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Mycotoxin-producing fungi in rotten tomatoes

Oridikitorusinyaa O.*, Amaechi G. and Emmanuel O. O.

Department of Microbiology, Rivers State University, P.M.B. 5080, Port Harcourt, Nigeria.

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ABSTRACT

This study investigated the presence and diversity of mycotoxin-producing fungi in rotten tomatoes collected from the Mile 3 market in Diobu, Port-Harcourt. Fungal isolates were characterized using morphological techniques. Total heterotrophic fungi counts (THFC) ranged from 1.8 x 10^4 to 3.2 x 10^4 CFU/g, indicating significant fungal contamination. The most prevalent fungi identified were *Aspergillus niger* (29.2%), *Fusarium* sp. (28.1%), *A. flavus* (15.6%), *Rhizopus stolonifer* (13.5%), *Penicillium* sp. (10.4%), *Cladosporium* sp. (2.2%), and *Mucor racemosus* (1.1%). Mycotoxin analysis confirmed the presence of aflatoxins, ochratoxins, fumonisins, deoxynivalenol, and zearalenone. Aflatoxin B_1 levels ranged from 3.5 to 6.1 µg/kg, while ochratoxin A concentrations varied from 1.8 to 2.7 µg/kg. Deoxynivalenol levels ranged from 1.0 to 1.7 mg/kg, zearalenone from 0.6 to 1.3 mg/kg, and fumonisin B_1 from 1.2 to 2.0 mg/kg. These levels raise concerns about the potential health risks of consuming spoiled tomatoes. Physicochemical analysis of the samples revealed conditions conducive to fungal growth and mycotoxin production, including high moisture content (91.0-94.0 %), favorable temperatures (23-28 °C), slightly acidic pH (4.6-5.0), and high water activity (0.97-0.99). These findings underscore the importance of proper storage and handling practices to mitigate fungal contamination and mycotoxin production in tomatoes. The study highlights the need for increased awareness among consumers and food handlers regarding the risks associated with consuming spoiled produce and the implementation of effective control measures throughout the food supply chain to safeguard public health.

Keywords: Fungi, mycotoxins, rotten tomatoes, health risks, food handlers.

*Corresponding author. Email: oridikitorusinyaa.odike@ust.edu.ng.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) belongs to the family Solanaceae and is the second most important vegetable crop, providing 40 % of the daily value (Piemontese et al., 2017). Tomatoes are among the most important and popular commercial vegetables grown globally and in Africa (Turner et al., 2019). It is rich in vitamins (A, B1, B2, and C), water, organic acids, minerals and dietary fiber (Food and Agriculture Organization, 2017). Tomato is used to make soup, puree, ketchup and juice, and can be consumed either raw or cooked (Robbins et al., 2000). Consumption of tomatoes decreases the risk of prostate, breast, head, and neck cancer (Eun-Sun and Phyllis, 2002). It also helps in the formation of blood vessels, a healthy immune system, and sperm production (Moss, 2008), as well as decreasing conditions like osteoporosis and cardiovascular diseases (Piemontese et al., 2017). Due to its high water content, which is estimated to be about 94 %, it is frequently susceptible to microbial contamination, especially fungi and bacteria, viruses and nematodes, which also enables fungi to produce mycotoxins that are detrimental to human health (Mateo et al., 2007).

Tomatoes are one of the most widely consumed and economically important vegetables globally, with a rich nutritional profile and versatility in culinary applications. However, the post-harvest spoilage of tomatoes poses significant challenges to food security and public health. Among the various factors contributing to tomato spoilage, the contamination of tomatoes by mycotoxin-producing fungi has emerged as a major concern (Richard, 2007).

Fungal contamination in tomatoes can occur at various stages of the supply chain, from cultivation to post-harvest handling and distribution. Mycotoxin-producing

fungi, such as *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp., *Cladosporium* sp., *Mucor* sp. and *Fusarium* spp., are known to colonize and infect tomatoes under suboptimal storage and handling condition (Akinmusire, 2011). The warm and humid climate of the Mile Three Market region can exacerbate fungal growth and mycotoxin production, making it imperative to understand the extent of this issue and its potential impact on public health (Marin et al., 2019). The specific challenges and characteristics of Mile Three Market, including regional climate, transportation logistics, and consumer behaviors, necessitate a specialized investigation on the isolation and identification of mycotoxin-producing fungi in spoiled tomatoes sold in this particular market.

Mycotoxins are toxic secondary metabolites produced by various molds, including *Aspergillus*, *Penicillium*, and *Fusarium* species (Ashiq et al., 2017). These toxins contaminate various agricultural products, including grains, nuts, and fruits (Alshannaq and Yu, 2017). Tomatoes, being susceptible to fungal infections, are not exempted from mycotoxin contamination. A diverse group of mycotoxins, including aflatoxins, ochratoxins, and fumonisins, have been reported in spoiled tomatoes (Awuchi et al., 2021). Mycotoxin was first used in the 1960s to describe the toxin associated with contaminated peanuts in animal feed and the loss of turkeys in England (Turkey-X-disease) (Battilani et al., 2016). This mycotoxin was later identified as the *Aspergillus flavus* toxin aflatoxin B1 (Bennett and Klich, 2018; Baker, 2006).

