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Polyphasic characterization reveals the first evidence of an atypical AFG1-dominant toxigenic profile of Aspergillus section Flavi in Uganda's cassava value chain

Elias Oyesigye
eliasoyesigye@must.ac.ug

Mbarara University of Science and Technology
Carla Cervini
Cranfield University
Angel Medina
Cranfield University
George Mahuku
International Institute of Tropical Agriculture

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Abstract

Understanding the diversity and toxigenic potential of *Aspergillus* section *Flavi* in cassava remains limited in sub-Saharan Africa despite increasing aflatoxin contamination. This study investigated fungal diversity across Uganda's cassava value chain using a polyphasic approach integrating morphology, chemotype profiling, ITS and calmodulin sequencing, and LC-MS/MS. A total of 288 samples comprising soil, fresh tubers, dried chips, and cassava flour were collected from eight major cassava-producing districts. *Aspergillus* was the predominant fungal genus (mean 4.82×10^5 CFU/g; P = 0.007), while *Aspergillus* section *Flavi* exhibited the highest abundance among Aspergillus sections (1.48×10^5 CFU/g; P = 0.021). Of 162 isolates screened, 77 (47.5%) were aflatoxigenic, with soil harbouring the greatest proportion of toxigenic isolates (22/27; 81%), identifying soil as the principal environmental reservoir of contamination. Polyphasic characterization of 52 representative isolates identified five species: *Aspergillus flavus* (64%), *A. parasiticus* (25%), *A. novoparasiticus* (8%), *A. minisclerotigenes* (1.9%), and *A. tamarii* (1.9%). Unexpectedly, AFG_1 rather than AFB_1 was the predominant aflatoxin produced by the isolates, with a significantly higher mean concentration (229.1 ± 21 µg/kg; range 0.68–1571.7 µg/kg; P = 0.0039) than AFB_1 (213.9 ± 3.3 µg/kg), indicating an atypical toxigenic profile of the *Aspergillus* section *Flavi* population associated with cassava production in Uganda. This is the first report of G-type aflatoxin predominance in Ugandan cassava-associated *Aspergillus* populations. The findings identify soil as a critical intervention point and demonstrate that reliance on AFB_1 alone may underestimate aflatoxin risk in cassava production systems.

1. Introduction

Uganda is a Sub-Saharan country situated in East Africa, astride the equator (1° S–4° N; 29°–35° E). This geographical positioning results in tropical climate of 18 to 28 ^{0}C and moderate humidity (50 to 76% (Nuwagira and Yasin, 2022). Under such conditions, filamentous fungi belong to phylum Ascomycota-particularly *Aspergillus*, *Penicillium*, and *Fusarium*-commonly colonise food and feed systems (Casu et al., 2024; Pitt & Hocking, 2022). This group is known for producing ascospores, with numerous spores for reproduction and septate, thread like hyphae that intertwin to form mycelium, enabling effective colonisation of substrates (Pitt et al., 2022). While other fungal genera such as *Cladosporium, Acremonium, Altenaria, Curvuraria, Trichoderma, Neurospora, Botrytis, Rhizopus* and Mucor exist, *Aspergillus, Penicillium*, and *Fusarium* are of significance due to their frequent presence in food crops mainly maize, wheat, peanuts, cassava, oats and beverages (Samson et al., 2019; Adeyeye, 2016) and production of mycotoxins (Eskola et al., 2020).

Mycotoxins pose both acute and chronic health impacts on humans and animals, including liver cancer, oesophageal cancer, nephrotoxicity, immunosuppression (Benkerroum, 2020; EFSA, 2020). Understanding the diversity and distribution of mycotoxigenic fungi is critical for identifying contamination hotspots and developing targeted mitigation strategies. Accurate species identification is particularly important, as mycotoxin production varies significantly within and between species. Consequently, polyphasic approaches integrating morphological, molecular, and chemical analyses are recommended for reliable characterisation of mycotoxigenic species (Samson et al., 2014; Baquião et al., 2013; Rodrigues et al., 2009). Cassava is the second most important staple food crop in Uganda (UBOS, 2023) and a suitable substrate for fungal colonisation and mycotoxin contamination (Oyesigye et al., 2025). Studies from other regions have demonstrated the dominance of *Aspergillus* and *Penicillium* species in cassava products, including a high prevalence of aflatoxigenic species such as *Aspergillus flavus* (Ekpakpale et al., 2021; Ono et al., 2023; Hartanti et al., 2021). However, in Uganda, evidence remains extremely limited. The only available study (Kaaya & Eboku, 2010) was restricted to a single region (Eastern Uganda) and relied solely on morphological identification, limiting species-level resolution and mycotoxigenic risk assessment. Since then, climate variability, changing agronomic practices, and evolving value chains may have significantly altered fungal ecology and contamination patterns. Recent findings indicate increasing aflatoxin contamination in Uganda, with markedly higher levels reported in Northern regions (Oyesigye et al., 2024), suggesting a shift in risk that is not yet well characterised.

Furthermore, current knowledge does not adequately capture fungal diversity across the cassava value chain, particularly the role of soil as a primary inoculum source. Therefore, this study addresses this critical gap by providing a comprehensive, multi-region analysis of fungal diversity and aflatoxigenic potential along the cassava value chain in Uganda. Specifically, it (i) determines fungal diversity in soil and cassava products (tubers, chips, and flour), (ii) identifies dominant fungal genera, (iii) evaluates the mycotoxin-producing potential of *Aspergillus* section *Flavi*, and (iv) applies a polyphasic approach to characterise aflatoxigenic species and their distribution across regions, value chain actors, and product types.

2. Materials and methods

Sample collection, inclusion, and exclusion criteria

Cassava is predominantly grown and consumed in Eastern and Northern Uganda (UBOS, 2023). A structured purposive sampling approach was employed to collect a total of 240 samples (each 500 g), comprising 48 soil samples, 48 cassava tubers, 48 cassava flour, and 48 cassava chips. For the processed cassava products (flour and chips), samples were equally obtained from farmers and wholesalers, with 24 samples per source in each region. Cassava chips refer to thinly sliced pieces (5–15 mm thick) that are dried and later processed into flour. Sampling targeted key value chain actors (farmers and wholesalers) across eight major cassava-producing districts representing contrasting agroecological conditions. These included Lira, Gulu, Oyam, and Kiryandongo in Northern Uganda, and Kamuli, Pallisa, Soroti, and Kumi in Eastern Uganda. These districts were selected to capture variability in rainfall patterns, temperature, and cassava production intensity.

Participants were selected based on predefined inclusion criteria. Farmers were required to have an active cassava garden and to store cassava chips and flour for at least 10–30. To better reflect typical rural storage conditions, samples were collected from locations at least 2 km away from main roads to minimize roadside sampling bias. Wholesalers were required to have at least two years of experience in cassava trade and to store cassava products for a minimum of 30 days.

2.1 Sample Collection

For each storage sack (lot), samples were collected using a sampling spear inserted into the top, middle, and bottom sections to ensure representative sampling. The subsamples obtained from these different points were combined to form a composite sample of approximately 500 g, following standard composite sampling procedures (Codex Alimentarius Commission guidelines). To prevent cross-contamination, the sampling spear was cleaned with 70% isopropanol and allowed to air dry between successive samples. Each composite sample was placed in a sterile polyethylene sampling bag, labelled with the date, district, and unique sample identification code. Samples were transported to Cranfield University, UK under cooled conditions using insulated containers with ice packs to maintain sample integrity. Upon arrival, samples were stored at − 20°C until further analysis. In addition, soil samples were collected from cassava fields associated with the sampled products to assess the potential environmental sources of contamination. At each sampling site, soil was collected from the topsoil layer (0–20 cm depth) using a sterile soil auger following a zigzag sampling pattern across the field to capture spatial variability. Multiple subsamples (5–10 per field) were collected and homogenized to form a composite soil sample of approximately 500 g. The auger was cleaned with 70% isopropanol between sampling points to avoid cross-contamination. Soil samples were placed in sterile polyethylene bags, properly labelled, and transported under similar cooled conditions as the cassava samples. Upon arrival at the laboratory, soil samples were air-dried, sieved through a 2 mm mesh to remove debris, and stored at 4°C prior to analysis.

