

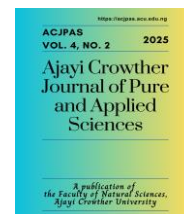
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Article

Mycological Assessment of Some Selected Fruits and Vegetables and Their Resistance Profile Against Some Antifungal Agents

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Abstract

Fruits and vegetables offer distinct nutritional benefits and health-enhancing properties, driving their growing popularity. This research aimed to evaluate the mycological characteristics and investigate the antifungal resistance patterns of pathogens from selected fruits and vegetables. Samples, including watermelon, tomato, cucumber, and garden egg, were obtained from fruit markets and home gardens. They were subjected to microbiological analysis using standard procedures. The isolates were examined to determine their ability to cause disease, contribute to spoilage, and produce aflatoxins. Isolated pathogens were further subjected to antifungal sensitivity testing using the agar well diffusion method with three (3) antifungal agents. A total of eighteen (18) fungi were isolated from the genera *Aspergillus* (33.3 %), *Candida* (11.1 %), *Cladosporium* (5.6 %), *Fusarium* (5.6 %), *Geotrichum* (11.1 %), *Penicillium* (5.6 %), *Pichia* (16.6 %) and *Saccharomyces* (11.1 %). Isolates with haemolytic potential were 7, while 11 had spoilage potential, and 12 were pathogenic. Of the 12 pathogenic fungi, 9 (75%) were resistant to at least one antifungal agent while 3 (25%) showed no resistivity; however, among the *Aspergillus* spp., 5 (83%) were aflatoxigenic. The overall mycological quality of the analysed samples is low, improved hygienic practices should be encouraged among farmers, fruit and vegetable handlers and consumers.

Keywords: Fruits, Vegetables, Aflatoxin, Antifungal resistance, Mycological assessment

1. Introduction

Fruits and vegetables are vital to human nutrition due to their health-enhancing qualities, including antioxidants that shield cells from free radicals, which are linked to the development of chronic conditions such as diabetes, obesity, and various micronutrient deficiencies, particularly in developing nations [1,2]. They also supply essential growth factors like vitamins (B, C, K), minerals (calcium, potassium, magnesium), and dietary fiber, which support proper metabolic function [3,4]. This nutritional value has boosted their consumption, but it has also coincided with a rise in foodborne illness outbreaks. Many fruits and vegetables create favorable environments for microbial growth, containing components like pectin, cellulose, hemicellulose, and polysaccharides, with starch serving as their main storage compound [5]. Microorganisms exploit these hosts by producing extracellular enzymes that break down these complex structures, releasing water and intracellular nutrients for their own use, as the inner tissues of fruits and vegetables are rich in sustenance [5]. Additionally, the abundance of vitamins, sugars, minerals, and amino acids, combined with a low pH, fosters the proliferation of diverse microorganisms, including various fungi [3].

Fungi are widely distributed in nature; they commonly contaminate agricultural commodities, foods and feeds including fruits and vegetables, causing high economic losses globally [6]. Fruits and vegetables can become contaminated with microorganisms at various stages—pre-harvest, harvest, or post-harvest—due to contact with soil, insects, animals, or humans. From farm to table, potential sources of contamination include fecal matter, human handling, harvesting tools, processing methods, transportation, and distribution [7]. Fungi commonly identified in raw fruits and vegetables include *Pichia* spp., *Candida* spp., *Rhodotorula* spp., *Saccharomyces* spp., *Zygosaccharomyces* spp., *Schizosaccharomyces pombe*, *Brettanomyces intermedius*, *Torulopsis holmii*, *Trichoderma* spp., *Aspergillus* spp., *Curvularia* spp., *Penicillium* spp., *Cladosporium* spp., *Fusarium* spp., *Geotrichum* spp., *Alternaria* spp., *Botrytis cinera*, *Rhizopus* spp. and *Mucor* spp. [8,9].

