INVESTIGATION OF THE MYCOFLORA OF DETERIORATING TOMATOES (Solanum lycopersicum Mill.) SOLD IN LOKOJA MARKETS, KOGI STATE, NIGERIA

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ABSTRACT
The deterioration of tomatoes poses significant risks to public health as it can lead to the growth of fungi that produce harmful mycotoxins. This study focused on investigating the mycoflora associated with deteriorating tomatoes sold in selected Lokoja markets, Kogi State, Nigeria. A total of eighteen (18) samples, each containing three deteriorating tomatoes were randomly collected from six vendors in three different markets. Standard microbiological methods were employed to analyze the samples. The results revealed the presence of Aspergillus niger, Microsphaeropsis arundinis, Penicillium sp. and Rhizopus arrhizus as the predominant fungi in the deteriorating tomato samples. Samples from Adankolo market yielded the highest mean fungal load (4.63 × 10^6 CFU/g) and was significantly different (p<0.05) from those of Old market (2.78 × 10^6 CFU/g) and Lokongoma market (2.67 × 10^6 CFU/g). Notably, A. niger had the highest occurrence (48.9 %) while R. arrhizus had the lowest occurrence (2.2 %). The presence of these fungal contaminants highlights the lack of fungi-free deteriorating tomatoes in Lokoja markets. The high occurrence of Aspergillus niger and the overall fungal load levels highlight the potential health risks associated with consuming these tomatoes. Consequently, the consumption of deteriorating tomatoes should be discouraged due to the potential health risks associated with mycotoxin production by these fungi. Strategies to mitigate fungal growth and spoilage of tomato in the markets as well as further research on mycotoxin production and health implications are fundamental for ensuring food safety and protecting public health.

Keywords: Deteriorating tomatoes, fungal load, Lokoja markets, mycotoxins.

INTRODUCTION
Tomato (Solanum lycopersicum Mill.) is one of the most widely consumed and economically important vegetable worldwide. It is a rich source of vitamins, minerals and antioxidants, making it a valuable component of a healthy diet. They are packed with essential nutrients such as vitamins C, A, K and B6 along with folate and minerals such as potassium, calcium and magnesium (Aslam et al., 2020). Tomatoes are particularly popular for their rich antioxidant content that include lycopene, beta-carotene and vitamin C which contribute to their potential health benefit (Tommonaro et al., 2012; Ali et al., 2020). These antioxidants combat oxidative stress, reduce the risk of certain cancers and promote overall well-being (Freeman and Reimers, 2010; Ali et al., 2020; Collins et al., 2022). In addition, tomatoes provide dietary fiber that aid digestion and promote healthy gut (Collins et al., 2022). With their diverse array of nutrients, tomatoes are a nourishing and appealing option for enhancing meals and promoting optimal health. However, tomatoes are highly perishable and their post-harvest handling and storage conditions greatly influence their quality and safety (Zewdie et al., 2021).

One of the major challenge in the post-harvest management of tomatoes is the occurrence of fungal contamination which can lead to deterioration and spoilage (Yahaya and Ay, 2019). Fungal contaminants not only reduce the shelf life of tomatoes but also pose significant health risks to consumers (Salehi et al., 2019; Okolo et al., 2022). Fungi are natural inhabitants of the environment whose presence on crops is unavoidable. When tomatoes are exposed to conditions such as high humidity, poor ventilation and improper storage, fungal growth and development are accelerated leading to rapid deterioration (Yahaya and Ay, 2019). Some genera of fungi such as Aspergillus, Penicillium and Fusarium species are known to produce mycotoxins, a secondary metabolites that can be harmful to human health when ingested in significant quantities (Kostoc et al., 2019). Consumption of mycotoxins in deteriorating tomatoes may lead to a range of health problems such as gastrointestinal disorders, allergic reactions and in severe cases, organ damage and risk of developing cancer with long-term exposure (Salehi et al., 2019; Darra et al., 2023).

Despite scientific evidence linking fungal contaminants to deteriorating tomatoes, their consumption remains prevalent in many developing countries including Nigeria where they are essential ingredient in various traditional dishes and are consumed by a large portion of the population. This preference for deteriorating tomatoes over fresh tomatoes despite the inherent risks and negative consequences can be attributed chiefly to affordability (Abera et al., 2020; Meloney et al., 2022). The consumption of deteriorating tomatoes contaminated with these fungi can thus have severe consequences on public health (Salehi et al., 2019). This raises concern about the level of awareness among consumers and the need for strategies to mitigate fungal contamination and promote the consumption of fresh tomatoes. Determination of fungal flora of deteriorating tomatoes is important to take effective control measures against their growth, impact, infections and ultimately safeguard consumers’ health. Therefore, this study was carried out to investigate the mycoflora of deteriorating tomatoes sold in the Lokoja markets, Kogi State, Nigeria.

