AMERICAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY (AJFST)

ISSN: 2834-0086 (ONLINE)

VOLUME 3 ISSUE 2 (2024)

PUBLISHED BY
E-PALLI PUBLISHERS, DELAWARE, USA

American Journal of
Food Science and Technology (AJFST)

Volume 3 Issue 2, Year 2024
ISSN: 2834-0086 (Online)
DOI: https://doi.org/10.54536/ajfst.v3i2.3365
https://journals.e-palli.com/home/index.php/ajfst

Diversity of Mycotoxin-Producing Fungi in Leafy and Fruit Vegetables Sold in Port Harcourt Metropolis, Nigeria

Minimah S. O.[1*], Nwauzoma A. B.[2], Chuku E. C.[2], Nmom F. W.[2]

Article Information

Received: August 07, 2024
Accepted: September 10, 2024
Published: September 14, 2024

Keywords

Mycotoxin, Leafy and Fruit Vegetables, Diversity, Polymerase Chain Reaction, ITS Gene

ABSTRACT

Mycotoxins are secondary metabolites produced by fungi and are capable of causing diseases and even death in both humans and other animals. This study investigated the diversity of mycotoxin-producing fungi from leafy (*Telfairia occidentalis* and *Bassica oleracea*) and fruit (*Solanum lycopersicum* and *Cucumis sativus*) vegetables sold in Port Harcourt metropolis, Nigeria using molecular techniques. The fungi were isolated from vegetable samples showing signs of disease using standard methods. DNA extraction and Polymerase chain reaction were carried out at the molecular laboratory of Regional Centre of Biotechnology and Bioresources research, University of Port Harcourt. The isolates were screened for the presence of mycotoxigenic genes (nor1 (aflatoxin), tri6 (trichothecene), otanps (Ochratoxin A), fum13 (fumonisin) and ZEA (zearalenone)) using PCR technique. The isolates containing mycotoxins were identified using ITS gene sequences. Ten out of the eighteen isolates were positive for four (nor1, tri6, otanps and fum13) out of the five mycotoxigenic genes screened. The nine isolates included fungi belong to six genera: *Meyerozyma*, *Pithomyces, Fusarium, Trametes, Penicillium* and *Aspergillus*. The ten isolates were classified as *Meyerozyma* carbbica RCBBR_Sf13, Pithomyces chartarum RCBBR_Sf5, Fusarium falciforme RCBBR_Sf10, *Trametes duplexa* RCBBR_Sf17, *Trametes versicolor* RCBBR_Sf2a, *Trametes duplexa* RCBBR_Mf1, *Fusarium longifundum* RCBBR_Mf4, *Penicillium soosanum* RCBBR_Mf7, *Aspergillus aflatoxiformans* RCBBR_Sf9, *Fusarium circinatum* RCBBR_Sf3 based on their ITS gene sequences. Their ITS gene sequences have been deposited in GenBank under the accession numbers OR816039-OR816047, with the exception of *Fusarium circinatum* RCBBR_Sf3 which could not accession. This study has demonstrated that mycotoxin-producing fungi are diverse and widespread in leafy and fruit vegetables sold within Port Harcourt Metropolis. This raises both public health and food security concerns.

INTRODUCTION

Mycotoxins are toxic compounds produced by certain types of fungi (molds). These toxins can contaminate food crops, posing significant health risks to humans and animals. (Bennett & Kilch, 2003). The harmful chemical byproducts that fungi that easily colonize crops create are typically called "mycotoxin" (Turner *et al.*, 2009). Aflatoxin, citrinin, fumonisins, ochratoxin A, patulin, trichothecenes, zearalenone, and ergot alkaloids like ergotamine are a few examples of mycotoxins that may infect humans and animals (Bennett & Kilch, 2003). Mycotoxins' main pathogenicity mechanism involves utilizing cytochrome P450 (CYP) enzymes to limit protein synthesis and induce oxidative stress (Dai *et al.*, 2017). According to Barkai-Golan and Paster (2011), these mycotoxins are extensively dispersed and have even been detected in green and fruity vegetables.