Fungi can cause spoilage in fruits and vegetables, these spoilage fungi can be toxigenic or pathogenic, causing infections or allergies in animals and humans [3]. While studies evaluating fungi as food-borne pathogens and assessing associated risks are limited, an approximate number of 150 million people have been infected with fungal infections annually, with approximately 1.7 million deaths reported annually due to fungal infections [31]. By the year 2023, more than 6.5 million people had suffered from fungal infections annually, of which more than 3.8 million deaths were recorded [32, 33]. The presence of toxigenic fungi in foods and crops can pose a serious health hazard to humans and animals through the production of some toxic secondary metabolites called mycotoxins. Mycotoxigenic fungi include *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria* and *Claviceps*. Producing toxins such as aflatoxins, fumonisins, ochratoxin A, and patulin, among others [10].

The use of antifungal agents for prolonged periods in treating fungal infections has resulted in the emergence of fungal resistance. Most fungi have developed several mechanisms to survive exposure to antifungal agents, hence, the need for antifungal susceptibility testing to available drugs for treatment and reduction in the spate of antifungal resistance [11]. This study aims to evaluate the mycological quality of selected fruits and vegetables and profile the antifungal resistance of isolated pathogens.

2. Materials and Methods

2.1 Sample collection

Four types of fresh fruits and vegetables that grow close to the soil and are commonly consumed raw were selected for this research. These included watermelon (n=3), cucumber (n=5), garden egg (n=16), and tomato (n=29). The samples were randomly collected from different fruit markets and home gardens in Oyo State, Ogun State, and Ekiti State in the South-Western region of Nigeria. All samples were transported to the Microbiology Laboratory, Department of Microbiology and Biotechnology, Ajayi Crowther University, Oyo, Nigeria, for mycological analysis.

2.2 Isolation of fungi

For each fruit and vegetable sample, one gram of the epicarp and mesocarp was measured and placed into stomacher bags (Seward, UK). This was then blended with 9 mL of sterile water using a stomacher (Stomacher 80 Biomaster, Seward, UK). Serial dilutions were prepared from the resulting homogenate and poured onto Potato Dextrose Agar (HiMedia, India) in duplicate. The inoculated plates were incubated at 25 ± 2 °C for five days. Following incubation, the total viable fungal count per gram was determined and reported as colony-forming units per gram (CFU/g).

2.3 Identification of fungi

The isolated fungi were identified according to their microscopic and macroscopic morphology using fungal identification keys. Young cultures of the fungal isolates were used. A drop of Lactophenol Cotton Blue was applied to clean slides, and a small fragment of each fungal mycelium was placed into the stain. The mycelium was gently spread apart using sterile inoculating needles, after which a clean coverslip was carefully placed over it to prevent air bubbles from forming. The prepared slides were

examined under the microscope using the scanning power objective ($\times 4$) followed by the low power ($\times 10$) and high power ($\times 40$) magnification objective lens for vegetative and reproductive structures including, the hyphae, conidia, sporangiophore etc.

2.4 Determination of haemolysin and amylase production in isolates

These experiments were conducted on all fungal isolates to evaluate their potential for pathogenicity and fruit spoilage, particularly in relation to starch metabolism. A haemolysis assay was carried out using freshly prepared blood agar (composed of 10% antibiotic-free human blood mixed with 100 mL of Potato Dextrose Agar, v/v), while an amylase test was performed using starch agar (w/v: 0.5 % peptone, 0.3 % yeast extract, 1.5 % agar-agar, 0.2 % soluble starch). For yeast isolates, a single streak was made from the young culture of each isolate at the centre of blood and starch agar plates. The plates were incubated at 25 ± 2 °C for 24 h. For mould isolates, a small portion of the mycelium of each isolate was placed at the centre of the blood and starch agar plates. The plates were incubated at 25 ± 2 °C for 3-5 days. The extent of starch hydrolysis was confirmed by flooding the starch agar plates with Lugol's iodine and observing colour changes around the colonies. The appearance of blue/black colouration indicates the inability of the organisms to utilize the starch. Beta haemolysis was indicated by a clear colourless zone surrounding the colonies, alpha haemolysis was indicated by greenish to brownish discolouration of the medium, while no clear zone and change in colouration of the medium indicates gamma haemolysis [12].