Traditionally, toxigenic fungi contaminating agricultural grains have been conventionally divided into two groups: “field” fungi (e.g., *Cladosporium, Fusarium, Alternaria*, etc.), which are thought to have access to seeds during plant development, and “storage” fungi, (e.g., *Aspergillus*, *Penicillium*, etc.), which proliferate during storage (Lee et al., 2015; Do et al., 2015). Currently, this division is not so strict because according to Richard (2007), four types of toxigenic fungi can be distinguished: Plant pathogens such as *F. graminearum* and *A. alternata*; Fungi that grow and produce mycotoxins on senescent or stressed plants, e.g., *F. moniliforme* and *A. flavus*; Fungi that initially colonize the plant and increase the feedstock’s susceptibility to contamination after harvesting, e.g., *A. flavus*; Fungi that are found on the soil or decaying plant material that occurs on the developing kernels in the field and later multiply in storage if conditions permit, e.g., *P. verrucosum* and *A. ochraceus* (Fog, 2021; Jeswal and Kumar, 2015).

The presence of mycotoxins in tomatoes is a significant concern due to the potential health risks they pose to consumers. These toxins not only affect the quality and marketability of tomatoes but have also been related to a range of severe health consequences, including hepatotoxicity, nephrotoxicity, carcinogenicity, and immunosuppression (Berger and Guss, 2015; EFSA, 2021). Therefore, identifying and mitigating mycotoxin contamination in tomatoes is essential for ensuring food safety and protecting public health.

Identifying the fungi responsible for mycotoxin contamination can inform targeted food safety measures and interventions to reduce mycotoxin levels in tomatoes sold at the Mile 3 market. Knowledge of the prevalent mycotoxin-producing fungi can help market vendors and regulatory authorities implement quality control measures to ensure the sale of safer and healthier tomatoes. Understanding the source of mycotoxin contamination in tomatoes can lead to better risk assessment and mitigation strategies, protecting consumers from potential health hazards.

MATERIALS AND METHODS

Study area

The study was conducted in Diobu, mile 3 market (Oroworukwo), Port Harcourt City Local Government Area, Rivers State. Its geographic location and coordinate of the LGA is latitude: 6°27' 59.4384" E.

Sample collection

Sterile gloves, sterile containers, a scalpel, sampling bags, and labels were used for the collection process. 10 different spoilt tomato samples were purchased from separate vendors in Mile 3 market, and each sample was labeled. The samples were then stored in sterile containers or sampling bags to prevent further contamination and transported to the laboratory.

Fungi isolation

Potato Dextrose Agar (PDA) plates, sterile Petri dishes, a laminar flow hood, an incubator, and a microscope were utilized to isolate the fungi. Small portions of the samples were placed on PDA plates under sterile conditions. The plates were incubated at 25-28°C for 5-7 days. Fungal growth was observed, and individual colonies were subcultured onto fresh PDA plates to obtain pure isolates.

Fungi identification

A microscope, lactophenol cotton blue stain, microscope slides, and coverslips were employed for fungi identification. Fungal mounts were prepared using lactophenol cotton blue stain. The morphological characteristics of fungi were examined under the microscope. Fungi were identified based on spore structure, hyphae, and colony morphology, as described by Ekeleme et al. (2021).

Mycotoxin extraction

Organic solvents (e.g., methanol, acetonitrile), a centrifuge, a rotary evaporator, and solid-phase extraction (SPE) columns were required for mycotoxin extraction. The fungal culture or contaminated sample was homogenized. Mycotoxins were extracted using an appropriate organic solvent. The extract was centrifuged to remove debris. The extract was then concentrated using a rotary evaporator. Finally, the extract was cleaned up using SPE columns to remove impurities. Using the method described by Kehinde et al. (2014).

Mycotoxin Detection

Using the High-Performance Liquid Chromatograph (HPLC) method, as illustrated by Rubert et al. (2012) for the detection of mycotoxins, the cleanup and preparation process was done as recommended by Greco et al. (2014). The cleaned extract was injected into the HPLC system. For chromatographic separation, Nexera X2 UHPLC (Shimadzu, Tokyo, Japan) was used. A suitable mobile phase consisting of 2.5 mM ammonium acetate acidified with 0.1 % acetic acid and methanol of 5 – 95 % within 8-9 min, the mobile phase was delivered at a flow rate of 0.4 ml/min and the column was maintained at 40^0C, and the injection volume of 2 L was used for mycotoxin separation. Mycotoxins were detected using a UV detector. For the Liquid Chromatography-Mass Spectrometry (LC-MS) method, the extract was injected into the LC-MS system. Mycotoxins were separated using liquid chromatography and then identified and quantified based on their mass spectra. Five milliliters (5 ml) of the cleaned extract sample was mixed with 20 ml of 25:75 (v/v) water/methanol, which was shaken for 30 min and centrifuged at 8500 g for 15 min, after which 5 ml of the supernatant was transferred to a 15 ml glass tube which was allowed to evaporate under a stream of water bath at 50^0C (Ruber et al., 2012).

