; *R. stol.* *Rhizopus stolonifera*; *Tric. spp.* *Trichoderma* spp.; *P. citr.* *Penicillium citrinum*; YN Yewa North; YS Yewa South; IP Ipokia

Effect of location on the fungi isolated from cowpea seeds at each market

Location significantly affected fungi isolated from cowpea seeds collected at each market across the three local governments (Table 6). The results showed that in Yewa north, *F. verticillioides*, was more prevalent in Ijoun, Imasayi and Ojaodan; in Yewa south, the fungus was more prevalent in Eredo, Sabo and Sayedero markets, while in Ipokia Local Government Area, *F. verticillioides* was more prevalent in all the markets under study. For *F. oxysporium*, the fungus was prevalent in all the markets across the three local government areas except at Tube in Ipokia local government. For *Colletotrichum sp.*, the fungus was highly prevalent in Ojaodan, Yewa north followed by Tube in Ipokia local government area (Table 6).

For *M. phaseolina*, the fungus was highly prevalent only in Imasayi in Yewa north, also prevalent in Sayedero and Sabo in Yewa south and highly prevalent in Ijofin and Ipokia in Ipokia Local Government Areas. For *A. flavus*, the fungus was highly prevalent in Imasayi and Ayetoro in Yewa north; Eredo in Yewa south and prevalent in Ijofin, Ipokia Local Government Areas. For *A. niger*, the fungus was highly prevalent in Tube, Ipokia LGA, Sayedero, Yewa south and slightly prevalent in Imasayi, Yewa North LGA. For *A. tubingensis*, the fungus was most prevalent in Ijofin and Idiroko in Ipokia LGA and more prevalent in Ayetoro, Oja odan and Ijoun in Yewa north and in Sayedero and Sabo in

Yewa South LGA. For *A. fumigatus*, fungus was most prevalent in Ojaodan in Yewa north, Sayedero and Eredo in Yewa south and in Idiroko and Ipokia markets for Ipokia Local Government Areas. For *Trichoderma sp.*, the fungus was more in Ojaodan and Imasayi in Yewa north, also at Sabo and Owode in Yewa South and Ipokia, in Ipokia LGA. For *P.citrinum*, fungi were highly prevalent in all the market across all the three Local Government Areas (LGA) except at Tube in Ipokia LGA (Table 6).

Table 6: Effect of location on frequency of fungi isolated from cowpea seeds collected

LGA	Market accession code	<i>F. ver.</i>	<i>F. oxy.</i>	<i>Coll. Sp</i>	<i>M. pha.</i>	<i>A. fla.</i>	<i>A. nig.</i>	<i>A. tub.</i>	<i>A. fum.</i>	<i>R. stol.</i>	<i>Tric. spp.</i>	<i>P. citr.</i>
YN	YN1	1.778 ^c	1.750 ^{ab}	-0.111 ^d	0.028 ^{de}	6.417 ^{abc}	4.454 ^{ef}	2.167 ^{ab}	1.954 ^{bc}	2.324 ^a	0.333 ^b	2.509 ^a
YN	YN2	2.778 ^{abc}	2.083 ^{ab}	2.556 ^a	1.028 ^{bc}	5.417 ^c	5.787 ^{cde}	2.167 ^{ab}	3.954 ^a	2.435 ^a	1.667 ^a	2.843 ^a
YN	YN3	3.778 ^a	2.750 ^a	0.889 ^{bc}	1.028 ^{bc}	5.750 ^{bc}	7.454 ^{ab}	2.167 ^{ab}	1.954 ^{bc}	2.324 ^a	0.333 ^b	2.176 ^a
YN	YN4	3.787 ^a	2.750 ^a	-0.111 ^d	2.361 ^a	8.083 ^a	6.343 ^{bcd}	0.167 ^d	1.287 ^{cd}	0.024 ^b	2.667 ^a	2.287 ^a
YS	YS1	2.694 ^{abc}	1.750 ^{ab}	0.806 ^{bc}	1.861 ^{ab}	6.417 ^{abc}	8.009 ^{ab}	2.167 ^{abc}	3.787 ^a	1.157 ^{ab}	0.083 ^b	2.954 ^a
YS	YS2	3.694 ^a	2.417 ^{ab}	0.806 ^{bc}	1.861 ^{ab}	6.083 ^{bc}	5.676 ^{cdef}	2.167 ^{ab}	0.787 ^{cd}	1.824 ^a	1.917 ^a	2.287 ^a
YS	YS3	3.694 ^a	3.083 ^a	0.806 ^{bc}	0.861 ^{cd}	8.083 ^a	5.676 ^{cdef}	1.167 ^{bd}	3.787 ^a	2.269 ^a	0.083 ^b	1.954 ^{ab}
YW	YS4	2.028 ^{bc}	2.083 ^{ab}	0.806 ^{bc}	-0.139 ^e	5.083 ^c	4.676 ^{def}	1.167 ^{bcd}	0.787 ^{cd}	2.157 ^a	1.917 ^a	2.620 ^a
IP	IP1	2.528 ^{abc}	2.167 ^{ab}	1.306 ^b	0.111 ^{cde}	5.833 ^{bc}	5.204 ^{def}	2.333 ^a	3.704 ^a	2.185 ^a	0.417 ^b	2.870 ^a
IP	IP2	3.528 ^{ab}	2.833 ^a	0.306 ^{cd}	2.111 ^a	7.500 ^{ab}	6.423 ^{bcd}	3.000 ^a	2.926 ^{ab}	1.519 ^{ab}	0.417 ^b	3.204 ^a
IP	IP3	3.528 ^{ab}	3.167 ^a	0.306 ^{cd}	2.111 ^a	6.167 ^{bc}	3.870 ^f	0.667 ^d	0.259 ^d	2.185 ^a	2.417 ^a	2.870 ^a
IP	1P4	2.528 ^{abc}	1.167 ^b	1.306 ^b	0.111 ^{cde}	6.167 ^{bc}	8.537 ^a	0.667 ^d	2.259 ^{bc}	1.519 ^{ab}	0.417 ^b	0.870 ^b
Mean Value		3.028	2.333	0.8056	1.111	6.417	6.009	1.667	2.287	1.852	0.917	2.454