2.5 Qualitative screening for the detection of aflatoxigenic moulds

All *Aspergillus* species isolated in this study were tested for aflatoxin production using palm kernel agar (PKA). The PKA was prepared from fresh palm kernel fruits sourced from local farms. The fruits were peeled to extract the kernels, and 20% (v/v) of these kernels were cleaned with warm water, then soaked in hot sterile water for 30 minutes. Afterward, the soaked kernels were blended aseptically for 10 minutes and filtered through four layers of muslin cloth. To the resulting filtrate, 2% agar was added, and the mixture was thoroughly mixed and sterilized at 121 °C for 15 minutes. Once cooled, it was poured evenly into sterile Petri dishes and allowed to solidify. A small piece of mycelium from each isolate was placed at the center of the PKA plates, which were then incubated at 25 ± 2 °C for 52 hours. Aflatoxin production by toxigenic molds was identified by the appearance of yellow-orange pigmentation on the reverse of the fungal cultures [13-15].

2.6 Antifungal susceptibility testing

After screening for haemolysin and amylase production, isolates that were either β -haemolytic, amylase producers or both β -haemolytic and amylase producers were termed pathogenic and were further subjected to antifungal susceptibility testing.

The agar well diffusion method was used on Potato Dextrose agar (HiMedia). The antimycotic drugs used belong to three classes: azoles (fluconazole – 10 mg/mL), polyenes (nystatin – 100 IU) and heterocyclic benzofuran (griseofulvin – 25 mg/mL). The fungal inoculum suspensions were made by suspending the mycelia and spores of 3-day-old cultures in 2 mL sterile water and adjusted to 0.5 McFarland's standard before testing. Each standardized inoculum was inoculated onto freshly prepared PDA by making a lawn using sterile swab sticks. Then, three wells of 6mm diameter were punched off from the agar medium aseptically with a sterile cork-borer, each well was filled with different antifungal agents under aseptic conditions and sterile water was used as a negative control. The plates were incubated in an upright position aerobically at 28 ± 2 °C for 24-72 h, and the diameter of the inhibition zones was measured in millimetres after incubation [16].

2.7 Statistical analysis

The data was analysed using GraphPad Prism, version 9.5.1. The results were expressed in mean \pm standard deviation. Unpaired t-tests were used to establish the difference between means following microbial load. A P value <0.05 was considered a significant difference for each sampling source.

3. Results

3.1 Total viable fungal count

The total viable count of fungi in selected retail and home-garden fruits and vegetables is presented in Table 1. The mean of the fungal count (log CFU/g) were observed to be high in the watermelon and cucumber samples from home gardens compared to the retail samples ($p < 0.05$). However, the mean fungal count in the tomato was higher in the retail samples compared to the home-garden samples ($p < 0.005$). Furthermore, there was no statistical difference between the garden-egg samples collected from retail and home garden sources (Table 1).

Table 1. Colony Counts of Retail and Home Garden Fruits and Vegetables Samples

Samples	Fungal colony count (log CFU/g)		P value
	Retail Mean \pm SD	Home-garden Mean \pm SD	
Watermelon	1.151 \pm 0.213	3.503 \pm 0.092	0.0023
Cucumber	1.360 \pm 0.392	3.289 \pm 0.080	0.0011
Garden-egg	3.1 \pm 0.174	3.153 \pm 0.066	0.6484
Tomato	3.492 \pm 0.199	2.619 \pm 0.151	0.0038

3.2 Characteristics of fungal isolates

Eleven (11) different fungal species were isolated in this study, they include; *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus terreus*, *Candida albicans*, *Candida krusei*, *Cladosporium* sp., *Fusarium dimerum*, *Geotrichum candidum*, *Penicillium* sp., *Pichia* spp., *Saccharomyces* spp. The colonial, cellular and morphological characteristics of the fungi isolated are shown in Table 2.