2.1.2 Determining moisture content of the samples

Moisture content was determined using the oven-drying method following standard procedures (AOAC, 2000). Approximately 5 g of finely ground and homogenized sample was weighed and analysed in triplicate. Samples were dried in a hot air oven at 105°C until constant weight (approximately 24 h), after which they were cooled in a desiccator containing silica gel to prevent moisture reabsorption. Moisture content (% MC) was calculated on a wet weight basis using the following equation:

$$\% \text{ M.C} = \frac{Wet\ weight\ of\ sample - dry\ weight\ of\ sample}{Wet\ weight\ of\ sample} \times 100$$

2.1.1 Isolation of fungi from soil, cassava tuber, flour and chips.

Fungal enumeration was performed using serial dilution techniques as described by Mohale et al. (2013). Due to the large number of samples analysed, performing full serial dilutions (10^{-1} to 10^{-5}) for all samples would have resulted in an excessive number of preparations. Therefore, a preliminary trial was conducted on representative samples to determine the appropriate dilution factor yielding countable colonies (5–50 per plate), as recommended by Al-Jaza et al. (2022). For each sample, 1 g was weighed in triplicate and transferred into sterile 15 mL falcon tubes containing 9 mL of sterile distilled water supplemented with Tween 80 (0.025% w/v) to obtain a 10^{-1} dilution. Subsequent serial dilutions (10^{-2} to 10^{-5}) were prepared by transferring 1 mL aliquots into four sterile dilution tubes each containing 9 mL of sterile diluent.

Fungal enumeration was performed using Dichloran Glycerol agar (DG18), a low water activity medium suitable for the isolation of xerophilic fungi (Wu et al., 2000). The medium (Thermo Fisher Scientific Oxoid Ltd, Basingstoke, UK) was prepared according to the manufacturer's instructions using deionised water (18 Ω·cm), and supplemented with 0.1% chloramphenicol (Fisher Bioreagents, UK) to inhibit bacterial growth. The medium was sterilized by autoclaving at 121°C for 15 minutes (15 psi). Approximately 20 mL of molten DG18 agar was poured into sterile 9 cm Petri dishes under aseptic conditions in a biological safety cabinet and allowed to solidify. Aliquots of 100 µL from appropriate dilutions were aseptically spread onto the surface of the agar using a sterile Drigalski spatula. All samples were plated in triplicate. The inoculated plates were incubated at 25°C for 7 days. After incubation, plates with 5–50 colonies were selected for enumeration, and fungal counts were expressed as colony-forming units per gram (CFU/g) of sample.

Fungal isolation and identification

After 7 days of incubation, plates were removed and examined for fungal growth. Plates with 5–50 colonies were selected, and colonies were counted using a digital colony counter. Fungal counts were expressed as colony-forming units per gram (CFU/g) of sample. A preliminary trial experiment indicated that the 10^{-3} dilution consistently yielded countable colonies (5–50 CFU per plate) across all sample types. Therefore, this dilution factor was selected for enumeration of fungal populations in all samples.

Fungal colonies were initially characterized at genus level based on macroscopic morphological traits. Colony colour (ranging from pale yellow green to dark green), conidial texture (smooth or rough), conidial arrangement (radiate or columnar), vesicle shape (globose, clavate, or ellipsoidal), and seriation (uniseriate or biseriate) were assessed following the descriptions by Baquião et al. (2013b), Samson et al. (2014), and Frisvad et al. (2019a). The most dominant fungal group (*Aspergillus* section *Flavi*) was isolated through repeated sub-culturing to obtain pure cultures, following the method described by Samson (2002). Pure isolates were obtained on Malt Extract Agar (MEA) (Thermo Fisher Scientific Oxoid Ltd, Basingstoke, UK) and incubated at 25°C for 7 days. Preliminary identification was based on both macroscopic and microscopic characteristics in accordance with Samson et al. (2019). For long-term preservation, spore suspensions were prepared by transferring spores into sterile 30% (v/v) glycerol/water solution. The suspensions were stored at − 20°C for subsequent analysis.

Aflatoxin production of isolated species

The aflatoxigenic potential and distribution across different sample types and geographical locations was assessed. Two complementary methods were employed to assess aflatoxin production. A qualitative screening was conducted using coconut cream agar (CCA) to detect aflatoxin-producing isolates, while quantitative analysis of aflatoxins was performed using liquid chromatography–tandem mass spectrometry (LC–MS/MS).

Coconut cream agar medium (CCA) was prepared following Dyer and McCammon (1994) with slight modifications. The medium consisted of 50% (v/v) coconut cream and 2% (w/v) agar. Preparation, sterilization, and plate pouring were carried out as above. A loopful of spore suspension from each isolate was aseptically inoculated at the centre of CCA plates, which were then incubated at 25°C for 7 days. Aflatoxigenic potential was assessed by examining plates under UV light (365 nm) in a dark cabinet. Isolates showing blue or green fluorescence on the obverse and reverse sides of the colony were considered putatively toxigenic. Reference strains of *Aspergillus flavus* SRKC-G1907 (USDA, USA) and *Aspergillus parasiticus* SSWT 2999 (Singh et al., 2019; Abdel-Hadi et al., 2011) were included as positive controls.

Mycotoxin etraction and quantification of aflatoxins by LC-MS/MS

Aflatoxins were extracted and quantified following Frisvad et al. (2019a) with minor modifications. Fungal isolates were cultured on yeast extract sucrose (YES) agar (2% yeast extract, 15% sucrose, 0.05% $MgSO_4$, and 2% agar; Thermo Fisher Scientific Oxoid Ltd, UK) and incubated at 25°C for 10 days. After incubation, 3–5 agar plugs (approximately 350–375 mg) were aseptically excised and transferred into pre-weighed 2 mL microcentrifuge tubes. Aflatoxins were extracted by adding 1.5 mL of extraction solvent (acetonitrile: water: acetic acid, 79:20:1, v/v/v), followed by shaking at 1500 × g for 60 min using a multi-tube vortexer (VWR VX-2500, USA). Samples were then centrifuged at 1500 × g for 2 min (Thermo Scientific Heraeus Labofuge 400 R, Germany).

An aliquot (350 µL) of the supernatant was transferred into amber glass vials containing 350 µL of dilution solvent (acetonitrile: water: acetic acid, 20:79:1, v/v/v). A 5 µL volume of each diluted extract was injected into an LC–MS/MS system (QTRAP 6500+, SCIEX, Foster City, CA, USA) equipped with an electrospray ionization (ESI) source. Chromatographic separation was achieved using an ACE 3 C18 column (2.1 × 100 mm, 3 µm; Hichrom) fitted with a guard cartridge (4 × 3 mm, Gemini, Agilent), maintained at 60°C. Quantification was performed using external calibration. A working standard solution was prepared by mixing 300 µL of a multi-analyte aflatoxin standard (AFB_1, AFB_2, AFG_1, AFG_2) with 200 µL of dilution solvent (20:79:1, v/v/v). A 10-point calibration curve (0.05–1000 µg/mL) was generated by serial dilution using acetonitrile: water: acetic acid (49.5:49.5:1, v/v/v).