Physiochemical analysis

The physicochemical analysis of spoiled tomatoes was conducted to determine the key parameters influencing mycotoxin production. Moisture content was measured using a moisture analyzer, with samples dried at 105°C until a constant weight was achieved. The temperature of the spoiled tomatoes was recorded using a calibrated digital thermometer inserted into the fruit's core. The pH levels were assessed by preparing a tomato puree and measuring it with a pH meter. Water activity was determined using a water activity meter on homogenized samples. Oxygen levels in the storage environment were monitored using an oxygen sensor. Storage time was tracked from the onset of visible spoilage until the point of examination. For each sample, approximately 100 grams of spoiled tomato tissue were collected, homogenized, and subjected to the various analyses. All measurements were performed in triplicate to ensure accuracy and reproducibility. The samples were handled aseptically to prevent cross-contamination. Data were recorded and analyzed to identify correlations between the physicochemical parameters and the observed mycotoxin levels. Statistical analysis was performed to determine significant differences among samples and establish relationships between the measured parameters and mycotoxin concentrations.

Pathogenicity Test

A pathogenicity or decay test was conducted to determine whether the isolated fungi were responsible for the spoilage of tomatoes bought from the Mile 3 market. This was done by using healthy tomato fruits, which were surface sterilized with 75% ethanol/alcohol. Cylindrical tissues were cut out from the tomato fruits using a sterilized 2 mm sized cork borer. Agar discs containing one-week-old fungal pure culture were aseptically placed in the holes, then covered and sealed with petroleum jelly. The procedure was repeated separately across each of the fungal pure isolates. The inoculated samples and the control were placed in sterile polythene bags and incubated at 28-39°c for 14 days. The inoculation of each type of fungus was examined and recorded. The diameter of the rotten portion of the tomato fruit was measured with a meter rule. The fungi were later re-isolated from the inoculated tomato fruits and compared with the initial isolates from the spoiled tomato samples.

RESULTS

At the end of mycological studies on spoiled tomato samples, the results obtained from this present study have indicated that tomatoes sold in Mile 3 market, Port Harcourt, are contaminated by fungal pathogens. The colony count results revealed that the tomato samples had Total Heterotrophic Fungi Counts (THFC) rangng from 1.8 x 10^4CFU/g to 3.2 x 10^4 CFUcfu/g, as shown in Table 1. The fungi isolated and identified from the spoiled tomatoes samples were *Aspergillus niger, Rhizopus stolonifer, Mucor racemosus, Penicillium* sp., *Cladosporium* sp., *Fusarium* sp. and *Aspergillus flavus* (Figure 1).

Table 2 shows the frequency and percentage of occurrence of fungi isolates. The frequency of occurrence indicates how often the fungi were observed in the samples, while the percentage of occurrence shows the proportion of samples in which the fungi were found. Table 3 shows the diameter of fungal isolates in millimeters. This data was collected to determine the

Table 1. Total fungal counts (TFC) from the spoiled tomato samples.

Tomatoes samples	THFC (CFU/g)(cu/g)
Sample A	2.7 x 10^4
Sample B	3.2 x 10^4
Sample C	1.8 x 10^4
Sample D	2.5 x 10^4
Sample E	3.1 x 10^4
Sample F	2.2 x 10^4
Sample G	2.9 x 10^4
Sample F	3.0 x 10^4
Sample G	3.1 x 10^4
Sample H	2.8 x 10^4

Key: **THFC** - Total Heterotrophic Fungi Count, **CFUg** - Colony forming units per gram.