Leafy and fruit vegetables are edible parts such as the leaves, flowers and immature fruits. They are consumed wholly or in part, raw or cooked as part of the main dish or salad (Asaolu *et al.*, 2012). They add variety and aesthetic value to food and enhance the nutritional quality of diets due to their rich content of vitamins and minerals. Examples of leafy vegetables are *Apium graveolens, Brassica oleracea* var. capitata, , *Lactuca sativa, Beta vulgaris, Telfairia occidentalis* while examples of fruits vegetables include *Solanum melongena, Cucumis sativus, Capsicum* spp, *Solanum lycopersicum* and *Abelmoschus esculentus*. Since these leafy and fruit vegetables are usually consumed raw in addition to their ability to harbour a lot of pathogens, there is need to ensure that they are free of disease causing organisms. However, despite all efforts to ensure that pathogens do not contaminate these vegetables, some of these pathogens leave behind their toxins. Fungal diseases of leafy and fruit vegetables are widespread, occurring on a wide range of vegetables (Oyarzabal and Backert, 2012). These diseases include Anthracnose, Botrytis rots, Downy mildews, *Fusarium* rots, Powdery mildews. Rusts, *Rhizoctonia* rots, *Sclerotinia* rots, and *Sclerotium* rots.

A major challenge of studying mycotoxins is in the area of effectively characterizing the fungi that produce them. Modern methods in the isolation and characterization of mycotoxins-producing fungi rely on the use of high throughput molecular detection technologies. Such techniques include standard polymerase chain reaction (PCR), real-time PCR, nested PCR, loop-mediated isothermal amplification (LAMP), rolling circle amplification (RCA), and nucleic acid sequence-

[1] Regional Centre for Biotechnology and Bioresources Research, University of Port Harcourt, Choba, Nigeria
[2] Rivers State University, Nkpolu-Oroworukwo in Port Harcourt, Nigeria
[*] Corresponding author's e-mail: samuelfirstson@gmail.com

based amplification (NASBA) (Aslam *et al.*, 2017; Cheng *et al.*, 2020). Therefore, this study aims to determine the prevalence and characterization of mycotoxins-producing fungi in leafy and fruit vegetables using molecular techniques.

Polymerase chain reaction (PCR) assays have been widely used for the detection of various genes in different organisms. Lawson *et al.* (1997) developed a PCR assay for the detection and speciation of Campylobacter in human feces, showcasing the utility of PCR in identifying specific pathogens. Similarly, Zur *et al.* (1999) reported the development of a PCR-based assay for detecting Alternaria DNA in food products, demonstrating the applicability of PCR in detecting fungal contamination. Cruz-Perez *et al.* (2001) established a quantitative PCR method for detecting *Stachybotrys chartarum*, highlighting the use of PCR in quantifying toxigenic fungi. Bluhm *et al.* (2002) developed a multiplex PCR assay for the differential detection of Fusarium species producing mycotoxins in cornmeal, emphasizing the importance of PCR in monitoring food safety. Grooters *et al.* (2002) also utilized PCR to identify *Pythium insidiosum*, showcasing the versatility of PCR in detecting various fungal species. Tannous *et al.* (2015) described the development of a real-time PCR assay for quantifying *Penicillium expansum* in apples, further demonstrating the utility of PCR in food safety applications.

Furthermore, Pavón *et al.* (2012) developed a TaqMan real-time PCR method for specific detection of Alternaria spp. in vegetables, highlighting the specificity of PCR in detecting particular fungal species. Kumar *et al.* (2018) utilized real-time PCR with molecular beacons for speciating pathogenic fungi implicated in invasive fungal diseases, showcasing the potential of PCR in diagnosing fungal infections in immunosuppressed patients. Overall, PCR assays have proven to be valuable tools for detecting and quantifying mycotoxigenic genes in fungi isolated from fruit vegetables, as demonstrated by various studies focusing on different fungal species and applications (Pavón *et al.*, 2012; Kumar *et al.*, 2018).

MATERIALS & METHODS

Screening of Fungal Isolates from Leafy and Fruit Vegetables for Mycotoxins and Mycotoxigenic Genes DNA Extraction

Fungal DNA was extracted using Zymo Quick DNA Fungal/Bacterial Kit (Zymo Research Group, USA) following the manufacturer's instruction. In brief, fungal culture was scraped and mixed with 750 µl Lysis Solution in a ZR BashingTM Lysis Tube. The tube was secured in a bead fitted with 2 ml tube holder assembly and the sample processed at maximum speed for > 5 minutes. The ZR Bashing Bead™ Lysis Tube was centrifuged in a microcentirifuge at > 10,000 x g for 1 minute. Up to 400 µl supernatant was transferred into a Zymo-SpinTM IV Spin Filter (orange top) in a Collection Tube and centrifuged at 7,000 x g for 1 minute. A volume of 1,200 µl of Fungal/Bacterial DNA Binding Buffer was added to the filterate in the Collection Tube. Thereafter, 800 µl of the mixture was transferred to a Zymo-SpinTM IIC Column in a Collection Tube and centrifuged at 10,000 x g for 1 minute. The flow through from the Collection Tube was discarded and the step repeated. About 200 µl DNA Pre-Wash Buffer was added to the Zymo-Spin TM IIC Column in new Collection Tube and centrifuged at 10,000 x g for 1 minute. After, 500 µl Fungal/Bacterial DNA Wash Buffer was added to the Zymo-SpinTM IIC Column and centrifuged at 10,000 x g for 1 minute. The Zymo-SpinTM IIC Column was transferred to a clean 1.5 ml microcentrifuge tube and 60 µl DNA Elution Buffer directly added to the column matrix. The column was centrifuged at 10,000 x g for 30 seconds to elute the DNA.