4.1.5 Molecular identification

Isolates were cultured on Yeast Extract Sucrose (YES) medium as described above. Genomic DNA was extracted from the aflatoxigenic isolates using the pepGOLD Plant DNA Mini Kit (VWR Life Science, Germany) according to the manufacturer's instructions. DNA concentration (ng/µL) and purity were determined using a Genova Nano spectrophotometer (Jenway, Stone, UK). DNA quality was assessed based on absorbance ratios at 260/280 nm and 260/230 nm. The 260/280 ratio was used to evaluate protein contamination, while the 260/230 ratio was used to assess contamination from organic compounds such as phenol and residual extraction reagents. The 260/280 ratios ranged between 1.8 and 1.9, indicating high-quality DNA suitable for downstream molecular analysis. The 260/230 ratios ranged between 2.0 and 2.2, consistent with minimal contamination. However, two samples exhibited consistently lower 260/230 ratios (1.8 and 1.7), indicating possible contamination; these samples were excluded from further molecular analysis.

4.1.5.1 Preparation of electrophoresis gel and TAE buffer

To prepare the gel for electrophoresis, 1X Tris-Acetate ethylenediaminetetraacetic acid (TAE) buffer was first prepared from stock concentration of 50X TAE. One g of molecular grade agarose was mixed with 100 mL of TAE buffer, and 10 µl of SafeView (10 µl/ 100 mL) was added. The mixture was heated at 60 0C until the agarose was dissolved and then poured into pre-prepared wells fitted with combs and kept undisturbed to solidify. Once solidified, 1 µl of genomic DNA was loaded into the solidified wells with 2 µl of 6× DNA loading dye (Thermo scientific), and 5 µl of 1 kb ladder was put in the first and last well. The gel was completely immersed in 1× TAE buffer and permitted to run at 100 volts for 60 minutes as described by Mohale et al. (2013).

4.1.5.2 Amplification with calmodulin and ITS region

To confirm species-level identity, two genetic markers were amplified: the Internal Transcribed Spacer (ITS) region and the calmodulin (Cmd) gene. The ITS region is the primary DNA barcode for fungi (Schoch et al., 2012; Schoch and Seifert, 2012), although it may lack sufficient resolution for closely related species within *Aspergillus* section *Flavi*. Therefore, the calmodulin gene, which provides higher discriminatory power, was used in combination with ITS for accurate species identification. A fragment of the calmodulin gene was amplified using primers Cmd5 (5'-CCG AGT ACA AGG AGG CCT TC-3') and Cmd6 (5'-CCG ATA GAG GTC ATA ACG TGG-3') (Samson et al., 2014), while the ITS region was amplified using primers ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') (Hong et al., 2006). PCR amplification was performed in a total reaction volume of 20 µL containing 1 µL template DNA (40 ng/µL), 2 µL each of forward and reverse primers, 5 µL Taq DNA polymerase (Takara, Kusatsu, Japan), and nuclease-free water (Thermo Scientific) to volume. Thermal cycling conditions consisted of an initial denaturation at 95°C for 4 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 60°C (Cmd) or 65°C (ITS) for 30 s, and extension at 72°C for 30 s, with a final extension at 72°C for 5 min (ITS) or 8 min (Cmd). PCR products were verified by electrophoresis on 1% agarose gels. Successful amplicons were subjected to bidirectional Sanger sequencing (GENEWIZ, Leipzig, Germany) using the same primers as for amplification. The resulting sequences were edited and assembled, and preliminary identification was performed using BLASTn searches against the NCBI GenBank database (https://blast.ncbi.nlm.nih.gov) and pairwise alignment in MycoBank (www.mycobank.org). Species identity was assigned based on highest sequence similarity and query coverage.

4.2 Data analysis

4.2.1 Preparation of data prior to analysis.

Mycotoxin data were acquired using Analyst® software (version 1.6.3) and quantified with MultiQuant™ software (version 3.0.3) (AB Sciex, Foster City, CA, USA). Peak integration was performed using a linear regression model with 1/x weighting, and two daughter ions per analyte were considered for quantification. The processed data were exported to Microsoft Excel for organization and preliminary handling. For molecular identification, raw sequence data were edited and assembled into consensus sequences using BioEdit software (version 7.2). The resulting sequences were subjected to similarity

searches using BLASTn against the NCBI GenBank database (https://www.ncbi.nlm.nih.gov/) and pairwise alignment in the MycoBank database (www.mycobank.org) to confirm species identity.

4.2.2 Statistical analysis

All statistical analyses were performed using Stata version 17 (StataCorp, College Station, TX, USA). Prior to analysis, data were tested for normality using the Shapiro–Wilk test and for homoscedasticity using the Breusch–Pagan test. Colony-forming unit (CFU) data violated assumptions of normality and homogeneity of variance even after transformation; therefore, non-parametric analysis was applied using the Kruskal–Wallis test at a significance level of $p < 0.05$ to assess differences across districts, value chain actors (farmers and wholesalers), and sample types (soil, cassava flour, chips, and tubers).

Mycotoxin (AFB_1) data met the assumptions of normality and homoscedasticity and were analysed using one-way analysis of variance (ANOVA) at a 95% confidence level. Where significant differences were detected ($p < 0.05$), Tukey's Honestly Significant Difference (HSD) test was applied for pairwise comparison of means. Agreement between LC–MS/MS and Coconut Cream Agar (CCA) methods for assessing aflatoxigenicity was evaluated using the Cohen's Kappa statistic (Dettori and Norvell, 2020). Kappa values range from − 1 to 1, where values close to 1 indicate strong agreement, values around 0 indicate agreement equivalent to chance, and negative values indicate disagreement. Higher Kappa values were interpreted as evidence of reliability of CCA as a screening method relative to LC–MS/MS.

4.3 Results

4.3.1 Fungal diversity in soil and cassava products (tubers, chips, and flour),

Fungi belonging to four genera including *Aspergillus* (84%), *Penicillium* (2.1%), *Fusarium* (3.6%), and *Rhizopus* (9.9%) were observed in the soil, and cassava product samples. The fungal load expressed as Colony Forming Units (CFU), varied significantly (P = 0.007) between the genera. The overall mean CFU was 1.42×10^5 CFU/g. Fungi belonging to the genus *Aspergili* exhibited the highest mean fungal load (4.82×10^5 cfu/g), followed by *Rhizopus* (0.56×10^5 cfu/g), *Fusarium* (0.21×10^5 cfu/g), and *Penicillium* (0.11×10^5 cfu/g) (Figure i).

Figure i

Summary of fungal populations expressed as total CFU/g x 10^5. Bars sharing the same letter indicate no significant difference ($p < 0.05$), while those that do not share the same letter are significantly different. Error bars represent standard deviation.

Based on morphological, macroscopic and microscopic identification, five sections belonging to *Aspergilli* were identified. These included *Aspergillus* section *Aspergillus* (formerly the genus *Eurotium*), *Aspergillus* section *Terrei*, *Aspergillus* section *Nigri*, *Aspergillus* section *Fumigati*, and *Aspergillus* section *Flavi*. The fungal load for the respective sections is presented in Figure ii. Results indicate a significant (P = 0.021) difference in mean CFUs for the sections belonging to *Aspergillus* genera. Species belonging to *Aspergillus* section *Flavi* exhibited the highest mean CFU of 1.48×10^5 cfu/g, followed by *Aspergillus* section *Nigri* (0.94×10^5 cfu/g), *Aspergillus* section *Aspergillus* (0.68×10^5 cfu/g), *Aspergillus* section *Terrei* (0.28×10^5 cfu/g) and *Aspergillus* section *Fumigati* (0.24×10^5 cfu/g).

Figure ii

Average CFUs for the observed five sections belonging to genera *Aspergillus* in all samples studied. Letters sharing the same letter indicate no significant difference ($p < 0.05$), while those that do not share the same letter indicate significant differences. Error bars represent standard deviation.