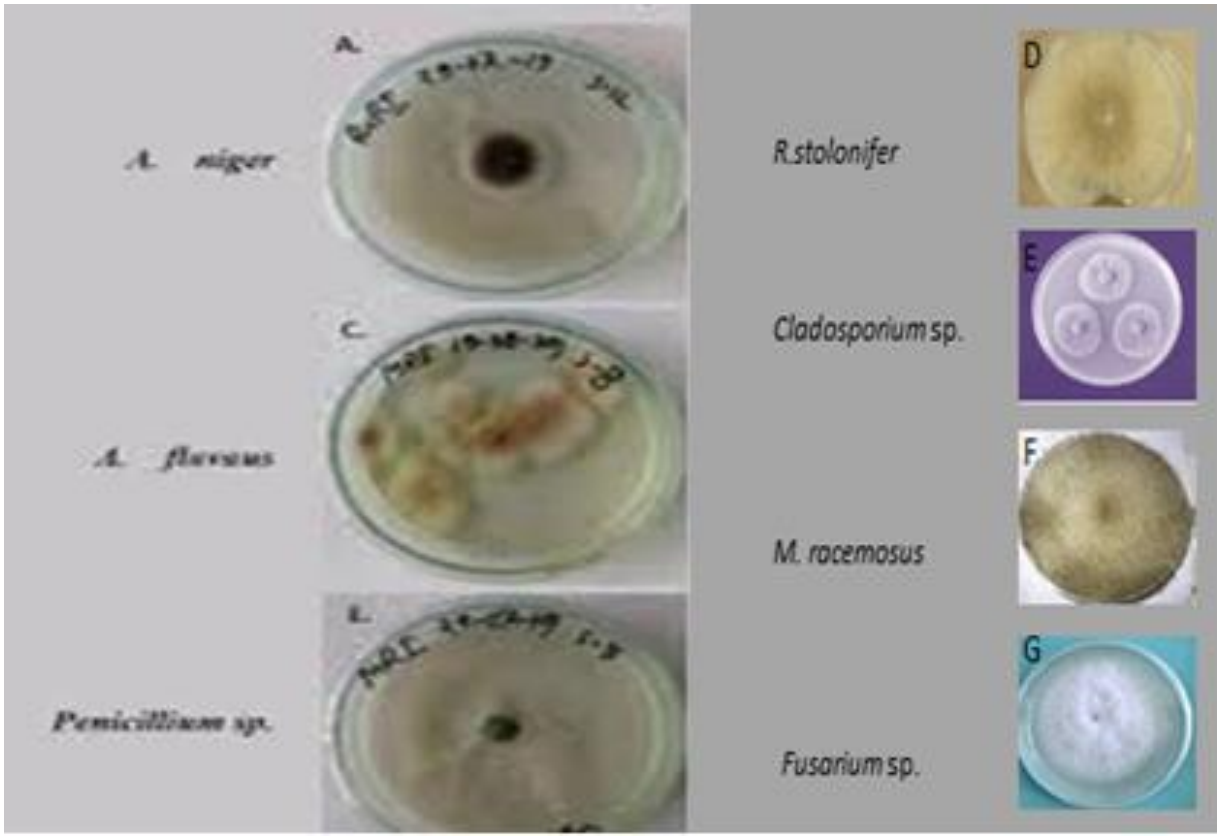

Figure 1. Images of pure cultures of the isolates.

growth rate of the fungi.

Table 4 shows the macroscopic and microscopic identification of fungal isolates. The macroscopic identification of fungi is based on their colony characteristics, such as shape, size, color, and texture. Microscopic identification refers to observing the fungi under a compound microscope to examine the hyphae, conidia, conidiophores, and arrangement of spores.

The quantification of mycotoxins in tomato samples revealed varying concentrations of several mycotoxins, including Aflatoxin B1, Ochratoxin A, Deoxynivalenol (DON), Zearalenone, and Fumonisin B1, as seen in Table 5. These findings highlight the importance of regular monitoring and control measures to ensure the safety of tomato products for consumer health.

All samples in Table 6 had a high moisture content of 91-94 %, typical for ripe and overripe tomatoes and supports fungal growth. The ideal temperature range for

Table 2. Frequency and percentage of occurrence of identified fungi associated with spoilage of tomatoes at Mile 3 market.

Fungal Isolates	Frequency of occurrence	Percentage of occurrence (%)
Aspergillus niger	28	29.2
Rhizopus stolonifer	13	13.5
Penicillium sp.	10	10.4
Cladosporium sp.	2	2.2
Aspergillus flavus	15	15.6
Fusarium sp.	27	28.1
Mucor racemosus	1	1.1
Total	**96**	**100**

Table 3. Measurement in millimeter of fungi isolated from spoiled tomato fruits sold in Mile 3 market after 14 days of incubation.

Fungal isolates	Diameter (mm)
Aspergillus niger	36.0
Rhizopus stolonifer	42.0
Aspergillus flavus	40.0
Mucor racemosus	25.0
Penicillium sp.	32.0
Cladosporium sp.	28.0
Fusarium sp.	30.0

Table 4. Macroscopic and microscopic identification of fungi isolates from spoiled tomato fruits sample.

Fungal isolates	Macroscopic	Microscopic
Aspergillus flavus	Green fungal colony that later turned greenish-yellow or pale green. Green colouration on the reverse side of the plate.	Conidiophore was thick walled, hyaline and slightly roughened, erect, long, aseptate with a resicle at the top with phialides and short conidial chains.
Rhizopus stolonifer	Whitish cottony growth, with greenish to blackish spots. Creamy power growth that later turned black.	Non septate unbranched sporangiophore with round head sporangia.
Aspergillus niger	Colonies widely spread, black with smooth edges and spongy surface densely packed and brown on the reverse side. Wooly velvet, whitish in colour but later turned black.	Septate hyphae. The conidiophore was long, erected from the base to the vesicle, smooth walled, hyaline with globes conidial head.
Cladosporium sp.	Radially furrowed dark-brown velvety growth with white periphery.	Septate hyphae, with septate conidiophores having short chains of conidia.
Mucor racemosus	White fluffy growth, with reverse white colour.	Non septate hyphae, with non septate sporangiophores.
Penicillium sp.	Powdery whitish surface but later turned bluish-green, whitish on reverse side and edges.	Septate hyphae with branched conidiophores bearing phialides. Conidia are arranged in chains on the phialides.
Fusarium sp.	White cuttony lawn-like growth, with reverse yellow colour	Septate hyphae, with presence ofs sickled shaped septate conidia., uniform micro-conidia are formed in long chains

the production of mycotoxin is 23-28°C. The samples' pH values are slightly acidic, falling between 4.6 and 5.0, favouring fungal growth and mycotoxin production. Water activity is very high at 0.97-0.99, further promoting fungal growth and toxin production, and aerobic conditions with 18-22% oxygen support fungal metabolism. Storage times vary from 5 to 10 days, allowing for fungal growth and mycotoxin accumulation.