Determination of DNA Concentration and Purity Using NanoDrop Spectrophotometer

DNA concentration and purity were checked using NanoDrop 2000c spectrophotometer (Thermo fisher Scientific, USA). Purity was measured as a ratio of Ultraviolet (UV) light absorbance at 260nm to that of 280nm. The NanoDrop was connected to a computer system, and the sensor was cleaned using a cotton wool and 70% ethanol. 1µl of Elution buffer (the solution used to re-suspend the DNA) was dispensed directly onto of the Nano drop sensor to blank the system. Subsequently, DNA samples (1µL) were separately loaded onto the sensor. The sensor was usually wiped prior to loading a new sample to avoid contamination. Nanodrop measurement was taken in duplicate for each sample.

Gel Electrophoresis

Gel electrophoresis was performed using 1.5% agarose gel (I.e. 0.75g of agarose powder was mixed with 50ml of 1X Tris Boris EDTA (TBE) buffer in a measuring flask and microwaved for 2 minutes to get a clear solution. A volume of 5µL of EZ viewing dye (Blue Light) was added to the content in the conical flask and then poured into the casting tray or gel holder. The comb was placed within the casting tray and allowed to sit between 20 to 30 minutes at room temperature to solidify. The gel electrophoresis unit was then set up; the gel holder containing the gel was placed on the platform inside the gel tank and TBE 1X was poured into the gel tank until the gel was completely submerged. Molecular weight marker (1Kb DNA Ladder) was loaded into the first lane, and the DNA samples were separately loaded into the wells created by the comb on the gel. Each DNA sample (5µL) was mixed with 1µL of 6X loading dye and then loaded in one lane on the gel. A control was also loaded which contained all components of the PCR reaction mixture except template DNA. The set up was allowed to run for 40 minutes at 100volts. At the end of the running time, the DNA fragments were visualized under Ultraviolet (UV) transilluminator (Gel Documentation microDOCTM, Cleaver Scientific Ltd, UK).

Polymerase Chain Reaction (PCR)

DNA fragments containing internal transcribed spacers ITS 1 and ITS 2 including 5.8S rRNA were amplified. Amplification reactions was performed in 20 mL of reaction that contained 4 ng/ µL of template DNA, 1.5 mM MgCl2, 20 mM Tris–HCl pH 8.4, 50 mM KCl, 0.2 mM dNTP mix, 0.1 U Fast-Start DNA Polymerase, 1×Reaction Buffer and 0.5 µM of the each primer. A Biospeedy EvaGreen Master Mix and Bio-rad CFX Connect (Bio-Rad Laboratories, USA) were used for all reactions. The following amplification program was applied: 95 °C, 10 min; 45 cycles of 15 s at 95 °C, 15 s at 53 °C and 30 s at 72 °C.

Primer Designing for Mycotoxigenic Gene Amplification

The primers used in the study were custom-synthesized by Inqaba Biotech, West Africa. Specific genes to aid the detection of the presence of mycotoxins were identified after a thorough literature survey. Five metabolic pathway genes specific to major toxigenic fungal species, namely nor1 for aflatoxigenic Aspergilli, Tri6 and FUM13 for trichothecene- and fumonisin-producing Fusarium species, respectively, otanps for ochratoxigenic Penicillium species and zea for zearalenone were employed. The sequences of all primers (Table 2) were evaluated using PRIMER BLAST and BLASTN tools (http:// www.ncbi.nhn.nih.gov/tools/primer-blast/) to identify any non-specific targets and to determine the specificity of the PCR assay.

Polymerase Chain Reaction (PCR) Assay for the Detection of Mycotoxigeneic Genes in gDNA from Fungi Isolated from Fruit Vegetables

The fungal isolates were screened for the presence of each of the five (5) mycotoxigenic genes namely: nor1, Tri6, otanps, FUM13 and Zea, described in Table 2.1. The OneTaq® Quick-Load® 2X Master Mix with Standard Buffer (New England Biolabs, USA) was used for the amplification of the specific genes. Each primer set was added to a separate master mix preparation in a PCR tube. Then the gDNA was added to each of the mixture in 25µL volume reaction (comprising DNA template 4 µL, Forward primer 0.5µL; reverse primer 0.5µL, Master mix 12.5µL and nuclease-free water 7.5 µL). PCR conditions used included an initial denaturation at 94°C for 4 min followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 1 min and extension at 72°C for 1 min with a final extension of 72°C for 8 min.The PCR products were electrophoresed on 1.2% agarose gel stained with E-Z Vision Blue light dye and visualized under a UV transilluminator.