Table 2. Colonial and Morphological Characteristics of Fungal Isolates

Colony appearance	Morphology characteristics	Probable identity
Yellowish-green colony surrounded by a white border, dry surface velvety texture, yellow to tan	Septate hyphae, long conidiophores, uniseriate and biseriate phialides	<i>Aspergillus flavus</i>
Greenish-blue, white colony dry surface, velvety texture, the reverse is white to tan	Septate hyphae, short conidiophores, round conidia, uniseriate phialides	<i>Aspergillus fumigatus</i>
Cinnamon-brown colony, velvety texture, sugar surface, the reverse is yellow to tan	Septate hyphae, short conidiophores, round conidia, biseriate phialides	<i>Aspergillus terreus</i>
Cream smooth colony	Spores round to oval, budding	<i>Candida albicans</i>
Cream colony, flat, dry, dull with mycelial fringe	Round to oval, with some elongated, pseudohyphae	<i>Candida krusei</i>
Greenish-brown to grey colony, velvety texture, reverse is black	Septate hyphae, short branched conidiophores, formed branching tree-like chains, oval conidia	<i>Cladosporium</i> sp.
Whitish-pink colony, velvety texture	Septate hyphae, convex-shaped conidia with septa	<i>Fusarium dimerum</i>
White colony, velvety texture	Rectangular segmented hyphae	<i>Geotrichum candidum</i>
Bluish-green with white border colony, the reverse is white	Septate hyphae, with branched and unbranched conidiophores	<i>Penicillium</i> sp.
Cream colony, smooth texture	Pseudohyphae, budding, ascus	<i>Pichia</i> sp.
Cream smooth round colony	Round ascospores	<i>Saccharomyces</i> sp.

3.3 Frequency of occurrence of fungal isolates

From the fruit and vegetable samples analysed, 18 fungi were isolated, part of which 11 (61.1 %) were moulds and 7 (38.9 %) were yeasts. Ten (10) (55.6 %) fungi were isolated from retail samples and 8 (44.4 %) from home garden samples. The varying occurrence of fungi in analysed samples is presented in Table 3.

Table 3. Frequency of Occurrence of Identified Fungal Isolates

Isolates	Samples obtained from	Number of Occurrence			Percentage (%)
		R	HG	Total	
<i>Aspergillus flavus</i>	Watermelon ^a , garden egg ^a , tomato ^b	2	1	3	16.6
<i>A. fumigatus</i>	Tomato ^a	1	0	1	5.6
<i>A. terreus</i>	Cucumber ^b , watermelon ^b	0	2	2	11.1
<i>Candida albicans</i>	Cucumber ^b	1	0	1	5.6
<i>Candida krusei</i>	Cucumber ^b	0	1	1	5.6
<i>Cladosporium</i> sp.	Watermelon ^a	1	0	1	5.6
<i>Fusarium dimerum</i>	Cucumber ^a	1	0	1	5.6
<i>Geotrichum candidum</i>	Tomato ^a , watermelon ^b	1	1	2	11.1
<i>Penicillium</i> sp.	Tomato ^a	1	0	1	5.6
<i>Pichia</i> spp.	Tomato ^a , cucumber ^b , garden egg ^b	1	2	3	16.6
<i>Saccharomyces</i> spp.	Cucumber ^a	2	0	2	11.1

Key: ^a – Retail samples

^b – Home garden samples

3.4 Haemolysin and amylase production in fungal isolates

The pathogenicity potential of fungal isolates tested by screening for haemolysin production on blood agar resulted in the classification of the 18 fungal isolates into either α -haemolytic, β -haemolytic or γ -haemolytic. Of the 7 yeasts isolated, 3 (16.7%) (2 from retail and 1 from home garden) were γ -haemolytic, 3 (16.7%) (1 from retail and 2 from home garden) were α -haemolytic and only 1 (5.6%) (*Candida albicans*) from home garden cucumber was β -haemolytic. All the isolated yeasts from this study were non-amylase producers. All moulds isolated were amylase producers, none was γ -haemolytic, 5 (27.8%) (4 from retail and 1 from home garden) were α -haemolytic and 6 (33.3%) (3 from retail and 3 from home garden) were β -haemolytic (Figure 3). In total, 11 (61.1%) fungi were amylase producers, 7 (38.9%) were non-amylase producers, 3 (16.7%) were γ -haemolytic, 8 (44.4%) were α -haemolytic and 7 (38.9%) were β -haemolytic as shown in Figure 1.



