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Identification, Pathogenicity, and Prevalence of Fungal Species Responsible for Post-Harvest Spoilage of Sweet Oranges (*Citrus sinensis*) in Dekina, Kogi State, Nigeria

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Abstract

Oranges (*Citrus sinensis*) are a vital source of nutrients and hold significant economic value in the global fruit industry. However, postharvest spoilage remains a major challenge, particularly in regions with limited infrastructure for storage and transportation. This study aims to identify the fungal pathogens responsible for the spoilage of sweet oranges in Dekina Local Government Area, Kogi State, Nigeria. A total of 162 sweet orange fruits were collected from three major markets in the area, with 54 fruits exhibiting visible signs of spoilage selected for further analysis. Fungal isolation was carried out using Potato Dextrose Agar (PDA), and the species were identified using a combination of morphological and molecular techniques. Six fungal species were isolated: *Aspergillus niger*, *Aspergillus fumigatus*, *Penicillium digitatum*, *Fusarium oxysporum*, *Aspergillus terreus*, and *Fusarium solani*. The occurrence, distribution, and pathogenicity of these fungi were evaluated, with *Aspergillus terreus* emerging as the most pathogenic (96 mm decay rate) and *Aspergillus niger* (22% occurrence) as the most prevalent across the markets. The findings underscore the critical need for improved postharvest handling, storage, and transportation practices to mitigate spoilage and reduce economic losses. Furthermore, the study highlights the importance of educating stakeholders within the citrus supply chain about the risks posed by fungal contamination and the necessary preventive measures. This research contributes valuable data to the limited body of scientific literature on postharvest spoilage of oranges in the region and provides a foundation for future efforts in developing effective management strategies. Additionally, the study offers crucial insights for agricultural extension services and potential investors in the orange juice industry, contributing to the sustainable development of citrus production in Nigeria.

Keywords: Pathogenicity, Fungal Species, *Aspergillus terreus*

1. Introduction

Sweet oranges (*Citrus sinensis*), a hybrid of pomelo (*Citrus maxima*) and mandarin (*Citrus reticulata*), hold a central place in the global fruit industry due to their high nutritional value, including vitamin C, dietary fiber, and antioxidant compounds (Seminara *et al.,* 2023) [16]. They are one of the most widely cultivated and economically significant citrus crops, contributing to the global agricultural economy. With over 3.8 million hectares dedicated to orange cultivation and a global yield of 75.5 million tons (FAOSTAT, 2020), countries such as Brazil, India, China, and the United States are the largest producers. In Nigeria, sweet oranges serve as a key fruit crop, supporting the livelihoods of millions and contributing to national nutritional needs. However, despite their economic importance, postharvest spoilage remains a major challenge, leading to significant losses in both domestic markets and the broader fruit industry. This issue is particularly exacerbated in tropical and subtropical climates, such as Nigeria, where limited storage infrastructure and poor transportation conditions facilitate fungal growth and subsequent fruit degradation.

Postharvest losses in sweet oranges, primarily due to fungal infections, are a widespread concern. In regions like Dekina Local Government Area of Kogi State, Nigeria, a substantial proportion of harvested oranges is wasted due to spoilage, driven

largely by inadequate handling, mechanical injuries, and poor sanitation during transportation and market display (Mukhtar *et al.,* 2019) [12]. Fungal pathogens, thriving in such environments, cause visible decay, off-odors, and textural breakdown, rendering the fruit unsuitable for consumption or sale (Chinyerum *et al.,* 2023) [5]. Among the common postharvest fungal pathogens, species of *Aspergillus*, *Penicillium*, *Fusarium*, *Rhizopus*, and *Mucor* have been identified as the primary contributors to spoilage (Mukhtar *et al.,* 2019; Abdul Rahaman *et al.,* 2023; Ezeonuegbu *et al.,* 2024) [12, 1, 6]. These fungi not only contribute to financial losses but also pose health risks to consumers, as certain species produce mycotoxins, such as ochratoxin, which have nephrotoxic and carcinogenic effects (Mailafia *et al.,* 2017) [10].