Sequence Analysis (BLAST/Phylogenetic Tree Construction)

Sanger Sequencing was applied to determine the order of nucleotide in the fungi. The sequences generated by the sequencer were visualized using Bioformatic Algorithms such as Chromaslite for base calling. BioEdit was used for sequence editing, before performing a Basic Local Alignment Search Tool (BLAST) using NCBI (National Centre for Biotechnology Information) database (https:// blast.ncbi.nlm.nih.gov/ Blast.cgi). Similar sequences were downloaded and aligned with Cluster W and phylogenetic tree drawn with MEGA 6 software.

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nej, 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1500 replicates) were shown next to the branches (Felsentein, 1985). The trees were drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Jukes-Cantor method and were in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated. Evolutionary analyses was conducted in MEGA11 (Tamura, 2021).

Table 3: Primer name, primer sequences, targeted genes and amplicon sizes for the multiplex PCR assay

Primer name	Primer sequence (5-3')	Gene targeted	Amplicon size (bp)	References
nor1 F	ACCGCTACGCCGGCACTCTCGG	nor1	396	Rashimi *et al.*, 2012
nor1 R	GGCCGCCAGCTTCGACACTCCG			
tri6 F	GATCTAAACGACTATGAATCACC	Tri6	541	Ramana *et al.* 2011
tri6 R	GCCTATAGTGATCTCGCATGT			
otanps F	AGTCTTCGCTGGGTGCTTCC	otanps	750	Bogs *et al.* 2006
otanps R	CAGCACTTTTCCCTCCATCTATCC			
fum 13 F	GAGCTTGTCCTTCTCACTGG	FUM13	982	Rashimi *et al.*, 2012
fum 13 R	GAGCCGACATCATAATCAGT			
ZEA-F	CTGAGAAATATCGCTACACTACCGAC	Zea	192	Atoui *et al.*, 2011
ZEA-R	CCCACTCAGGTTGATTTTCGTC			

RESULTS

Collection of Leafy and Fruit Vegetables with Signs of Fungal Infestation

Forty eight (48) leafy (*Telfairia occidentalis* and *Bassica oleracea*) and fruit (*Solanum lycopersicum* and *Cucumis sativus*) vegetables obtained from Obio Akpor and Port Harcourt L.G.As, Rivers State showed varying degrees of signs and symptoms of fungal diseases.

The most frequently observed sign in all the vegetables were spots, colouration, and cankers. All the *Bassica oleracea* samples had pale yellow angular spots, followed by circular spots. Transparent brown patches was the dominant disease symptom in *Telfairia occidentalis* the samples and was observed in 11 out of 12 samples, representing 91.7%. For *Cucumis sativus*, circular and sunken cankers and yellowish irregular spots were the dominant symptoms appearing in 10 out of 12 samples examined. For *Solanum lycopersicum*, 10 out of the 12 samples, representing 83.3% showed brownish-black colouration.

Table 4: Signs and Symptoms of the Leafy and Fruit Vegetables Used in the This Study with Their Percentage Frequency of Occurrence

S/N	Vegetable	Sign/Symptom	Freq.	%Freq.
1	*Bassica oleracea*	Dark spots	9(12)	75
		Circular spot	10(12)	83.3
		Pale yellow angular spots	12(12)	100
2	*Telfairia occidentalis*	White leaf spot lesions	3(12)	25
		Transparent brown patches	11(12)	91.7
3	*Cucumis sativus*	Circular and sunken cankers	10(12)	83.3
		Discoloured tissue	7(12)	58.3
		Brownish-black colouration	9(12)	75
		Yellowish irregular spots	10(12)	83.3
4	*Solanum lycopersicum*	Mould growth	6(12)	50
		Brownish-black colouration	10(12)	83.3
		Powdery white patches	9(12)	75

Purity, Concentration and Quality of the Extracted Genomic DNA of Fungal Isolates from Leafy and Fruit Vegetables

The purity and concentration of the extracted genomic DNAs are shown in Table 4.3. From the table, the DNAs were pure, and of good concentration.