Figure 1. Haemolysin Production in *Aspergillus terreus* Isolated from Home-garden Fruits

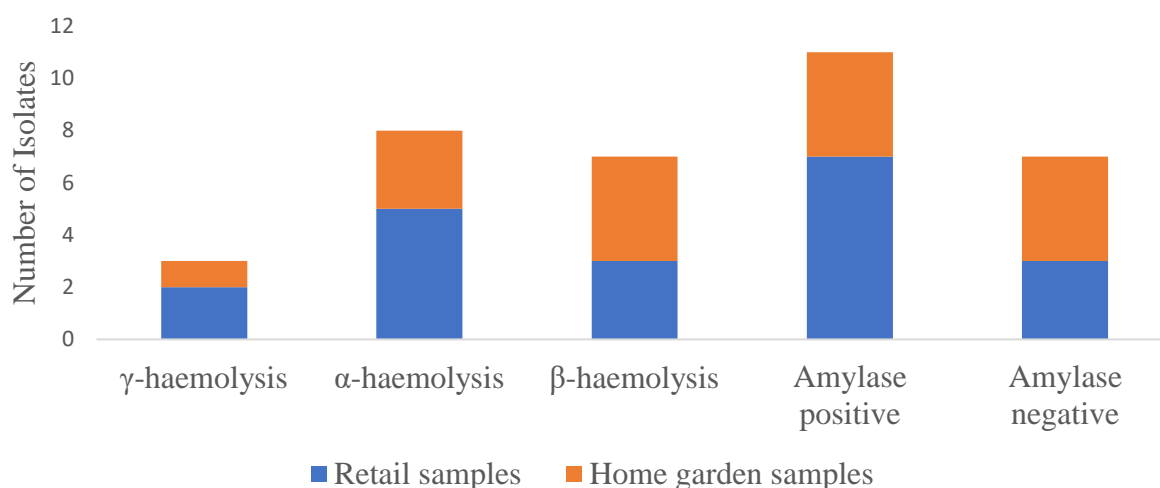


Figure 2. Haemolysin and Amylase Production in Fungal Isolates from Retail and Home Garden Fruits and Vegetables

3.5 Detection of aflatoxigenic moulds

The result of aflatoxigenic potential of *Aspergillus* spp. isolates determined by screening of Palm Kernel Agar is represented in Table 4. Five (83%) out of the six *Aspergillus* spp. subjected to this screening were aflatoxigenic. Watermelon, tomato and garden egg of retail source, and cucumber, watermelon, and tomato of home garden source were contaminated with aflatoxigenic moulds.

Table 4. Detection of Aflatoxigenic Moulds (Production of Pigments on Palm Kernel Agar)

S/N	Isolates	Samples obtained from	Pigments
1	<i>Aspergillus flavus</i>	Watermelon ^a	+
2	<i>Aspergillus terreus</i>	Cucumber ^b	+
3	<i>Aspergillus terreus</i>	Watermelon ^b	+
4	<i>Aspergillus fumigatus</i>	Tomato ^a	-
5	<i>Aspergillus flavus</i>	Garden egg ^a	+
6	<i>Aspergillus flavus</i>	Tomato ^b	+