4.3.2 Dominance of *Aspergili* section in soil and cassava products

Significant differences in mean CFU counts among *Aspergillus* sections were observed across cassava products and soil (Table i). In cassava tubers, *Aspergillus* section *Flavi* had the highest mean CFU ($1.48 \pm 0.21 \times 10^5$ CFU/g), followed by section *Aspergillus* ($0.39 \pm 0.07 \times 10^5$ CFU/g), while species belonging to section *Fumigati* were not detected. In soil samples, mean CFU counts across the five *Aspergillus* sections were not significantly different ($P = 0.09$). However, section *Terrei* ($1.05 \pm 0.20 \times 10^5$ CFU/g) and section *Flavi* ($0.65 \pm 0.01 \times 10^5$ CFU/g) exhibited relatively higher counts compared to other sections. In cassava chips, CFU counts varied significantly among sections, with section *Nigri* having the highest mean CFU ($2.48 \pm 0.14 \times 10^5$ CFU/g), had high section *Flavi* ($2.05 \pm 0.26 \times 10^5$ CFU/g). No species from section *Terrei* were detected in cassava chips. Similarly, in cassava flour, section *Flavi* had the highest mean CFU ($1.73 \pm 0.14 \times 10^5$ CFU/g), followed by section *Aspergils* ($0.95 \pm 0.04 \times 10^5$ CFU/g). Overall, the highest mean CFU counts were observed in cassava chips ($1.14 \pm 0.02 \times 10^5$ CFU/g), followed by cassava flour ($0.94 \pm 0.07 \times 10^5$ CFU/g), soil ($0.42 \pm 0.10 \times 10^5$ CFU/g), and cassava tubers ($0.39 \pm 0.05 \times 10^5$ CFU/g).

Table i

The distribution of fungal load (CFU) in soil and cassava products, and regions.

Aspergillus section	Mean cfu/g ×10^5 in soil and cassava products				Mean cfu/g ×10^5 in two regions			
	Tuber	**Soil**	**Chips**	**Flour**	**Northern**	**Eastern**	**Northern**	**Eastern**
A. section *Flavi*	**1.48 ± 0.21^{b}**	0.65 ± 0.01	2.05 ± 0.26^{ab}	**1.73 ± 0.14^{b}**	**1.91 ± 0.1 ^{b}**	1.04 ± 0.9 ^{ab}	Total mean cfu	
A. section *Nigri*	0.06 ± 0.02 ^{a}	0.12 ± 0.12	**2.48 ± 0.14^{b}**	1.10 ± 0.06^{ab}	0.77 ± 0.01^{ab}	**1.13 ± 0.75 ^{b}**	0.85 ± 0.15	0.60 ± 0.11
A. section *Aspergillus*	0.39 ± 0.07 ^{a}	0.11 ± 0.03	1.07 ± 0.07 ^{ab}	1.14 ± 0.12 ^{ab}	0.95 ± 0.04 ^{ab}	0.40 ± 0.1 ^{ab}		
A. section *Terrei*	0.03 ± 0.001 ^{a}	**1.05 ± 0.2**	-	0.03 ± 0.01^{c}	0.34 ± 0.01 ^{a}	0.21 ± 0.03 ^{a}		
A. section *Fumigati*	-	0.18 ± 0.01	0.10 ± 0.04^{a}	0.68 ± 0.02^{ac}	0.26 ± 0.11 ^{a}	0.22 ± 0.09 ^{a}		
	P = *0.015*	P = *0.09*	P = *0.015*	P = *0.002*	P = *0.0026*	P = *0.0048*	P = *0.049*,	T = 1.67

Means sharing the same letter are not statistically different from each other (P < 0.05).

4.3.2.1 Diversity of *Aspergillus* section in soil and cassava products.

Overall, samples from the Northern region (Gulu, Kiryandongo, Lira, and Oyam districts) exhibited significantly higher mean CFU counts (0.85 ± 0.15 × 10^5 CFU/g) compared to those from the Eastern region (Kumi, Pallisa, Kamuli, and Soroti districts) (0.60 ± 0.11 × 10^5 CFU/g; *P* = 0.049) (Table i). Within regions, *Aspergillus* section *Flavi* had the highest mean CFU in the Northern region (1.91 ± 0.10 × 10^5 CFU/g), whereas in the Eastern region, section *Nigri* had the highest mean CFU (1.13 ± 0.75 × 10^5 CFU/g). All five *Aspergillus* sections were detected in both regions, although abundance varied significantly (*P* < 0.05).

4.4.1 Toxigenic potential of isolated *Aspergillus* section *Flavi* isolates

4.4.1.1: Identification of toxigenic isolates using coconut cream agar media (CCA)

The ability to fluoresce blue (for B_1, B_2) or green (for G_1, G_2) indicates aflatoxin producing ability of the isolate (Teja, 2017). A total of 162 isolates were screened for fluorescence on CCA. Out of these, 82 (50.6%), fluoresced in CCA (Figure iii). Among the fluorescent isolates, 11 emitted green fluorescence, while 71 emitted blue. Soil-derived isolates exhibited the highest fluorescence with 81% (22/27) fluorescencing on CCA. This was followed by isolates from flour, where 61.9% (26/42) had fluorescent, and tubers, with 33.3% (20/60) showing fluorescence. The lowest incidence of fluorescence was observed in isolates from chips 16.7% (14/84).

Figure iii

Blue and green fluorescence of isolates on CCA viewed under 365 nm UV light.

4.4.1.2 Mycotoxin quantification with LC-MS/MS

Out of 162 isolates analysed with LC-MS/MS for aflatoxin production (AFB1, AFB2, AFG1, AFG2), 77 (47.5%) produced aflatoxins. All the 77 isolates produced AFB1, and of these, 3 isolates did not produce AFB2. Eleven isolates produced all types of aflatoxins. The mean concentrations of aflatoxin types were significantly different (P = 0.0039). AFG1 had the highest (mean of 229.1 ± 21 µg/kg) concentration, ranging from 0.68–1571.7 µg/kg. Eleven isolates produced all types of aflatoxin (total aflatoxins) with a mean of 936.8 ± 7.1 µg/kg and ranging from 100 to 2610 µg/kg. This was followed by isolates producing a combination of AFB1 + AFB2 (mean concentration of 338.7 ± 4.8 µg/kg). The mean concentration of AFB1 was 213.9 ± 3.3 µg/kg and range of 1.92–944.2 µg/kg. Both AFB2 and AFG2 were produced in lower amounts compared to AFG1and AFB1 (Table ii).

Table ii

Concentration of aflatoxins extracted from isolates of *Aspergillus* section *Flavi.*

Aflatoxin type	Number of positive isolates	Mean concentration (µg/kg)	Concentration range (µg/kg)
AFB1	77	213.9 ± 3.3 [b]	1.92–944.2
AFB2	74	13.1 ± 0.64 [a]	0.16–104.8
AFG1	41	229.1 ± 21[b]	0.68–1571.7
AFG2	36	31 ± 2.1 [ab]	1.76–73.9
AFB1 + AFB2+AFG1 + AFG2	11	936.8 ± 7.1 [a]	100–2610
AFB1 + AFB2	72	338.7 ± 4.8 [c]	626 – 2.47
AFB1 + AFB2+AFG1	41	39.3 ± 3.3 [b]	111.3–2.99
P = 0.0039			

Further results indicate that 81% (22/27) of isolates extracted from soils were aflatoxigenic followed by those from cassava flour (57%). Cassava chips and tubers contained isolates with least aflatoxigenic species at 16 and 33% (Figure iv).

Figure iv

Prevalence of aflatoxigenic samples isolated from soil, and cassava products.