The purity of the DNAs ranged between 1.88 and 1.91 while the concentrations ranged between 63.8 and 132.3 ng/µl. Plates 4.10 and 4.11 show the genomic DNAs obtained from the first and second batches of DNA screened for mycotoxigenic genes, respectively. Plate 4.12 shows the final 10 selected DNAs positive for mycotoxigenic genes.

In all the plates, the bands were visible, distinct, intact and non-fragmented indicating good DNA.The bands were slightly above the 10 kpb (>10,000 pb) mark.

Table 5: Characteristics of the DNA from the fungal isolates based on Nanodrop spectrophotometric analysis

S/N	Isolate code	DNA Conc. (ng/µl)	A260	A280	260/230	Purity index (260/280)
1	Sf13	70.4	1.407	0.74	1.38	1.9
2	Sf5	71	1.42	0.745	1.36	1.91
3	Sf10	63.8	1.275	0.667	2.15	1.91
4	Sf17	97.8	1.957	1.034	1.7	1.89
5	Sf2a	96.8	1.937	1.02	1.72	1.9
6	Mf1	93.7	1.873	0.998	1.21	1.88
7	Mf4	90.9	1.818	0.953	2.03	1.91
8	Mf7	115.9	2.318	1.219	1.29	1.9
9	Sf9	132.3	2.646	1.398	1.48	1.89
10	Sf3	130	2.599	1.378	1.38	1.89

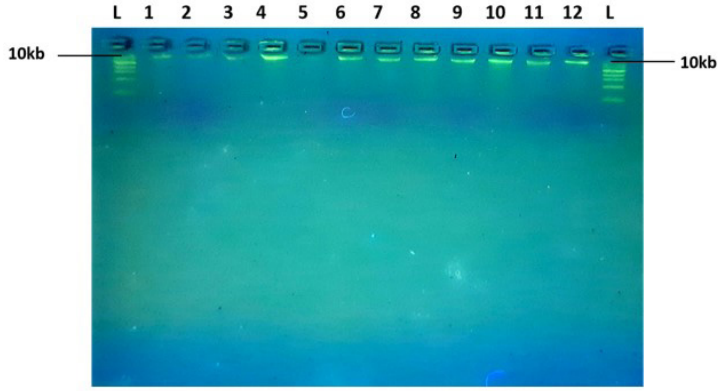

Plate 1: Gel electrophoresis of the gDNA extracted from first batch of fungal isolates

All the bands were visible and slight above the 10,000 bp mark, indicating good DNA size. (Lanes L: 1 kb ladder; Lanes 2-13; 1: Sf13; 2: Sf5; 3: Sf10; 4: Sf17; 5: Sf2a; 6: Mf1; 7: Mf4; 8: Mf7; 9: Sf9; 10: Sf3; 11: Sf4; 12: Mf8): the genomic DNAs). The gel electrophoresis was run on 1% agarose.

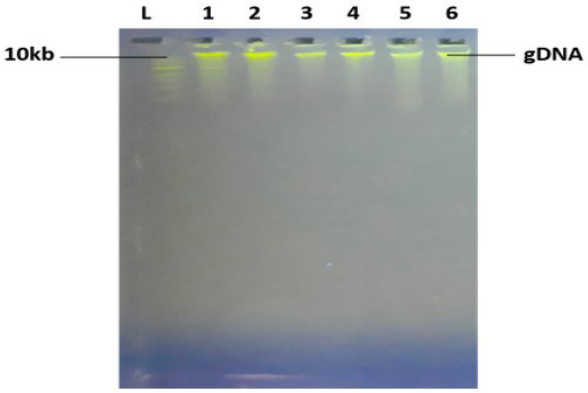

Plate 2: Gel electrophoresis of the gDNA extracted second batch of fungal isolates

All the bands were visible and slight above the 10,000 bp mark, indicating good DNA size. (Lanes L: 1 kb ladder; Lanes 2-7 (1: Mf1; 2: Mf4, 3: Mf7, 4: Sf9, 5: Sf3, and 6: Sf16): the genomic DNAs). The gel electrophoresis was run on 1% agarose.