Key: ^a – Retail samples ^b – Home garden samples

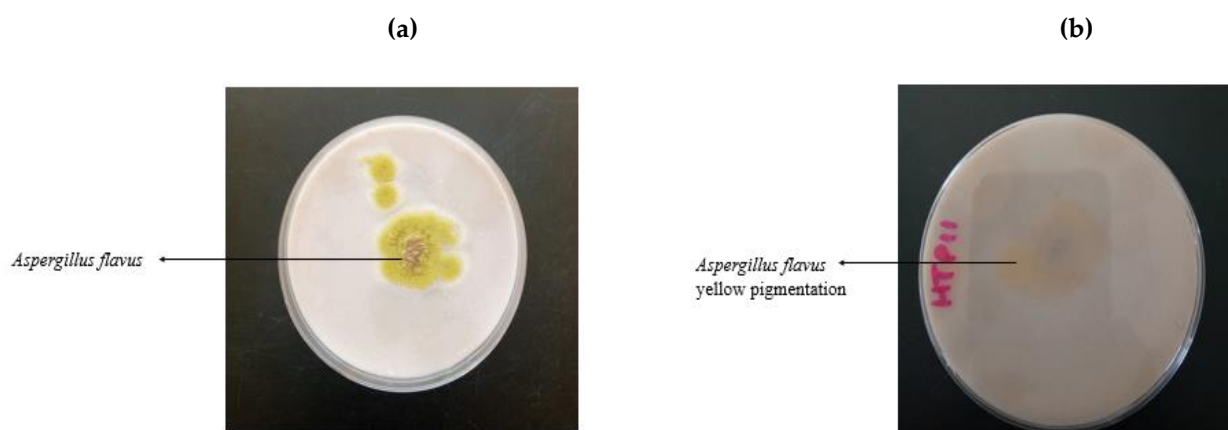


Figure 3. *Aspergillus flavus* isolated from home-garden vegetable on palm kernel agar, (a) Surface (b) Reverse pigmentation indicating aflatoxin production

3.6 Antifungal Susceptibility Test

The result of the antifungal resistance profile of tested isolates is presented in Table 12. A total number of 12 pathogenic fungal isolates were tested constituting 11 moulds and 1 yeast. The yeast tested was susceptible to griseofulvin and resistant to fluconazole and nystatin. The majority of the moulds (83.3 %) were susceptible to fluconazole and nystatin, and only 33.3 % were susceptible to griseofulvin (Table 5).

Table 5. Antifungal Susceptibility Test of Fungi Isolates (mm)

S/N	Isolates	Samples obtained from	Zone of inhibition (mm)		
			FLU	GRI	NYS
1	HCP11	Home-garden cucumber	50	NZ	NZ
2	CWAjP41	Retail watermelon	26	20	20
3	CWAp61	Retail watermelon	52	30	16
4	HWP23	Home-garden watermelon	68	24	38
5	HWP13	Home-garden watermelon	NZ	NZ	20
6	CTAKP11	Retail tomato	22	NZ	46
7	HCP23	Home-garden cucumber	40	NZ	30
8	CTAjP13	Retail tomato	43	NZ	21
9	CTAjP11	Retail tomato	18	NZ	17
10	CCAp21	Retail cucumber	38	NZ	NZ
11	CGP32	Retail garden-egg	40	NZ	17
12	HTP11	Home-garden tomato	40	NZ	18

Where: FLU = Fluconazole

GRI = Griseofulvin

NYS = Nystatin

NZ = No zone

4. Discussion

The mycological safety study carried out revealed that the mean fungal count (yeasts and moulds) from cucumber and watermelon were higher in the home garden compared to the retail source which is significantly revealed in the mean and standard deviation. However, the fungal count in retail tomatoes was higher compared to home gardens. The reason for this might be because the majority of the organisms in the home-garden samples were at their viable state since the samples were harvested fresh. Also, some organisms from the retail samples would have lost viability during the process of transportation and the long storage period before sales. From all the samples analysed in this study, watermelon, cucumber and tomato had a very high count, this could be a result of the high moisture content of the samples. Feroz *et al.* [17] reported higher fungal counts from fruits and vegetables collected from Dhaka, Bangladesh.