4.4.1.3 Distribution and prevalence of AFB1 within region, value chain, and products.

AFB1 is the most carcinogenic mycotoxin and for this reason further results were presented based on this type of aflatoxin in cassava flour. While no significant differences (P = 0.4125) in AFB1 was observed between isolates collected from two regions, the concentration of AFB1 varied significantly across districts. Isolates from Pallisa contained significantly highest concentrations of AFB1 (875.7 ± 12.1), followed by those from Kiryandongo (332.3 ± 0.62) and Kamuli (318.7 ± 0.56). Although isolates from flour, soil, and tubers exhibited higher levels of AFB1, the differences were not statistically significant. Likewise, isolates from wholesaler showed higher concentrations of AFB1 compared to those from farmers, but the difference was not statistically significant (Table iii).

Table iii

Distribution of mean concentration of AFB1 by region, product and value chain

Region	Mean conc. AFB1(µg/kg)	District	Mean conc. AFB1(µg/kg)	Product	Mean conc. AFB1(µg/kg)	Value chain actor	Mean conc. AFB1(µg/kg)
Northern	182.1 ± 0.58	Gulu	120.6 ± 0.66 [ab]	Soil	205.6 ± 0.67	Farmer	183.3 ± 0.58
Eastern	248.4 ± 0.78	Kamuli	318.7 ± 0.56 [b]	Chips	66.2 ± 0.92	Wholesaler	223.2 ± 0.51
		Kiryandongo	332.3 ± 0.62 [b]	Flour	227.6 ± 0.58		
		Kumi	116.9 ± 1.41[ab]	Tubers	161 ± 12.3		
		Lira	177.1 ± 0.76 [ab]				
		Oyam	91 ± 0.67 [ac]				
		Pallisa	875.7 ± 12.1[a]				
		Soroti	20.8 ± 0.82 [c]				
	P = 0.4125		*P = 0.0354*		*P = 0.2716*		*P = 0.5401*

Mean conc.= mean concentration. Means sharing the same letter are not statistically different from each other (P < 0.05).

4.3.1.4 Comparison between two methods (coconut cream agar media and LC-MS/MS).

Results from kappa statistic, which measures the level of agreement between two categorical variables, indicated a fair agreement (0.389) between the coconut cream agar media (CCA) and LC-MS/MS method. This agreement was statistically significant (P = 0.0004), with 78.9% of observation consistent across both methods. These findings underscore the reliability of the CCA method as a qualitative screening tool for aflatoxin production.

4.4 Polyphasic identification of *A*. section *Flavi* isolated from soil, tubers, flour and chips

Morphology

Results from kappa statistic, which measures the level of agreement between two categorical variables, indicated a fair agreement (0.389) between the coconut cream agar media (CCA) and LC-MS/MS method. This agreement was statistically significant (P = 0.0004), with 78.9% of observation consistent across both methods. These findings underscore the reliability of the CCA method as a qualitative screening tool for aflatoxin production. Based on colony morphology, microscopic and macroscopic observations, pure isolates were grouped to reduce possible duplicates. A total of 77 *Aspergillus* section *Flavi* isolates (with some presented in Figure v) possessed unique features from one another and were retained for further molecular identification.

Figure v

Morphological diversity of *Aspergillus* section *Flavi* isolates cultured on MEA at 25°C for 7 days. These representative isolates were obtained from soil samples. Distinct colony morphotypes suggest variability in putative species within the *Flavi* section.

4.4.1 Chemotypes

The isolates were categorised in VIII chemotype groups as described by Giorni et al. (2007). Of the eight chemotypes, the isolates in this study belonged to four (I, V, VI, VII) (Table iv). Chemotype V included non-aflatoxin producing species. Majority (33/52) of the isolates belonged to chemotype I characterised by high AFB1 concentrations ranging between 1.92–605.2 µg/kg, and low AFB2 ranging between 0.18-23 µg/kg. The second dominant (18/52) chemotype was VII, characterised by ability for the isolates to produce AFB1, AFB2, AFG1, AFG2, with no CPA. Isolates in this chemotype group produced the highest AFB1 (range:52–944 µg/kg), AFB2 (range:0.73–105 µg/kg), AFG1 (range:0.68–1572 µg/kg), AFG2 (range:3.8–74 µg/kg). Only one isolate belonged to chemotype VI, where all the four aflatoxins and CPA were produced.

Table iv: Characterizing the types of aflatoxins according to the eight chemotypes.

Chemotype	AFB	AFG	CPA	No. of Isolates	AFB1 (µg/kg)	AFB2 (µg/kg)	AFG1 (µg/kg)	AFG2 (µg/kg)	Total AFs (µg/kg)
I (B1 > B2)	+	-	+	33(43%)	158.2 (1.92–605.2)	6.7(0.18-23)	nd	nd	164 (1.9–627)
II(B1 < B2)	+	-	+	nd	nd	nd	nd	nd	nd
III	+	-	-	nd	nd	nd	nd	nd	nd
IV	-	-	+	nd	nd	nd	nd	nd	nd
V	-	-	-	25(32%)	-	-	-	-	-
VI	+	+	+	1(1%)	53.6	2.56	42.4	1.76	100.3
VII	+	+	-	18(23%)	494.5(52–944)	38.1(0.73–105)	370.5(0.68–1572)	33.9(3.8–74)	934(69-2610)
VIII	-	+	+	nd	nd	nd	nd	nd	nd

nd: not detected, +: presence. Total AFs-Total aflatoxins.

Based on classification on chemotypes, Table v presents a summary of observed chemotypes and species within the *Aspergillus* section *Flavi* that may produce such pattern as reported by Frisvad et al., 2019. According to this classification, the 33 isolates that belonged to chemotype I, are likely to be *Aspergillus flavus*, 18 that belonged to chemotype VIII, are putatively *Aspergillus parasiticus*, and 1 is likely to be *A. minisclerotigenes/A. sergii/A. aflatoxicoformans*. To further confirm the identity of these isolates, they were further amplified with ITS and calmodulin genes and results are presented in Table vi.

Table v

Summarised classification according to chemotypes.

Species in *Aspergillus* section *Flavi*	Chemotypes					
	AFB1	AFB2	AFG1	AFG2	CPA	Total species
A. flavus	+	+	-	-	+	33
A. parasiticus	+	+	+	+	-	18
A. novoparasiticus	+	+	+	+	-	4
A. minisclerotigenes	+	+	+	+	+	1
A. tamari	-	-	-	-	+	1
Total aflatoxigenic species						**52**

4.4.2 Identification based on calmodulin gene and ITS region.

To further confirm the identity of these isolates, they were further amplified with ITS region and calmodulin gene and results are presented in Table vi. The identification was based on Cmd and ITS sequences, blasted in two software (NCBI and Mycobank) considering a similarity threshold of ≥ 90% with at least 80% query coverage (Frisvad et al., 2019). Results (Table vi) from molecular identification were in agreement with the chemotype classification reported in Table v. Table vi, indicate that, of the 52 isolates that were aflatoxigenic, 64% (33/52) were *Aspergillus flavus*, 25% (13/52) were *Aspergillus parasiticus*, 8% (4/52) were *Aspergillus novoparasiticus*, while 1.92% (1/52) isolate was *Aspergillus minisclerotigenes*, and another (1.92%) was *Aspergillus tamarii*.

Table vi

Identity of *Aspergillus* section *Flavi* isolates based on calmodulin gene and ITS region.