Study Area
The study was carried out in Lokoja. Samples were obtained from major markets (Old, Lokongoma and Adankolo markets) in Lokoja Kogi State, Nigeria (Figure 1).
Lokoja is a confluence city where River Niger and River Benue meet in the north central part of Nigeria. It is situated geographically at latitude 7.8023°N and longitude 6.7333°E, 55m above the sea level. The rainfall in the region was reported as 1016–1524 mm with mean temperature of 27.7°C every year (Alabi, 2009; Adetunji, 2018). The markets were selected because of their significance in the local tomato trade and their widespread distribution of deteriorating tomatoes. The markets are also known for their diverse vendor profile and varied storage and handling practices which makes them appropriate study area to assess fungal contamination.

Sample Collection
A systematic random sampling approach was used to collect the tomato samples from six vendors operating in the three markets. A total of 18 samples, each consisting of three deteriorating tomatoes were collected. The samples were obtained by identifying visibly deteriorated tomatoes with signs of rot, mold growth or discoloration. Special care was taken to ensure representation from various market sections to capture potential variations in fungal contamination. The collected samples were labeled and transported in ice-packed containers to the Biological Sciences laboratory of Federal University Lokoja for microbiological analysis.

Isolation and Identification of Fungal Contaminants
The collected tomato samples were aseptically processed to obtain the fungal isolates. The tomato samples were homogenized separately using mortar and pestle. One gramme (1g) of each sample was transferred into sterile test tube that contain 9 mL of sterile distilled water. Serial dilutions were carried out and 0.1 mL of appropriate dilutions (10⁻³ and 10⁻⁴) were spread-plated onto sterile Potato Dextrose Agar (PDA) plates using a sterile spreader. The inoculated plates were incubated at 28°C for 5 days to allow fungal growth. After incubation, fungal colonies were then enumerated using colony counting machine to determine fungal loads in colony forming units per gramme of the deteriorating tomatoes. The representative colonies with distinct morphological characteristics were subcultured onto fresh PDA plates for pure culture isolation. The pure fungal cultures were subjected to microscopic examination using lactophenol cotton blue stain to observe morphological features such as spore type, conidiophore arrangement and hyphal characteristics. The observed cultural and microscopic morphological characteristics of each stained fungal isolates were compared with standard reference keys and atlas for their probable identities as reported earlier (Alexopoulos and Mims, 1979; Fawole and Oso, 1988; Jay, 1998; De Hoog et al., 2000; Kidd, et al., 2016).

Data Analysis
The data collected (fungal load and fungal species distribution) were analyzed and presented with descriptive statistics such as mean and standard deviation. The frequencies were calculated to summarize the data. The means of the samples were compared for the different locations using analysis of variance (ANOVA) to obtain the significant difference among variables at p=0.05.

RESULT AND DISCUSSION
In developing countries including Nigeria, there is an observed trend of increasing preference for deteriorating tomatoes over fresh tomatoes, despite the inherent risks and negative consequences. Hence, this study evaluated the mycoflora of deteriorating tomatoes sold in three major markets within Lokoja metropolis, Kogi State, Nigeria. Table 1 presents the fungal load of deteriorating tomato samples obtained from three markets (Adankolo market, Lokongoma market and Old market) within Lokoja metropolis. From the results, Adankolo market had the highest mean fungal load. On the other hand, Lokongoma market displayed a higher standard deviation of 3.06 × 10⁵ CFU/g, implying relatively consistent fungal load levels within the samples obtained from this market. The variability within the samples from Adankolo market and Lokongoma market was more pronounced, indicating a wider range of fungal contamination levels compared to Old market. The fungal loads obtained in this study were much higher than those reported from Awka (Onuorah and Orji, 2015),...
Dutsin-Ma Metropolis (Kutawa et al. 2020) and Wukari (Ogodo et al., 2020). The difference may be as a result of difference in the level of freshness of the tomatoes, storage conditions, hygiene practice and market temperature. This is in line with previous submissions that attributed the difference in levels of contamination of commercial fruits and vegetables to the difference in freshness, sources of the products, handling practices and sanitary conditions of the market (Buck et al., 2003; Akinlade et al., 2011; Akinmusire, 2011; Lemma et al., 2014; Ugwu et al., 2014; Wogu and Ofuase, 2014; Sanyaolu, 2016; Onuorah and Orji, 2015; Dimphna, 2016; Mailafia et al., 2017; Obeng et al., 2018; Sani et al., 2018; Kutawa et al., 2020; Ogodo et al., 2020). The high fungal load obtained from the deteriorating tomato samples are of concern because they indicate higher risk of fungal contamination which can affect the quality and safety of the tomatoes.