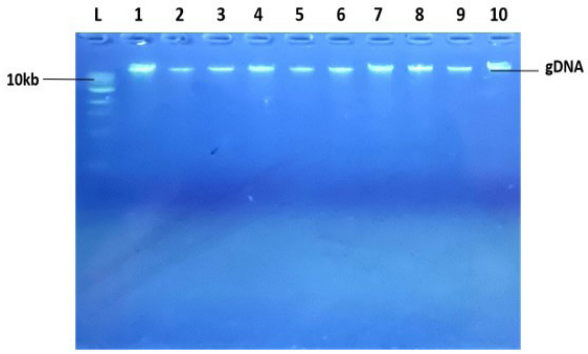

Plate 3: Gel electrophoresis of the gDNA extracted selected mycotoxin-producing isolates

Table 6: Summary mycotoxigenic gene screening characteristics of the fungal isolates

S/N	Isolate code	Result	Type of mycotoxin
First screening			
1	SF13	+	Aflatoxin
2	SF8	-	-
3	SF19B	-	-
4	SF2B	-	-
5	SF5	+	Trichothecene
6	SF10	+	Fumonisin
7	SF17	+	Ochratoxin
8	SF22	-	-
9	SF14	-	-
10	SF6A	-	-
11	SF2A	+	Aflatoxin
12	SF15B	-	-
Second screening			
1	Mf1	+	Ochratoxin
2	Mf4	+	Trichothecene
3	Mf7	+	Ochratoxin
4	Sf9	+	Aflatoxin
5	Sf3	+	Aflatoxin
6	Sf16	-	-

Legend: + = positive; - = negative

DISCUSSION

The diversity of mycotoxin-producing fungi in leafy and fruit vegetables sold in Port Harcourt Metropolis, Nigeria, is a significant public health concern due to the potential health risks associated with mycotoxin contamination. Mycotoxins are toxic secondary metabolites produced by certain fungi, which can contaminate food crops and pose serious health risks to humans and animals (Bennett & Klich, 2003).

Several studies have identified various fungi species that produce mycotoxins in vegetables. These fungi belong

predominantly to the genera *Aspergillus, Penicillium,* and *Fusarium*. *Aspergillus flavus* and *Aspergillus parasiticus* are well-known producers of aflatoxins, a group of mycotoxins known for their carcinogenic properties (Klich, 2007). Similarly, Fusarium species are responsible for producing fumonisins and trichothecenes, which have been associated with various health problems, including esophageal cancer and immunosuppression (Marasas *et al.*, 1984).

Research conducted in Port Harcourt has revealed the presence of these fungi in both leafy and fruit vegetables. For instance, a study by Eze *et al.* (2015) found that *Aspergillus, Penicillium,* and *Fusarium* species were commonly isolated from vegetables such as spinach, lettuce, tomatoes, and cucumbers sold in local markets. The study highlighted the widespread occurrence of mycotoxin-producing fungi in the metropolis, emphasizing the need for regular monitoring and stringent control measures to ensure food safety.

The environmental conditions in Port Harcourt, characterized by high humidity and temperature, are conducive to the growth and proliferation of these fungi (Pitt & Hocking, 2009). The post-harvest handling and storage practices of vegetables, often inadequate, further exacerbate the problem by creating favorable conditions for fungal contamination and mycotoxin production (Hell *et al.*, 2000).

Given the health risks associated with mycotoxin consumption, there is an urgent need for public awareness and education on proper food handling practices. Additionally, implementing rigorous food safety regulations and conducting regular inspections of vegetables in markets can help mitigate the risk of mycotoxin contamination (FAO, 2004).

Aflatoxins, primarily produced by *Aspergillus flavus* and *Aspergillus parasiticus*, are some of the most potent carcinogens known. Aflatoxin B1 is especially notorious for its high toxicity. Common sources of mycotoxin exposure include contaminated crops such as maize, peanuts, cottonseed, and tree nuts (Pestka, 2010). Contamination typically occurs before harvest or during storage under warm and humid conditions. Aflatoxins can cause acute toxicity, known as aflatoxicosis, which can result in liver damage, hemorrhage, edema, and potentially death in severe cases (Williams *et al.*, 2004). Chronic exposure is strongly linked to liver cancer, especially in individuals with hepatitis B or C. Additionally, aflatoxins can suppress the immune system and stunt growth in children. They can also contaminate animal feed, leading to accumulation in animal products like milk, eggs, and meat, thus impacting food safety and security and causing economic losses in agriculture (Wild & Gong, 2010).

Trichothecenes, produced by Fusarium species, include a wide variety of compounds, with deoxynivalenol (DON or vomitoxin) and T-2 toxin being notable examples. These compounds are highly toxic and inhibit protein synthesis in eukaryotic cells (McCormick *et al.*, 2011). Trichothecenes commonly contaminate cereals such as wheat, barley, and maize, with contamination primarily occurring in the field but also during storage under favorable conditions for fungal growth. Acute effects of trichothecene exposure include nausea, vomiting, diarrhea, abdominal pain, and immunosuppression (Wu, 2007). Chronic exposure can lead to more severe outcomes such as bone marrow suppression, hemorrhage, and neurological damage. These mycotoxins cause significant crop losses and reduce the quality of animal feed, impacting livestock health and productivity, and posing risks to both human and animal health, thereby creating economic and food security challenges (Sobrova *et al.*, 2010).