	Average % of similarity												
	NCBI		**Mycobank**			**Distribution in soil and cassava products**				**Main value chain actors**		**Region of collection**	
A. section Flavi	**ITS**	**Cmd**	**ITS**	**Cmd**	**Total**	**Soil**	**Tuber**	**Chips**	**Flour**	**Farmer**	**Wholesale**	**Northern**	**Eastern**
A. flavus	87.8	98.8	94.5	95.4	33(64%)	6(35%)	3(75%)	8(80%)	16(76%)	18(78%)	15(52%)	24(73%)	9(47%)
A. novoparasiticus	92.6	98.7	88.7	96.2	4(8%)	3(18%)	-	-	1(5%)	1(4%)	3(10%)	4(12%)	-
A. parasiticus	94.5	97.4	94.5	96.1	13(25%)	7(41%)	1(25%)	2(20%)	3(14%)	4(17%)	9(31%)	4(12%)	9(47%)
A. tamari	94.6	99.4	96.2	99.6	1(1.9%)	1(6%)	-	-	-	-	1(3%)	1(3%)	-
A. minisclerotigenes	92.1	99.4	94.7	99.6	1(1.9%)	-	-	-	1(5%)	-	1(3%)	-	1(5%)

4.4.3. Distribution of Aspergillus section Flavi species in soil and cassava products, value chain and region of collection.

The distribution of *A.* section *Flavi* varied across the different substrates from which they were isolated (Table vi). In soil, *A. parasiticus* was the most dominant species (41%), followed by *A. flavus* (35%). In cassava chips (80%), tubers (75%), and flour (76%), *A. flavus* was the dominant species. In tubers and chips, the only identified species were *A. flavus*, and *A. parasiticus*. *A. minisclerotigenes* was only detected in cassava flour and *A. tamari* was found solely in soil. The distribution of identified species within value chain indicated almost a similar trend. *A. flavus* was the dominant species at both stages of the value chain, with a higher prevalence at farm level (78%) compared to wholesale stage (52%). *A. parasiticus* was the second most common species, with a greater occurrence in samples collected from the wholesaler (31%) than those at farm level (17%). It should be noted that whereas all the five species belonging to *A.* section *Flavi* were all present in samples collected at wholesale stage, only 3 species (*A. flavus, A. parasiticus*, and *A. novoparasiticus*) were present at farm level (Table vi).

5. Discussion

The objectives of this study were to determine the fungal diversity and prevalence in soil and cassava products (tubers, chips, and flour) collected in contrasting regions of Uganda, isolate the most prevalent fungi, utilise the polyphasic approach to characterise and identify aflatoxigenic *Aspergillus* section *Flavi* species in order to understand their distribution, and prevalence within region, main value chain actors (farmers and wholesalers), and various substrates. For the first time in Uganda, this comprehensive study utilises a polyphasic approach to identify the dominant fungal species in cassava, expanding on the foundational work by Kaaya et al. (2000), who assessed the fungal load (CFU), but did not conduct an in-depth characterisation. In this study, *Aspergillus, Rhizopus, Penicillium, Fusarium*, were the most dominant fungi isolated from soil, and cassava products. The genus *Aspergillus* had the highest xerophilic fungal load of 4.82×10^5 cfu/g. The species belonging to *Aspergillus, Penicillium, Fusarium* are important in relation to food safety, as they produce mycotoxins that are harmful to humans and animals (Pandey et al., 2023; Syamilah et al., 2022). To-date, fungi from these genera are responsible for more than 86% of food spoilage (Twarużek, Soszczyńska and Kwiatkowska-Giżyńska, 2021). When conditions are favourable, they can produce highly potent mycotoxins with carcinogenic, nephrotoxic, hepatoxic, immunosuppressive, neurotoxic, and teratogenic effects, leading to various chronic and acute complications (Benkerroum, 2020). The presence of these fungi in cassava raises significant concerns (IARC, 2002). The observed dominance of *Aspergillus* genera could be attributed to several factors mainly related to adaptability and environmental preferences. Uganda lies in the tropics with an average temperature of 25 ^{0}C, and average relative humidity of 63% (Nuwagira and Yasin, 2022). These conditions are conducsive for growth of *Aspergillus* species. Unlike genera such as *Penicillium* which prefer, more temperate conditions, many *Aspergillus* species are xerophilic in nature, allowing them to thrive in warmer conditions with low water activity (García-Cela et al., 2014). This adaptability enables *Aspergillus* species withstand desiccation more effectively compared to *Penicillium* and other genera (García-Cela et al., 2014; Sklenář et al., 2017). The warmer and relatively dry conditions in Uganda, especially the Northern part of the country, are more conducive for the proliferation of *Aspergillus* (Hawkins, Windham and Williams, 2005; Magan and Aldred, 2007; Magan and Lacey, 1984).

The same genera were reported in Uganda's cassava by Kaaya & Eboku (2010), with *Rhizopus* (66.7%), *Mucor* (37%), *Penicillium* (22.2%), *Aspergillus* (20.4%), and *Fusarium* (5.5%) observed in 75 cassava samples, showcasing the concurrence of our results with previous researcher. However, in this study we report a higher overall mean CFU of 1.42×10^5 cfu/g compared to CFU of 5.0×10^4 cfu/g by Kaaya & Eboku (2010) on MEA media. The highest CFUs were reported in cassava chips (2.48 ×105 cfu/g) and in Northern Uganda (1.91 ×105 cfu/g). Cassava chips spend 5–90 days in storage and are transformed into flour by retailers depending on demand. Therefore, cassava chips spend more time in stores which are characterised with high moisture, poor ventilation and un-hygienic conditions, making them prone to contamination with *Aspergillus* (Oyesigye et al., 2024a). These conditions provide a conducive environment for

fungal growth and are likely to be the reason for higher CFUs observed in cassava chips. Additionally, cassava chips are sometimes inadequately dried which encourages fungal growth. Moreover, cassava chips are normally stored in huts and other non-moisture absorbing bags which sometimes leak in rainy seasons, creating damp conditions that favour fungal growth (Kaaya and Eboku, 2010b). Soil harbours significant microorganisms, including *Aspergillus* species. In this study, 81% of species belonging to *Aspergillus* section *Flavi* isolated from soil were toxigenic. Previous studies have shown that 61% (72/119) of farmers and wholesalers dried cassava on bare ground, and 51.6% peel cassava on bare ground (reference). This suggests a high likelihood of contamination during these processes, since the drying surface has been identified as a major reservoir for aflatoxigenic fungi. This underscores the importance of enforcing practices that minimise contact between cassava and soil during peeling and drying or explore other innovations like solar dryers that reduce ground drying. Kaaya & Eboku (2010) reported drying on bare ground as a key factor contributing to high CFUs and high aflatoxin levels. This study provides strong evidence that soil in Uganda's major cassava production areas is a significant reservoir for aflatoxigenic fungi and should be avoided during the peeling and drying.

Remarkably, while it is commonly reported in other studies that most aflatoxigenic isolates predominantly produce AFB1 in highest concentrations (Akinola, Ateba and Mwanza, 2019; Hove et al., 2016; Lai et al., 2015; Sultan and Magan, 2010) our study revealed that AFG1 was the aflatoxin produced in highest abundance, with a mean concentration of 229.1 ± 21 µg/kg and ranging from 0.68–1571 µg/kg. A few studies (Gabal, Hegazi and Hassanin, 1994; Wokorach et al., 2022) have reported similar findings. Although exact reasons remain unclear, this deviation from the typical B-type aflatoxin dominance may be attributed to a combination of genetic and environmental factors. Aflatoxin biosynthesis is governed by a well-characterized gene cluster, including regulatory genes such as aflR and aflS, and structural genes like aflP (omtA) and aflQ (ordA). These genes drive a shared biosynthetic pathway, with branching steps that lead to either B- or G-type aflatoxins. The production of AFG1 and AFG2, in particular, involves additional oxidation steps catalyzed by enzymes such as NADPH-dependent oxidoreductases (Cary et al., 2006). Notably, our isolates were cultured on YES (Yeast Extract Sucrose) medium, which is known to be highly conducive for aflatoxin biosynthesis due to its rich carbohydrate and nitrogen content. Several studies have shown that the composition of the growth medium, including carbon-to-nitrogen ratio, significantly influences the expression of aflatoxin biosynthetic genes and the relative abundance of different aflatoxins (Passone et al., 2010; Magan et al., 2003). It is therefore possible that the YES medium, in combination with specific isolate genotypes, favored the enzymatic pathways leading to G-type over B-type aflatoxins. Similar study by Wokorach et al. (2022) found a similar trend of isolates in Uganda producing higher AFG1 than AFB2.