Means followed by the same letter in any vertical column are not significantly different at p = 0.05 according to Duncan's Multiple Range Test.

Key: *F. ver.* *Fusarium verticillioides*; *F. oxy.* *Fusarium oxysporum*; *Coll. sp* *Colletotrichum* sp.; *M. pha.* *Macrophomina phaseolina*; *A. fla.* *Aspergillus flavus*; *A. nig.* *Aspergillus niger*; *A. tub.* *Aspergillus tubingensis*; *A. fum.* *Aspergillus fumigatus*; *R. stol.* *Rhizopus stolonifera*; *Tric. spp.* *Trichoderma* spp.; *P. citr.* *Penicillium citrinum*; YN Yewa North; YS Yewa South; IP Ipokia

Discussion

This study investigated the seed borne fungi associated with the cowpea seed collected from twelve markets in selected Local Government areas of Ogun West Senatorial District, Ogun State. Using the conventional agar plate method, eleven potential mycotoxigenic fungal species from seven genera- *Fusarium verticillioides*, *Fusarium oxysporum*, *Colletotrichum sp*, *Macrophomina phaseolina*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus tubingensis*, *Aspergillus fumigatus*, *Rhizopus stolonifera*, *Penicillium citrinum* as well as a Hyper- pathogenic fungus; *Trichoderma spp* were recovered from cowpea seeds in this investigation.

The majority of the examined seeds had seed-borne fungal organism infections, which might lower the proportion of cowpea seeds that germinate (Akande, 2007; Khare *et al.* 2016).

Some pathogenic organisms like *Fusarium verticillioides*, *Fusarium oxysporum*, *Colletotrichum sp*, *Macrophomina phaseolina* found prevalent in humid areas of Yewa north, Yewa south and Ipokia Local Government area suggests that the prevalence of these pathogens is being induced by longer alternating periods of warmth and moisture in the region. The result is in line with Martin *et al.* (2022) who reported that post-harvest moisture content play a significant role in fungal infections. Saprophytic organisms like *Aspergillus flavus* and *Aspergillus fumigatus* found prevalent in Eredo in Yewa south, *Aspergillus niger* and *Aspergillus tubingensis* found in Idiroko and Ijofin in Ipokia local government may also be due to high humidity caused by high rainfall.

Fusarium verticillioides and *Fusarium oxysporum* which are fungi recovered from seeds are linked to both saprophytic and pathogenic fungi. Among the fungi found on South African cowpea are *Fusarium equiseti*, *F. semitectum*, *F. subglutinans*, *F. chlamydosporum*, *F. sambucium*, and *F. graminearum* (Kritzing *et al.*, 2003). Seed samples collected from Northern Nigerian markets were found to include *Ascochyta sp.*,

Colletotrichum lindemuthianum, *Rhizoctonia solani*, *F. oxysporum*, *F. solani*, *Macrophomina phaseolina*, *Septoria vignae*, and *Corticium rolfsii* (Emechebe and McDonald, 1979).

Mogle and Maske (2012) isolated *Rhizoctonia solani*, *Aspergillus flavus*, *Cladosporium sp.*, *Aspergillus niger*, *Penicillium sp.*, *Fusarium oxysporum*, *F. solani*, *F. semitectum*, *Trichoderma viride*, *Curvularia lunata*, *Mucor sp.*, and *Verticillium sp.* from the seed samples collected from Maharashtra, India. *Alternaria longissima*, *A. flavus*, *A. fumigatus*, *A. niger*, *Botryodiplodia theobromae*, *Colletotrichum sp.*, *Mucor himelis*, *M. phaseolina*, and *R. oryzae* were all isolated by Amadi and Oso in 1996 using agar and blotter methods. Embaby and Abdel-Galil (2006) detected species of *Alternaria*, *Aspergillus*, *Epicoecum*, *Fusarium*, and *Trichoderma* from cowpea and lupine seed samples from Egypt; the amount of protein, carbohydrate, fat, fiber, and ash decreased when the seeds were intentionally infected with aflatoxin. Ahmad *et al.* (1993) discovered *Fusarium moniliforme*, *Curvularia lunata*, and *Colletotrichum dematium* on Pakistani cowpea seeds.