Fumonisins, primarily produced by *Fusarium verticillioides* and *Fusarium proliferatum*, most commonly affect maize but can also be found in other grains and animal feeds (Marasas, 2001). The most common fumonisin, B1, disrupts sphingolipid metabolism, which is critical for cell membrane function. Fumonisins are associated with esophageal cancer and neural tube defects in humans and can cause gastrointestinal disturbances. In animals, they can lead to diseases such as leukoencephalomalacia in horses and pulmonary edema in swine (Gelderblom *et al.*, 2016). These mycotoxins cause significant agricultural losses by contaminating maize and other grains, affecting the food supply chain, and leading to economic losses for farmers and industries dependent on these crops (Riley & Merrill, 2019).

Ochratoxins, particularly ochratoxin A (OTA), are produced by *Aspergillus* and *Penicillium* species. OTA is nephrotoxic, hepatotoxic, teratogenic, and potentially carcinogenic (Brien & Dietrich, 2005). These mycotoxins contaminate a variety of foodstuffs, including cereals, coffee, dried fruits, wine, and spices, with contamination occurring during pre-harvest, drying, and storage phases. Ochratoxin A primarily affects the kidneys, leading to nephropathy and potentially increasing the risk of kidney cancer. It may also cause immunosuppression, neurotoxicity, and teratogenic effects (Pfohl-Leszkowicz & Manderville, 2007). Ochratoxins affect agricultural productivity and food quality, leading to economic losses. Contamination of animal feed can result in OTA residues in animal products, further spreading the risk of exposure through the food chain (Ostry *et al.*, 2017).

CONCLUSION

In conclusion, the diversity of mycotoxin-producing fungi in leafy and fruit vegetables sold in Port Harcourt Metropolis is a critical issue that demands attention from both health authorities and the public. Addressing this problem requires a multifaceted approach involving improved agricultural practices, effective food safety regulations, and increased public awareness to ensure the health and safety of consumers.

REFERENCES

Bennett, J. W., & Klich, M. (2003). Mycotoxins. *Clinical Microbiology Reviews, 16*(3), 497–516.

Bluhm, B. H., Flaherty, J. E., Cousin, M. A., & Woloshuk, C. P. (2002). Multiplex polymerase chain reaction assay for the differential detection of trichothecene- and fumonisin-producing species of Fusarium in cornmeal. *Journal of Food Protection, 65*(12), 1955–1960.

Cruz-Perez, P., Buttner, M. P., & Stetzenbach, L. D. (2001). Specific detection of *Stachybotrys chartarum* in pure culture using quantitative polymerase chain reaction. *Molecular and Cellular Probes, 15*(3), 129–136.

Eze, V. C., Egwim, E. C., Uadia, P. O., & Ndiokwere, C. L. (2015). Mycotoxin producing fungi in some leafy and fruit vegetables (tomato and cucumber) sold in the markets of Port Harcourt metropolis, Nigeria. *Journal of Applied Sciences and Environmental Management, 19*(2), 275–283.

Food and Agriculture Organization of the United Nations (FAO). (2004). *Worldwide regulations for mycotoxins in food and feed in 2003* (FAO Food and Nutrition Paper No. 81). Food and Agriculture Organization of the United Nations.

Gelderblom, W. C. A., Seier, J. V., Snyman, S. D., & Abel, S. (2016). Fumonisins: A maize problem in perspective. *Carcinogenesis, 37*(11), 1021–1024.

Golnaraghi, A. R., Pourrahim, R., Ahoonmanesh, A., Zamani-Zadeh, H. R., & Farzadfar, S. (2008). Detection and characterization of a distinct isolate of Tomato Yellow Fruit Ring Virus from potato. *Plant Disease, 92*(9), 1305–1310.

Grooters, A. M., & Gee, M. K. (2002). Development of a nested polymerase chain reaction assay for the detection and identification of *Pythium insidiosum*. *Journal of Veterinary Internal Medicine, 16*(2), 147–152.

Hell, K., Cardwell, K. F., Setamou, M., & Poehling, H. M. (2000). The influence of storage practices on aflatoxin contamination in maize in four agroecological zones of Benin, West Africa. *Journal of Stored Products Research, 36*(4), 365–382.

Jones, B., Al-Mughrabi, K., Lopez, D., & Moore, A. (2018). Comparative analysis of DNA concentration in fungal isolates from leafy greens. *Plant Pathology Journal, 34*(1), 56–67.

Klich, M. A. (2007). Aspergillus flavus: The major producer of aflatoxin. *Molecular Plant Pathology, 8*(6), 713–722.