These parameters correspond to the mycotoxin levels in Table 5. Sample D, showing the highest mycotoxin levels, has the highest moisture content, temperature and longest storage time. Conversely, sample C, with generally lower mycotoxin levels, has lower moisture content, lower temperature and shorter storage time. The

Table 5. Concentration of Mycotoxins in tomato samples.

Samples	Aflatoxin B_1 (µg/kg)	Ochratoxin A (µg/kg)	Deoxynivalenol (mg/kg)	Zearalenone (mg/kg)	Fumonisin B_1 (mg/kg)
Sample A	5.2	2.0	1.2	0.8	1.5
Sample B	4.8	2.5	1.5	1.0	1.8
Sample C	3.5	1.8	1.0	0.7	1.2
Sample D	6.1	2.7	1.7	1.1	2.0
Sample E	5.0	2.3	1.4	0.9	1.6
Sample F	4.2	1.9	1.1	0.6	1.3
Sample G	5.5	2.1	1.3	0.8	1.7
Sample H	4.9	2.4	1.6	1.1	1.9
Sample I	3.8	2.3	1.3	1.3	1.4
Sample J	4.1	2.0	1.5	1.1	1.3

Table 6. Physiochemical parameters of spoilt tomatoes.

Samples	Moisture content (%)	Temperature (^oC)	PH	Water Activity	Oxygen (%)	Storage Time (days)
Sample A	92.2	25	4.8	0.98	20	7
Sample B	93.0	27	4.7	0.99	21	8
Sample C	91.0	23	5.0	0.97	19	15
Sample D	94.0	28	4.6	0.99	22	10
Sample E	92.8	26	4.7	0.98	20	7
Sample F	91.5	24	4.9	0.97	18	16
Sample G	93.2	26	4.7	0.98	21	8
Sample H	93.5	27	4.6	0.99	21	9
Sample I	92.0	25	4.8	0.98	20	7
Sample J	91.8	24	4.9	0.97	19	6

other samples fall between these extremes, with their physicochemical parameters correlating to their respective mycotoxin levels. This data set demonstrates how variations in these parameters can influence mycotoxin production in spoiled tomatoes.

Table 7 summarizes four key variables: Total Fungal Count, Aflatoxin B1 concentration, Moisture Content, and pH. For each variable, the table provides the mean, standard deviation, and coefficient of variation. Table 8 specifically addresses mycotoxin concentrations, covering Aflatoxin B_1, Ochratoxin A, Deoxynivalenol, Zearalenone, and Fumonisin B_1. The table includes the mean, standard deviation, coefficient of variation, minimum and maximum values, and units of measurement for each mycotoxin.

Table 7. Statistical analysis of key variables.

Variable	Mean	Standard Deviation	Coefficient of Variation (%)
Total Fungal Count	2.73×10^4	0.48×10^4	17.58
Aflatoxin B1 (µg/kg)	4.71	0.79	16.77
Moisture Content (%)	92.5	0.94	1.02
PH	4.77	0.13	2.73

Table 8. Statistical analysis of mycotoxin concentrations.

Mycotoxin	Mean	Standard Deviation	Coefficient of Variation (%)	Min	Max	Units
Aflatoxin B_1	4.71	0.79	16.77	3.5	6.1	µg/kg
Ochratoxin A	2.20	0.28	12.73	1.8	2.7	µg/kg
Deoxynivalenol	1.36	0.22	16.18	1.0	1.7	mg/kg
Zearalenone	0.94	0.22	23.40	0.6	1.3	mg/kg
Fumonisin B_1	1.57	0.27	17.20	1.2	2.0	mg/kg

DISCUSSION

The study revealed significant fungal contamination in spoiled tomato samples sold at the Mile 3 market. Total fungal counts ranged from $1.8x10^4$ to $3.2x10^4$ CFU/g, indicating substantial fungal growth. These levels are consistent with previous studies on fungal contamination in spoiled fruits and vegetables (Hegazy, 2017).

The most frequently isolated fungi were *Aspergillus niger* (29.2%), *Fusarium* sp. (28.1%), and *Aspergillus flavus* (15.6%). This distribution aligns with earlier findings by Abhinaba (2009), who reported similar predominant fungi in spoiled tomatoes from open markets. Several physicochemical parameters contribute to fungal growth and subsequent mycotoxin production in spoiled tomatoes. The samples had moisture contents ranging from 91.0 to 94.0 %, providing an ideal environment for fungal growth. This is consistent with findings by Onuorah and Orji (2015), who noted that moisture contents above 90 % significantly promote fungal proliferation in fruits. The recorded temperatures (23-28°C) fall within the optimal range for most of the fungal species identified. This temperature range enhances mycotoxin production, particularly for *Aspergillus* and *Fusarium* species (Suleiman et al., 2023).

The slightly acidic pH (4.6-5.0) of the samples favors the growth of many fungal species, especially *Aspergillus* and *Penicillium* (Wilson et al., 2002). The water activity values of 0.97-0.99 are highly conducive to fungal growth and mycotoxin production. These levels exceed the minimum water activity required for the growth of most mycotoxigenic fungi (Lopez-Diaz and Flannigan, 1997). The aerobic conditions (18-22% oxygen) support fungal metabolism and secondary metabolite production, including mycotoxins (Suleiman et al., 2023; Logrieco et al., 2019). The storage times ranging from 6 to 16 days allow ample opportunity for fungal growth and mycotoxin accumulation. Longer storage times correlate with higher mycotoxin levels (Hegazy, 2017; Yin et al., 2018).