The results on distribution of AFB1 revealed that, although cassava flour exhibited lower CFUs, compared to chips, isolates from cassava flour produced higher levels of AFB1 (227.6 ± 0.58 µg/kg). Since cassava flour is the final product consumed either in its pure form or as a composite flour, the higher levels of AFB1 produced by aflatoxigenic species are particularly concerning, especially in Northen Uganda where conditions favour aflatoxin production. Additionally, isolates from samples collected from wholesaler produced more AFB1 (223.2 ± 0.51 µg/kg) than those collected at farm level (183.3 ± 0.58), highlighting a critical point of contamination that warrants increased attention. Results from polyphasic characterisation, majorly based on chemotype and molecular identification revealed that aflatoxins in Uganda's cassava are produced by *A. flavus* (33/52), *A. parasiticus* (13/52), *A. novoparasiticus* (4/52), *A. minisclerotigenes* (1/52) and *A. tamari* (1/52). *A. flavus* was the dominant species along the value chain, in the different regions of sample collection, and in cassava products. These results align with previous studies in Uganda about characterisation of *Aspergillus* section Flavi species isolated from other crops (maize, peanuts, and soil). For instance, Wokorach et al. (2021) reported that 63% of the isolated species were *A. flavus*, and Sserumaga et al.(2020a) noted an incidence of 89.8% for *A. flavus*. In this study, 63.3% of the aflatoxigenic species, were *A. flavus*, providing more justification that in Uganda, *A. flavus* is responsible for aflatoxin contaminations, thus should be more targeted during mitigation strategies in food products. However, it is noteworthy that in soil, *A. parasiticus* was the most dominant species and the highest producer of aflatoxins. Compared to other species in *Aspergillus* section *Flavi*, *A. parasiticus* thrives in slightly higher moisture conditions and warmer temperatures, both of which are prevalent in Uganda's climate (Magan et al., 2012). This species is also known to produce a wide range of aflatoxins, especially AFB1 and AFB2, which makes it particularly adapted to contaminate crops like cassava. As indicated in this study, *A. parasiticus* produced the highest total aflatoxins concentration 69-2610 µg/kg, highlighting the considerable risk of soil contamination along the cassava production chain. Characterisation of *Aspergillus* section *Flavi* by Frisvad et al. (2019) and Norlia et al. (2019) indicated that *A. tamari* does not produce AFB or AFG toxins but produces CPA.

Conclusion

This study provides the first comprehensive polyphasic characterisation of *Aspergillus* section *Flavi* associated with the Ugandan cassava value chain, revealing a diverse and highly toxigenic fungal population. *Aspergillus* was the predominant fungal genus, with *Aspergillus* section *Flavi* representing the most abundant section, and soil identified as a major reservoir of aflatoxigenic isolates (81%). Molecular and chemotype characterisation demonstrated that *A. flavus* was the dominant species, alongside other aflatoxigenic species including *A. parasiticus*, *A. novoparasiticus*, *A. minisclerotigenes*, and *A. tamarii*. A key finding of this study was the unexpected predominance of AFG_1 production over AFB_1 among cassava-associated *Aspergillus* section *Flavi* isolates, representing the first evidence of G-type aflatoxin dominance in Ugandan cassava systems. This finding challenges the common assumption that AFB_1 is the primary indicator of aflatoxin risk and suggests that AFG_1 should be incorporated into risk assessment and surveillance frameworks. Overall, these findings identify soil as a critical intervention point and provide new insights into the ecology and toxigenic diversity of *Aspergillus* populations affecting cassava production in Uganda.

Declarations

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Author Contributions:

Elias Oyesigye: conceptualization, data collection and curation, formal analysis, validation, visualization, funding acquisition, resources, writing-original draft, writing-review and editing; Carla Cervini: conceptualization, investigation, supervision, writing-review and editing; George Mahuku: conceptualization, supervision, writing-review and editing; Angel Medina: conceptualization, supervision, writing-review and editing. All authors contributed to the interpretation of results, critically revised the manuscript for important intellectual content, and approved the final version for submission.

Competing Interests

The authors declare that they have no competing interests, whether financial or non-financial, that are directly or indirectly related to the work submitted for publication.

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Generative AI tools were used only for minor language editing, including paraphrasing and spelling corrections. The authors reviewed and verified all content and take full responsibility for the accuracy and integrity of the manuscript.

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Figures

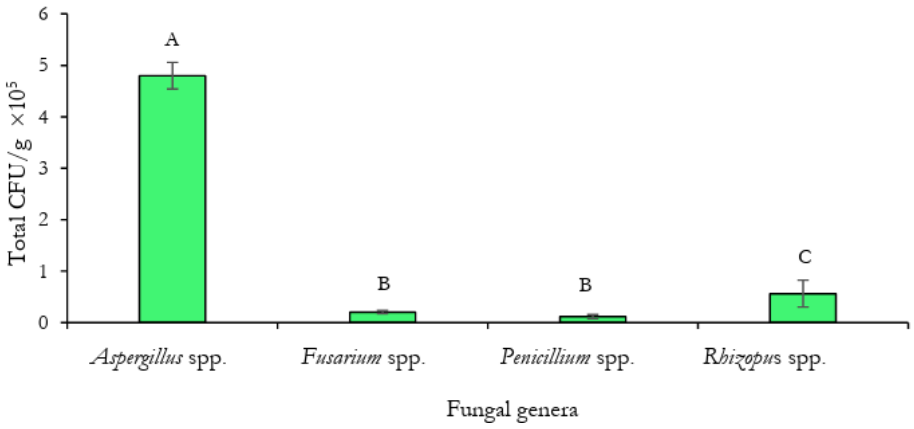


Figure 1

Summary of fungal populations expressed as total CFU/g x 10^5. Bars sharing the same letter indicate no significant difference ($p < 0.05$), while those that do not share the same letter are significantly different. Error bars represent standard deviation.

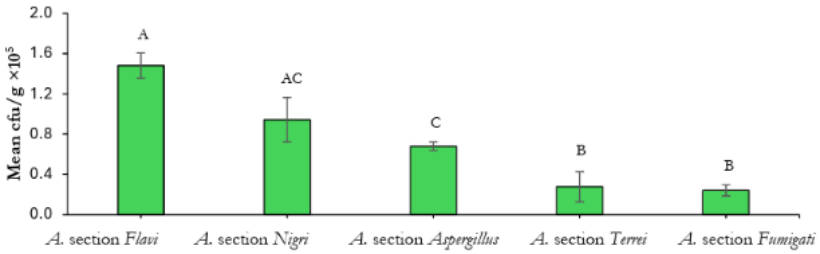


Figure 2

Average CFUs for the observed five sections belonging to genera *Aspergillus* in all samples studied. Letters sharing the same letter indicate no significant difference ($p < 0.05$), while those that do not share the same letter indicate significant differences. Error bars represent standard deviation.