Table 1: Fungal load of the deteriorating tomato samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Adankolo Market</th>
<th>Lokongoma market</th>
<th>Old market</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$5.1 \times 10^6$</td>
<td>$8.0 \times 10^5$</td>
<td>$3.3 \times 10^6$</td>
</tr>
<tr>
<td>2</td>
<td>$5.1 \times 10^5$</td>
<td>$2.1 \times 10^6$</td>
<td>$2.2 \times 10^6$</td>
</tr>
<tr>
<td>3</td>
<td>$5.6 \times 10^5$</td>
<td>$1.8 \times 10^6$</td>
<td>$3.8 \times 10^5$</td>
</tr>
<tr>
<td>4</td>
<td>$4.9 \times 10^5$</td>
<td>$3.0 \times 10^5$</td>
<td>$3.2 \times 10^6$</td>
</tr>
<tr>
<td>5</td>
<td>$2.8 \times 10^5$</td>
<td>$1.2 \times 10^6$</td>
<td>$1.0 \times 10^6$</td>
</tr>
<tr>
<td>6</td>
<td>$2.7 \times 10^5$</td>
<td>$9.0 \times 10^5$</td>
<td>$3.4 \times 10^6$</td>
</tr>
<tr>
<td>Mean</td>
<td>$4.63 \times 10^5$</td>
<td>$2.87 \times 10^5$</td>
<td>$2.78 \times 10^6$</td>
</tr>
<tr>
<td>SD</td>
<td>$1.10 \times 10^5$</td>
<td>$3.06 \times 10^5$</td>
<td>$1.02 \times 10^6$</td>
</tr>
</tbody>
</table>

*Mean values with similar alphabet are not significantly different (p<0.05)

The colonial morphology and microscopic characteristics of the fungal isolates obtained from Adankolo, Lokongoma and Old markets within Lokoja metropolis, Kogi State are shown in Table 2. The appearance and structure of the colonies as well as the microscopic features of the fungal species identified are described. The findings from the distinct colonial morphology and microscopic characteristics helped in the identification of the four predominant fungal isolates: Aspergillus niger, Microsphaeropsis arundinis, Rhizopus arrhizus, and Penicillium sp. It is worth noting that the fungal isolates identified in this study are consistent with common fungal species found in various food commodities including fruits and vegetables in Nigeria and other African countries (Mailafia et al., 2017; Yahaya and Ay, 2019; Kutawa et al., 2020; Zewdie et al., 2021; Yusuf et al., 2022; Bano et al., 2023).

Table 2: Colonial morphology and microscopic characteristics of the fungal isolates from the deteriorating tomato samples

<table>
<thead>
<tr>
<th>Colonial Morphology</th>
<th>Microscopic Characteristic</th>
<th>Probable fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black powdery colonies</td>
<td>Simple upright conidiophore terminating in a globose conidia head</td>
<td>Aspergillus niger</td>
</tr>
<tr>
<td>Dense greenish-gray colonies</td>
<td>Pigmented septate hyphae with brown conidia</td>
<td>Microsphaeropsis arundinis</td>
</tr>
<tr>
<td>Fast growing white cottony colonies that turn brownish grey</td>
<td>Smooth-walled, non-septate, simple sporangiospores</td>
<td>Rhizopus arrhizus</td>
</tr>
<tr>
<td>Green colonies</td>
<td>Smooth hyaline conidiophore with phialides that have conidia attached to them</td>
<td>Penicillium sp.</td>
</tr>
</tbody>
</table>