The study detected various mycotoxins in the spoiled tomato samples. Aflatoxin B_1 levels ranged from 3.5 to 6.1 µg/kg, with Sample D showing the highest concentration. These levels are concerning, as aflatoxins are potent carcinogens (IARC, 2012). Ochratoxin A concentrations varied from 1.8 to 2.7 µg/kg. Ochratoxin A is known for its nephrotoxic and potentially carcinogenic effects (EFSA, 2020). Deoxynivalenol (DON) levels ranged from 1.0 to 1.7 mg/kg. DON can cause gastrointestinal distress and immune system dysfunction (Pestka, 2007). Zearalenone concentrations varied from 0.6 to 1.3 mg/kg. Zearalenone is an estrogenic compound that can disrupt endocrine function (Malekinejad et al., 2005). Fumonisin B_1 levels ranged from 1.2 to 2.0 mg/kg. Fumonisins have been linked to esophageal cancer and neural tube defects (Marasas et al., 2004; Trucksess and Scott, 2018).

The presence of these mycotoxins in spoiled tomatoes poses significant health risks to consumers. Chronic exposure to even low levels of mycotoxins can lead to various health issues, including carcinogenicity, immunosuppression, reproductive and developmental issues, gastrointestinal problems, and nephrotoxicity. Aflatoxins and potentially ochratoxin A are known carcinogens (IARC, 2012). Several mycotoxins, particularly aflatoxins and DON, can compromise the immune system (Bondy and Pestka, 2000). Zearalenone can interfere with reproductive health, while fumonisins have been associated with birth defects (Cortinovis et al., 2013). DON and other trichothecenes can cause vomiting, diarrhea, and other gastrointestinal disturbances (Pestka, 2007). The most common mycotoxins that raise concerns for human or animal health include aflatoxins, ochratoxin A and Fusarium toxins such as deoxynivalenol (EFSA 2024; Veprikova et al., 2015). The statistical analysis of mycotoxin concentrations in the tomato samples reveals several important implications. Aflatoxin B_1 emerges as the most prevalent mycotoxin, exhibiting the highest mean concentration and absolute variability. This suggests that Aflatoxin B_1 contamination is a significant concern in these samples, potentially posing the highest risk among the analyzed mycotoxins. The high variability also indicates that contamination levels can differ substantially between samples, highlighting the need for consistent monitoring and quality control measures.

Zearalenone, while present in lower absolute concentrations, shows the highest relative variability. This implies that its presence is less predictable across samples, which could complicate risk assessment and management strategies. The inconsistent levels of Zearalenone may be due to varying environmental conditions or fungal growth patterns affecting its production (Marasas et al., 2004). Ochratoxin A demonstrates the most consistent levels across samples, as indicated by its lower coefficient of variation. This relative stability could suggest more uniform contamination patterns or environmental conditions favoring its production. However, its presence, even at more consistent levels, still warrants attention in food safety protocols.

The presence of multiple mycotoxins in varying concentrations underscores the complexity of fungal contamination in tomatoes. It highlights the need for comprehensive testing protocols capable of detecting and quantifying a range of mycotoxins, rather than focusing on a single contaminant. The diversity of mycotoxins suggests that multiple fungal species are involved in the spoilage process, each potentially responding differently to environmental conditions and storage practices (Marasas et al., 2004). These findings emphasize the importance of implementing robust quality control measures throughout the tomato supply chain. They also underscore the need for further research into the factors

influencing mycotoxin production in tomatoes, such as storage conditions, handling practices, and environmental factors. Understanding these influences could lead to more effective strategies for preventing or minimizing mycotoxin contamination, enhancing food safety and reducing potential health risks associated with consuming contaminated tomatoes.

This study highlights the significant fungal contamination and mycotoxin production in spoiled tomatoes sold in the Mile 3 market. The physicochemical parameters of the spoiled tomatoes provide an ideal environment for fungal growth and mycotoxin production. The presence of multiple mycotoxins at concerning levels emphasizes the potential health risks associated with the consumption of spoiled tomatoes. These findings underscore the need for improved storage conditions, regular quality control measures, and public awareness about the dangers of consuming spoiled produce.

CONCLUSION

This study reveals significant fungal contamination and mycotoxin production in spoiled tomatoes sold at the Mile 3 market. The findings highlight a serious public health concern, with total fungal counts ranging from $1.8x10^4$ to $3.2x10^4$ CFU/g and the presence of various mycotoxins at concerning levels. The predominant fungi identified were *Aspergillus niger*, *Fusarium* sp. and *Aspergillus flavus*, which are known to produce harmful mycotoxins.

The physicochemical parameters of the spoiled tomatoes, such as high moisture content, favorable temperature, slightly acidic pH, and high water activity, provide an ideal environment for fungal growth and mycotoxin production. These conditions, combined with extended storage times, contribute to the accumulation of dangerous levels of mycotoxins, including Aflatoxin B_1, Ochratoxin A, Deoxynivalenol, Zearalenone and Fumonisin B_1. The health implications of consuming these contaminated tomatoes are severe and wide-ranging.