Mycotoxin levels increase with the quantity of fungus isolated from a seed sample. Mycotoxin levels are secondary metabolites produced by fungi (Rahim *et al.*, 2013). Mycotoxins generated by *Aspergillus* species were found in cowpea seed samples by Ibeh *et al.* (1991). However, Houssou *et al.* (2009) observed aflatoxin in cowpea seed samples from West Africa, whereas Habish (1972) detected aflatoxin in samples taken from Sudan. According to Rahim *et al.* (2010) and Agarwal and Sinclair (1996), the nutritional quality, viability, and germination of seeds decrease as the quantity of storage fungus rises.

Aspergillus was found to be the most prevalent mold in melon that was sold in stores by Bankole *et al.* (2004) during their examination of melon seeds. The most frequent fungus found to infect plant-based products was *Aspergillus* (Lugauskas, 2005; Fagbohun *et al.*, 2010; Gautam and Bhadauria, 2010). The government needs to develop a good road network in the area to improve the hygienic conditions around these markets. Also, there is the need for

awareness on the microorganisms associated with cowpea seed contamination and on the health hazards associated with consumption of fungal infected seeds. People should be taught basic handling of cowpea seeds before it is considered safe for planting and consumption.

Pathogenic fungi e.g., *F. verticillioides*, *F. oxysporium*, *Colletotrichum* sp., and *M. phaseolina* are dominant in Ijoun, Imasai, Sabo, Eredo, Ipokia, and Ijofin (Yewa North, Yewa South, Ipokia).

Saprophytic fungi such as *A. flavus*, *A. fumigatus*, *A. niger*, *A. tubingensis* are dominant in Eredo, Idiroko, and Ijofin. However, the primary mycotoxins produced by the most dominant species identified are *Aspergillus flavus*, produces Aflatoxins; *Fusarium verticillioides*, Fumonisin; while *Penicillium citrinum*, produces Citrinin in the affected crop (Osibe *et al.*, 2025). *Trichoderma* species are more widely recognized in agriculture and industry as biocontrol agents (hyper-parasites of other fungi) and for their capacity to promote plant growth. The prevalence of key mycotoxigenic species—specifically *Aspergillus flavus* (aflatoxin producer) and *Fusarium verticillioides* (fumonisin producer)—necessitates a shift from traditional plating methods to rapid molecular screening e.g., qPCR Assay

Development: The specific identification of these fungal species allows for the immediate design of quantitative Polymerase Chain Reaction (qPCR) primers targeting their unique DNA sequences. Furthermore, morphologically similar species, such as *Aspergillus niger* and *A. tubingensis*, cannot be reliably differentiated without molecular tools. In addition, confirmation of *Fusarium verticillioides* requires TEF-1α sequencing. Thus, Further biotechnology research should focus on developing inexpensive, user-friendly Lateral Flow Devices (LFDs) or ELISA-based immuno-assays specific to Aflatoxins and Fumonisin. These portable diagnostic tools would empower local market inspectors and co-operatives to conduct on-the-spot safety checks on cowpea, particularly in high-prevalence areas like Eredo and Ijofin. Also, Strategic Biocontrol and Biopesticide Development: The presence of *Trichoderma* spp., a recognized biocontrol agent, suggests that local microbial resources can be exploited. Researchers would need to prioritize screening

these local *Trichoderma* strains for antagonistic activity against the dominant pathogens (*F. verticillioides* and *A. flavus*). Commercializing the most effective strain as a biopesticide or seed-treatment product offers an environmentally friendly alternative to chemical fungicides.

For advanced control, future biotechnology efforts could explore using RNA interference (RNAi) to silence the genes in *Aspergillus* and *Fusarium* responsible for mycotoxin synthesis. This novel biopesticide approach would prevent the fungi from producing toxins even if their growth is not completely arrested, drastically improving food safety.

Data Integration and Precision Agronomy: The location-specific data (e.g., higher prevalence in highly humid areas like Ijoun and Imasai, allows for the integration of data science with agricultural policy. Integrating the market-specific prevalence data with regional climatic and humidity records via Geographic Information Systems (GIS) is essential. This integration will create fungal risk maps for Ogun West, allowing agricultural extension services to predict when and where contamination is most likely occurring. This predictive data can then guide the targeted distribution of preventive measures, such as hermetic storage bags, to the most vulnerable communities.

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