Figure 2 presents the percentage of occurrence of each fungal species isolated from the deteriorating tomato samples from Adankolo, Lokongoma and Old market in Lokoja, Kogi State. The finding indicate that A. niger had the highest occurrence percentage, accounting for 48.9 % of the fungal isolates. This suggests that A. niger is the most prevalent fungal species among the samples examined. The high occurrence of A. niger is consistent with its wide distribution and ability to colonize a variety of substrates including fruits and vegetables (Mailafia et al., 2017). Its opportunistic nature, ability to produce mycotoxins and resistance to various environmental conditions contribute to its prevalence in food spoilage and post-harvest produce (Simonovivcova, et al., 2021). M. arundinis had the next highest occurrence percentage, accounting for 28.9% of the fungal isolates. This suggests that M. arundinis is also a significant contributor to fungal contamination in the deteriorating tomato samples. Although less studied compared to some other fungal species, M. arundinis has been associated with soft tissue plant diseases and may play a role in post-harvest deterioration (Pendle et al., 2004). Further research is necessary to understand the specific impact of M. arundinis on tomato quality and safety. Penicillium sp. accounted for 20.0 % of the fungal isolates. Penicillium is a well-known post-harvest pathogen of citrus fruits that cause green mold decay. However, it can also be found in other fruits such as tomatoes where it contribute to spoilage (Deepa and Sreenivas, 2019; Selah and Al-Thani, 2019). The presence of Penicillium species highlights the need for proper storage and handling practices to prevent fungal contamination during transportation and storage. R. arrhizus had the lowest occurrence percentage, accounting for only 2.22 % of the fungal isolates. While less prevalent in this study, R. arrhizus is a common post-harvest pathogen known to cause soft rot in various cereals, fruits and vegetables (Omolaran et al., 2016). Its low occurrence suggests that other fungal species may be more dominant in the deteriorating tomato samples examined in this study. The fungi isolated from the tomato fruits are in consonance with the report of Kutawa et al. (2020), who reported the presence of Aspergillus niger and Rhizopus stolonifer in spoil tomato fruits sold within Dutsin-Ma Metropolis, Katsina State, Nigeria. Similar genera of fungi were also reported from Lagos State (Sanyaolu, 2016) and Nsukka (Amuji et al., 2013). However, our findings differ slightly from the work of Onuorah and Orji (2015) who in addition to our isolates reported the presence of Saccharomyces cerevisiae, Alternaria alternata and Geotrichum candidum from post-harvest tomato fruits sold in major markets in Awka, Nigeria. In the same vein, Dimphna (2016) reported the isolation of Penicillium and Cladosporium species from postharvest decaying tomatoes sold in Abakaliki market, Nigeria. Penicillium and Mucor spp were reported from different tomato markets in Abuja (Mailafia et al., 2017; Mukhtar et al., 2019). Contrary isolates including Rhodotorula species, Mucor species and Saccharomyces cerevisiae were reported from Wukari, Nigeria (Ogodo et al., 2020). The common handling practices, transportation and prevailing storage environment of the farmers and marketers could account for varying type of fungal isolates from commercial tomatoes (Okojie and Isah, 2014).
Figure 2: Occurrence of each fungal isolate from the deteriorating tomato samples

Table 3 displays the results of the fungal occurrence in each market. According to the data in Table 3, the highest percentage of fungal isolates was obtained from Old market with a percentage of 46.7%. Adankolo market had a percentage of occurrence of 31.1%, while Lokongoma market had the lowest percentage of occurrence of 22.2%. These results indicate that the order of fungal contaminations in the markets was as follows: Old Market > Adankolo Market > Lokongoma Market. These findings support the earlier observations of fungal loads of deteriorating tomatoes from each market. The percentage of occurrence of the identified fungal species emphasizes the significance of implementing effective post-harvest handling, storage, and hygiene practices to minimize fungal contamination and subsequent spoilage. It is essential to address the specific factors contributing to fungal contamination in each market through further studies. This would enable the development of targeted interventions and the implementation of preventive measures to reduce fungal contamination effectively.

Table 3: Percentage of occurrence of the fungi isolated from the markets

<table>
<thead>
<tr>
<th>Samples</th>
<th>Adankolo Market</th>
<th>Lokongoma market</th>
<th>Old market</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. niger</td>
<td>7</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>M. arundinis</td>
<td>4</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>P. digitatum</td>
<td>3</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>R. arrhizus</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>14 (31.1 %)</td>
<td>10 (22.2 %)</td>
<td>21 (46.7 %)</td>
</tr>
</tbody>
</table>

Conclusion

This study investigated the mycoflora associated with deteriorating tomatoes sold in the Lokoja markets of Kogi State, Nigeria. The results revealed the presence of Aspergillus niger, Microsphaeropsis arundinis, Penicillium sp. and Rhizopus arhizus as the predominant fungi in the deteriorating tomato samples. Adankolo market had the highest mean fungal load, indicating a higher risk of fungal contamination compared to Lokongoma market and Old market. Aspergillus niger was the most prevalent fungus identified. The presence of these fungal contaminants highlights the lack of fungicide-free deteriorating tomatoes in Lokoja markets and the potential health risks associated with consuming these tomatoes. The high occurrence of Aspergillus niger and the overall fungal load levels underscore the importance of implementing strategies to mitigate fungal growth and spoilage of tomatoes sold in markets. Further research on mycotoxin production and its health implications is crucial for ensuring food safety and protecting public health. It is necessary to discourage the consumption of deteriorating tomatoes due to the potential risks associated with mycotoxin production by these fungi. Measures should be taken to raise awareness amongst consumers.

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