These findings emphasize the urgent need for improved storage conditions, regular quality control measures, and increased public awareness about the dangers of consuming spoiled produce. Future research should focus on developing effective strategies to minimize fungal contamination and mycotoxin production in tomatoes and other susceptible fruits and vegetables. Additionally, stricter regulations and monitoring of products sold in open markets are required to safeguard public health.

RECOMMENDATIONS

Based on the findings presented in the study, here are some recommendations:

1. Implement better storage practices for tomatoes, including temperature-controlled environments and proper ventilation to reduce moisture accumulation. This can help slow down fungal growth and mycotoxin production.

2. Establish regular inspections and testing protocols for tomatoes sold in the Mile 3 market and similar venues. This should include visual inspections for signs of spoilage and periodic laboratory testing for fungal contamination and mycotoxin levels.

3. Develop and enforce regulations on the maximum permitted levels of fungal contamination and mycotoxins in market-sold tomatoes and other vegetables.

4. Conduct awareness campaigns to inform vendors and consumers about the risks associated with spoiled tomatoes and how to detect symptoms of fungal contamination.

5. Encourage faster turnover of produce to minimize storage time and reduce the opportunity for fungal growth and mycotoxin accumulation.

6. Train farmers and distributors in proper harvesting, handling, and transportation techniques to minimize damage and contamination of tomatoes.

7. Support further studies on effective methods to prevent fungal growth and mycotoxin production in tomatoes and other susceptible produce.

REFERENCES

Abhinaba G, **2009**. Identification of microorganisms responsible for spoilage of tomato (*Lycopersicum esculentum*) Fruit. J Phytol, 1(6): 414-416.

Akinmusire OO, **2011**. Fungi species associated with the spoilage of some edible fruits in Maiduguri, North Eastern Nigeria. Adv Environ Biol, 5: 157-161.

Alshannaq AF, **Yu** JH, **2017**. Occurrence, toxicity, and analysis of major mycotoxins in food. Int J Environ Res Public Health, 14(6): 632.

Ashiq S, Hussain M, Ahmad B, **2017**. Natural occurrence of mycotoxins in medicinal plants: a review. Fungal Genet Biol, 66: 1–10.

Awuchi CG, Ondari EN, Eseoghene IJ, Twinomuhwezi H, Amagwula IO, Morya S, **2021**. Fungal growth and mycotoxins production: Types, toxicities, control strategies, and detoxication. In Fungal reproduction and growth. London, UK: IntechOpen. https://doi.org/10.5772/intechopen.100207, 2021.

Baker S, **2006**. *Aspergillus niger* genomics: Past, present and into the future. Med Mycol, 44: S17-21.

Battilani P, Toscano P, Van der Fels-Klerx HJ, **2016**. The role of climate in the occurrence and distribution of mycotoxins in Europe. J Sci Food Agric, 96(6): 2061-2070.

Bennett JW, **Klich** M, **2018**. Mycotoxins. Clin Microbiol Rev, 16(3): 497-516.

Berger KJ, **Guss** DA, **2015**. Mycotoxins revisited: Part I. J Emerg Med, 28(1): 53–62.

Bondy GS, **Pestka** JJ, **2000**. Immunomodulation by fungal toxins. J Toxicol Environ Health, Part B, 3(2): 109-143. DOI:

10.1080/109374000281113.
Cortinovis C, Pizzo F, Spicer LJ, Caloni F, **2013**. *Fusarium* mycotoxins: Effects on reproductive function in domestic animals - A review. Theriogenology, 80(6): 557-564. DOI: 10.1016/j.theriogenology.2013.06.018
Do KH, An T J, Oh SK, Moon Y, **2015**. Nation-based occurrence and endogenous biological reduction of mycotoxins in medicinal herbs and spices. Toxins, 7(10): 4111–4130.
European Food Safety Authority (**EFSA**), 2020. Risk assessment of ochratoxin A in food. EFSA J, 18(5): 6113.
Ekeleme IK, Makat MD, Owuna JE, Obiekezie SO, Muhammed FH, **2021**. Cetric Acid Production by *Aspergillus niger* and *Aspergillus awamori* Isolated from Soil in Keffi, Nigeria. Innov Appl Sci, 6(3): 44-48.
European Food Safety Authority (**EFSA**), **2021**. Scientific Opinion on the risks for animal and public health related to the presence of Alternaria toxins in feed and food. EFSA Journal, 9(10): 2407-2411.
Fog NK, **2021**. Mycotoxin production by indoor molds. Fungal Genet Biol, 39 (2): 103–117.
Greco MV, Franchi M, Hxed A, Luisa SL, Rico-Golba A, Pando G, Pose GN, **2014**. Mycotoxins and Mycotoxigenic Fungi in Poultry Feed for Food-Producing Animals. Sci World J, 9-17.
Hegazy EM, **2017**. Mycotoxin and fungal contamination of fresh and dried tomato. Ann Res Rev Biol, 17(5): 1-9.
International Agency for Research on Cancer (**IARC**), **2012**. Chemical Agents and Related Occupations. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Volume 100F.
European Food Safety Authority (**EFSA**), **2024**. Mycotoxins. https://www.efsa.europa.eu/en/topics/topic/mycotoxins.
Eun-Sun H, **Phyllis** EB, **2002**. Tomatoes or Lycopene in Cancer Risk Reduction. Integr Cancer Ther, 1(2): 121-132.
Jeswal P, **Kumar** D, **2015**. Mycobiota and Natural Incidence of Aflatoxins, Ochratoxin A, and Citrinin in Indian Spices Confirmed by LC-MS/MS. Int J Microbiol, 1–8.
Kehinde M, Oluwafemi F, Itoandon E, Orji F, Ajayi O, **2014**. Fungal Profile and Aflatoxin Contamination in Poultry Feeds Sold in Abeokuta, Ogun State, Nigeria. Nig Food J, 32(1): 73-79.
Lee HB, Patriarca A, Magan N, **2015**. Alternaria in food: Ecophysiology, mycotoxin production and toxicology. Mycobiology, 43(2): 93-106.
Logrieco A, Moretti A, Ritieni A, **2019**. Occurrence and significance of mycotoxins in fruits. In Mycotoxins in Fruits and Vegetables (pp. 1-20). Elsevier.
Lopez-Diaz TM, **Flannigan** B, **1997**. Production of patulin and cytochalasin E by 4 strains of *Aspergillus clavatus* during malting of barley and wheat. Int J Food Microbiol, 35: 129-136.
Malekinejad H, Maas-Bakker RF, Fink-Gremmels J, **2005**. Bioactivation of zearalenone by porcine hepatic biotransformation. Vet Res, 36(5-6): 799-810. DOI: 10.1051/vetres:2005034.
Marasas WF, Riley RT, Hendricks KA, Stevens VL, Sadler TW, Gelineau-van Waes J, Merrill Jr AH, **2004**. Fumonisins disrupt sphingolipid metabolism, folate transport, and neural tube development in embryo culture and in vivo: a potential risk factor for human neural tube defects among populations consuming fumonisin-contaminated maize. J Nutr, 134(4): 711-716.
Marin S, Ramos AJ, Cano-Sancho G, **2019**. Mycotoxins: Occurrence, toxicology, and exposure assessment. Food Chem Toxicol, 60: 218-237.
Mateo R, Medina A, Mateo EM, Mateo F, Jiménez M, **2007**. An overview of ochratoxin A in beer and wine. Int J Food Microbiol, 119(1–2): 79–83.
Moss MO, **2008**. Fungi, quality and safety issues in fresh fruits and vegetables. J Appl Microbiol, 104(5): 1239–1243. DOI: 10.1111/j.1365-2672.2007.03705x.
Onuorah S, **Orji** MU, **2015**. Fungi Associated with the Spoilage of Post-Harvest Tomato Fruits Sold in Major Market in Awka, Nigeria. Univers J Microbiol Res, 3(2): 11-16.
Pestka J, **2007**. Deoxynivalenol: toxicity, mechanisms and animal health risks. Anim Feed Sci Technol, 137(3-4): 283-298. DOI: 10.1016/j.anifeedsci.2007.06.006.
Piemontese L, Brera C, Miraglia M, Scarcella C, **2017**. Impact of mycotoxins on human health: a comprehensive review of the literature. Toxins, 9(12): 1-36.
Richard JL, **2007**. Some major mycotoxins and their mycotoxicosis: an overview. Int J Food Microbiol, 119(1–2): 3–10.
Robbins CA, Swenson LJ, Nealley ML, Gots RE, Kelman BJ, 2000. Health effects of mycotoxins in indoor air: a critical review. App Occup Environ Hyg, 15(10): 773–784. DOI: 10.1080/10473220050129419.
Rubert J, Soler C, Manes J, **2012**. Application of HPLC-MS/MS method for mycotoxins analysis in commercial baby foods. Food Chem, 133(1): 176-183.
Suleiman HA, Owuna JE, Makut MD, Yahaya I, Ekeleme IK, Abdullahi ZT, **2023**. Assessment of mycotoxin producing fungi isolated from dried tomato chips sold in Keffi, Nigeria. World J Adv Eng Technol Sci, 4(2): 235-241.
Trucksess MW, **Scott** PM, **2018**. Mycotoxins in botanicals and dried fruits: A review. Food Addit Contam, 25(2): 181–192.
Turner NW, Subrahmanyam S, Piletsky SA, **2019**. Analytical methods for determination of mycotoxins: a review. Anal Chim Acta, 632(2): 168–180.
Veprikova Z, Zachariasova M, Dzuman Z, Zachariasova A, Fenclova M, Slavikova P, Vaclavikova MK, Hengst D, Hajslova J, **2015**. Mycotoxins in Plant-Based Dietary Supplements: Hidden Health Risk for Consumers. J Agric Food Chem, 63(29): 6633–6643.
Wilson DM, Mubatanhema W, Jurjevic, Z, **2002**. Biology and ecology of mycotoxigenic *Aspergillus* species as related to economic and health concerns. Adv Exp Med Biol, 504: 3-17. DOI: 10.1007/978-1-4615-0629-42.
Yin YN, Yan LY, Jiang JH, Ma ZH, **2018**. Biological control of aflatoxin contamination of crops. J Zhejiang Univ Sci Biol, 9(10): 787–792.

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