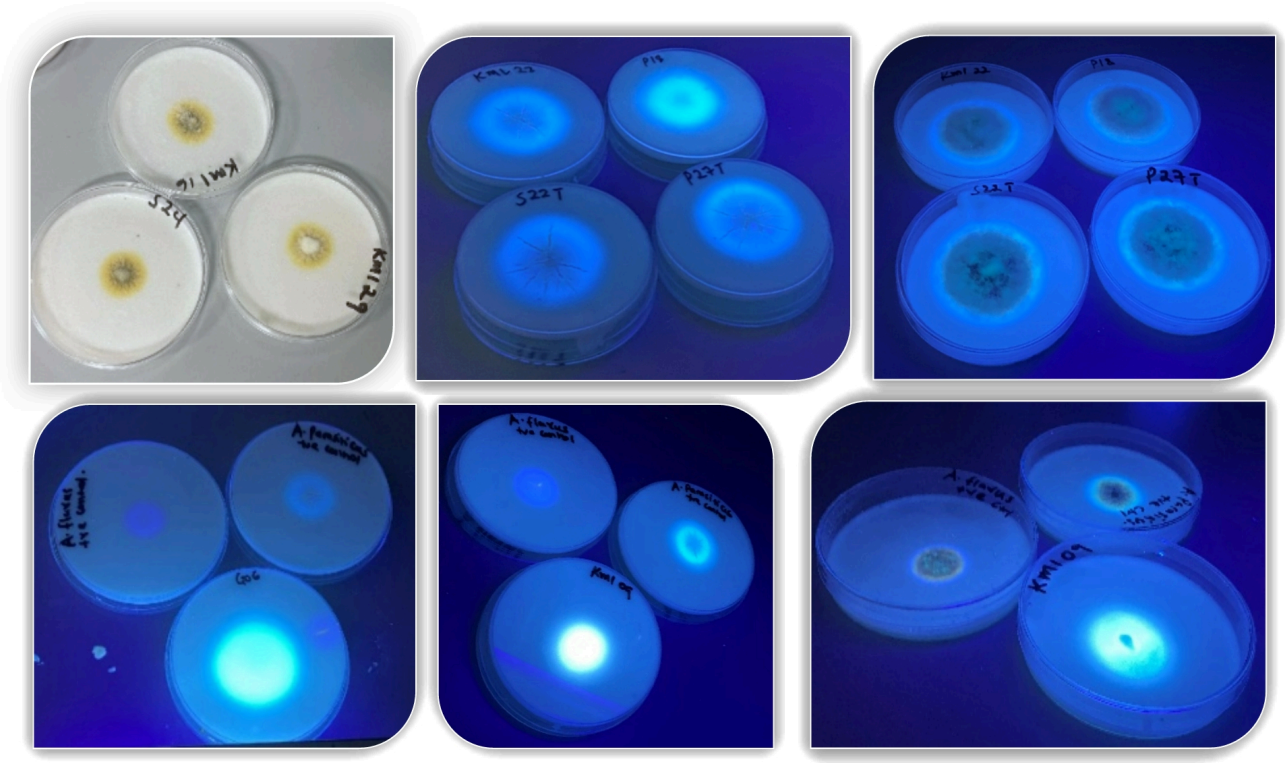


Figure 3

Blue and green fluorescence of isolates on CCA viewed under 365 nm UV light.

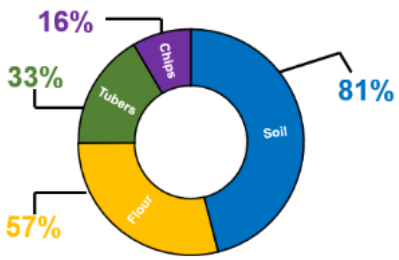


Figure 4

Prevalence of aflatoxigenic samples isolated from soil, and cassava products.

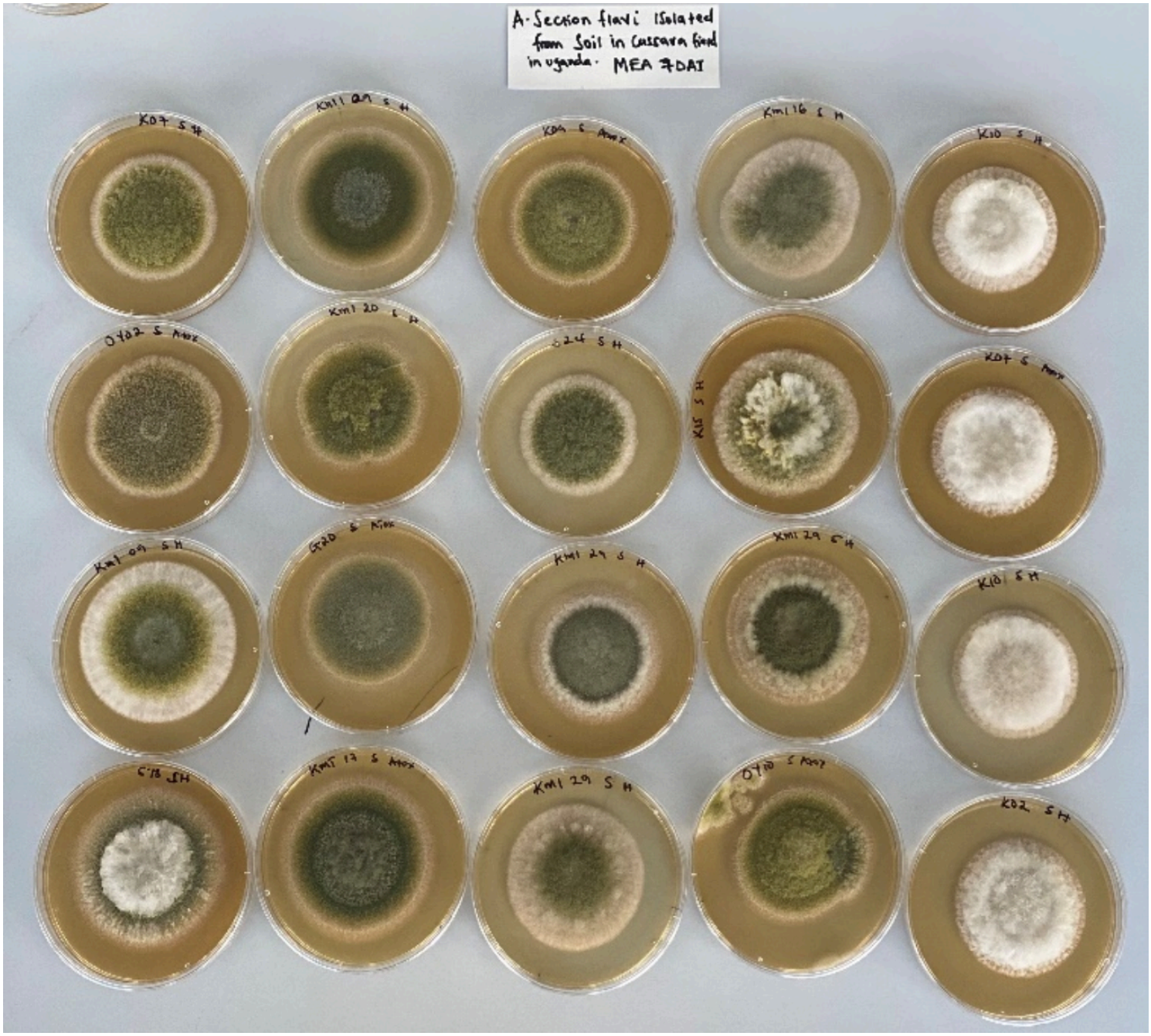


Figure 5

Morphological diversity of *Aspergillus* section *Flavi*isolates cultured on MEA at 25°C for 7 days. These representative isolates were obtained from soil samples. Distinct colony morphotypes suggest variability in putative species within the *Flavi* section.