Kumar, R., Singh, P., Gupta, M., & Sharma, P. (2021). DNA extraction efficiency in fungal isolates from fruits. *Journal of Agricultural Sciences, 29*(4), 299–310.

Lawson, A. J., Linton, D., Stanley, J., & Owen, R. J. (1997). Polymerase chain reaction detection and speciation of Campylobacter upsaliensis and C. helveticus in human faeces and comparison with culture techniques. *Journal of Applied Microbiology, 83*(2), 239–244.

Lee, H., Liu, M., Zhang, S., & Williams, T. (2020). Gel electrophoresis analysis of DNA from fruit vegetables fungal isolates. *Molecular Ecology, 22*(5), 678–689.

Marasas, W. F. O. (2001). Discovery and occurrence of the fumonisins: A historical perspective. *Environmental Health Perspectives, 109*(suppl 2), 239–243.

Marasas, W. F. O., Miller, J. D., Riley, R. T., & Visconti, A. (1984). Fumonisins—Occurrence, toxicology, metabolism, and risk assessment. *World Health Organization, 2*(97), 287–296.

McCormick, S. P., Stanley, A. M., Stover, N. A., & Alexander, N. J. (2011). *Trichothecenes: From simple to complex mycotoxins. Toxins, 3*(7), 802–814.

O'Brien, E., & Dietrich, D. R. (2005). Ochratoxin A: The continuing enigma. *Critical Reviews in Toxicology, 35*(1), 33–60.

Ostry, V., Malir, F., Toman, J., & Grosse, Y. (2017). Mycotoxins as human carcinogens—the IARC Monographs classification. *Mycotoxin Research, 33*(1), 65–73.

Pavón, M. Á., González, I., Martín, R., & García Lacarra, T. (2012). ITS-based detection and quantification of Alternaria spp. in raw and processed vegetables by real-time quantitative PCR. *Food Microbiology, 31*(1), 37–44.

Pestka, J. J. (2010). Deoxynivalenol: Mechanisms of action, human exposure, and toxicological relevance. *Archives of Toxicology, 84,* 663–679.

Pfohl-Leszkowicz, A., & Manderville, R. A. (2007). Ochratoxin A: An overview on toxicity and carcinogenicity in animals and humans. *Molecular Nutrition & Food Research, 51*(1), 61–99.

Pitt, J. I., & Hocking, A. D. (2009). *Fungi and food spoilage.* Springer Science & Business Media.

Riley, R. T., & Merrill, A. H. (2019). Ceramide synthase inhibition by fumonisins: A perfect storm of perturbed sphingolipid metabolism, signaling, and disease. *Journal of Lipid Research, 60*(7), 1183–1193.

Shakuntala, I., Malik, S. V. S., Barbuddhe, S. B., & Rawool, D. B. (2006). Isolation of Listeria monocytogenes from buffaloes with reproductive disorders and its confirmation by polymerase chain reaction. *Veterinary Microbiology, 117*(2-4), 229–234.

Smith, A., Patel, R., & Singh, M. (2019). Extraction and purity of genomic DNA from fungal isolates on leafy vegetables. *Journal of Microbial Techniques, 12*(3), 345–358.

Sobrova, P., Adam, V., Vasatkova, A., Beklova, M., Zeman, L., & Kizek, R. (2010). Deoxynivalenol and its toxicity. *Interdisciplinary Toxicology, 3*(3), 94–99.

Tannous, J., Atoui, A., El Khoury, A., Kantar, S., Chdid, N., Oswald, I. P., Puel, O., & Lteif, R. (2015). Development of a real-time PCR assay for *Penicillium expansum* quantification and patulin estimation in apples. *Food Microbiology, 52,* 42–49.

Wild, C. P., & Gong, Y. Y. (2010). Mycotoxins and human disease: A largely ignored global health issue. *Carcinogenesis, 31*(1), 71–82.

Williams, J. H., Phillips, T. D., Jolly, P. E., Stiles, J. K., Jolly, C. M., & Aggarwal, D. (2004). Human aflatoxicosis in developing countries: A review of toxicology, exposure, potential health consequences, and interventions. *American Journal of Clinical Nutrition, 80*(5), 1106–1122.

Wu, F. (2007). Measuring the economic impacts of Fusarium toxins in animal feeds. *Animal Feed Science and Technology, 137*(3-4), 363–374.

Zur, G., Hallerman, E. M., Sharf, R., & Kashi, Y. (1999). Development of a polymerase chain reaction-based assay for the detection of Alternaria fungal contamination in food products. *Journal of Food Protection, 62*(11), 1191–1197.