Fungi are the leading contributors to plant diseases, being both highly prevalent and highly damaging to plants as well as posing risks to human health [18]. Contamination with fungi was observed in fruits and vegetables analysed in this study, spoilage and pathogenic fungi such as *Aspergillus*, *Candida*, *Penicillium*, *Pichia*, and *Geotrichum*. *Cladosporium*, *Fusarium* and *Saccharomyces* were isolated, this correlates with the findings of Shehu and Waziri [19]. Certain fungi, such as *Aspergillus*, *Penicillium*, and *Fusarium*, can inflict significant losses on fruits and vegetables during packing, transportation, and storage by producing mycotoxins. These toxins make the produce unsafe for human consumption and diminish its nutritional quality. While many spoilage and pathogenic fungi begin infecting crops in the field prior to harvest and storage, the symptoms of these infections often become evident during storage. This leads to deterioration and damage, resulting in substantial losses for farmers and potential disease outbreaks among consumers [19]. Hence, their presence in both retail and home garden samples. As a result, fruits and vegetables can become contaminated on farms due to factors such as irrigation with sewage-contaminated water, the use of organic manure as fertilizer, dirty transportation and storage equipment, unsanitary cutting surfaces and tools, and unhygienic handling by workers [20]. Some species of the genus *Candida* cause infection in humans (candidiasis) if growth becomes out of control or enters deep into the body [21]. The most prevalent is *Candida albicans* which was isolated in this study, this is of food safety concern.

Microbial cells that generate the enzyme haemolysin can break down or destroy red blood cells, a trait that highlights their potential to cause disease [22]. This finding emphasizes the health hazards linked to eating ready-to-eat fruits and vegetables. The presence of amylase enzymes plays a key role in the spoilage of sugar-rich foods like fruits and vegetables [23]. In this study, about 61% of the 18 fungal isolates tested were found to produce amylase, suggesting their ability to cause fruit spoilage. However, food spoilage depends not only on the presence of spoilage-causing microbes but also on their population reaching levels where the decline in quality becomes evident. These haemolytic, amylase-producing fungi present an underrecognized threat to consumers, as they may attach and grow within the gastrointestinal tract, potentially leading to acute gastroenteritis [12].

Aflatoxins are highly toxic secondary metabolites produced by fungal species, including *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius* [24]. These fungi commonly infect plants, posing significant risks to human and animal health. Exposure to aflatoxins can lead to severe complications such as hepatotoxicity, teratogenicity, and immunotoxicity [25-27]. Contamination with aflatoxigenic fungi can occur in the field as a result of poor agronomic practices or during harvest, transport, storage of fruits and/or climatic conditions [24]. Aflatoxins were detected in retail watermelon and garden eggs from fruit markets and cucumber, watermelon and tomato from home gardens. This result correlates with that of Okigbo *et al.* [28] who detected aflatoxin B1 and B2 in pineapple, watermelon, and cucumber in varying concentrations. The presence of aflatoxins in these ready-to-eat fruits poses a significant health risk to the general public, as these fruits are a staple in the daily diet.

The fungal isolates exhibited varying antimicrobial resistance patterns to the three antifungal agents used. Most fungal isolates were resistant to griseofulvin, this finding is similar to that of El-Hamd *et al.* [29]. Fluconazole and nystatin had activity with most isolates (91%), however, fluconazole had wider zones of inhibition in isolates than nystatin. This result contradicts the findings of El-Hamd *et al.* [19] and Kulkarni *et al.* [30]. It was observed that retail cucumber and watermelon, and home-garden cucumber were resistant to two of the antimycotic agents used in this study. The resistance of these fungi to commonly used antifungal agents might be as a result of the presence of innate resistance genes to these antifungal agents or these genes might be horizontally transferred from their interaction with soil fungi. The presence of these antifungal resistance fungi in ready-to-eat fruits and vegetables is of public health concern.

5. Conclusion

Contamination with pathogenic, spoilage and aflatoxigenic fungi was discovered in analysed selected samples purchased from fruit markets and home gardens. Overall, contamination was higher in retail samples than in home garden samples. Most of the isolates were resistant to at least one antifungal agent. These results indicate the low mycological quality of the analysed fruits and vegetables. It is

therefore imperative for farmers, fruit and vegetable sellers and consumers to keep good hygienic practices to avoid contamination.

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