Understanding the species composition, distribution, and pathogenicity of these fungal pathogens is crucial to developing effective strategies for mitigating postharvest losses. Previous studies have identified *Aspergillus niger*, *Penicillium digitatum*, and *Fusarium oxysporum* as dominant fungi in orange spoilage (Abdul Rahaman *et al.,* 2023; Ezeonuegbu *et al.,* 2024) [1, 6]. Notably, *Aspergillus terreus* has emerged as the most pathogenic species, while *Aspergillus niger* is the most prevalent across markets (Mailafia *et al.,* 2017) [10]. Despite these findings, comprehensive studies on the prevalence and pathogenicity of fungi responsible for orange spoilage in Kogi State, Nigeria, are scarce. This gap in knowledge underscores the importance of this research, which aims to identify and assess the fungal species involved in the spoilage of sweet oranges in the Dekina region.

The economic implications of fungal spoilage are profound, contributing not only to direct financial losses for farmers and marketers but also to broader concerns of food security and nutritional deficits in urban areas, where fresh fruits are already in short supply. Effective postharvest management, including proper handling, storage, and transportation practices, along with targeted fungal control measures, are essential to curbing these losses (Mukhtar *et al.,* 2019) [12]. This study provides a critical examination of the fungal pathogens associated with sweet orange spoilage in Dekina, Kogi State, offering valuable data that can inform agricultural extension services, improve storage practices, and guide the development of fungal management strategies in the region. Additionally, this work contributes to the limited body of knowledge on the postharvest challenges facing orange producers in Nigeria, paving the way for further research and interventions to enhance the sustainability of the citrus industry in the country.

2. Materials and Methods

2.1 Materials

A total of 162 sweet oranges (*Citrus sinensis*) were used for the study. The laboratory apparatus and materials used for fungal isolation and analysis were sourced from reputable manufacturers, including Leica Cameras, Hovers ABS, Pyrex, Titan Bio-Technology, and Surehatch. Additional materials and equipment included beakers, test tubes, borers (2 mm, 4 mm), cotton wool, ethanol, and petri dishes. These materials were utilized for the collection, isolation, and identification of fungal species contributing to the postharvest spoilage of the oranges.

2.2 Samples Collection

A total of one hundred and sixty-two (162) sweet oranges fruits (Citrus sinensis) were obtained from Anyigba, Egume and Abocho markets in Dekina Local Government. Fifty-four (54) oranges already showing signs of spoilage were obtained and used for test samples while One hundred and eight (108) healthy orange fruits were later obtained and used for pathogenicity test (fifty-four for the test samples and the other fifty-four as control samples). Potato dextrose Agar was used for the purpose of this research; 39g of the powder was weighed and dissolved in 1000ml of water in a conical flask. The content was then sterilized in the autoclave for 121°C for 15 min and allowed to cool, after which 2% chloramphenicol was added to the agar and later plated into sterile petri dishes (Akintobi *et al.,* 2011 [3] and Tafinta *et al.,* 2013).

2.3 Isolation and Identification of Fungi

The spoilt sweet orange fruits were surface sterilized with cotton wool soaked in 70% alcohol and cut into two using sterile scalpels. The segments were carefully homogenized and serially diluted with sterile distilled water. One milliliter of each homogenized suspension was dispensed into sterile potato dextrose agar (PDA). The contents were properly mixed and thereafter incubated at 28° C for 72hrs. The colonies that developed was later purified by sub culturing onto sterile potato dextrose agar plates to obtain pure cultures and then stored on sterile PDA slants for identification (Akintobi *et al.,* 2011 [3] and Tafinta *et al.,* 2013). The pure cultures of the fungi were identified based on cultural and morphological features such as colony growth pattern, conidial morphology and pigmentation using the slide culture technique and microscopic examination (Akintobi *et al.,* 2011 [3] and Tafinta *et al.,* 2013).

2.4 Slide Culture Technique

A small portion of the aerial mycelia was picked using a sterile inoculating needle and inoculated on a slide containing prepared potato dextrose agar, which was incubated at room temperature for 24 hours after which it was viewed under the microscope. The identity of the fungi was confirmed with the aid of a mycological atlas (Akintobi *et al*. 2011 [3] and Tafinta *et al.* 2013). Microscopic Examination Microscopic examination was also carried out using the lacto phenol cotton blue stain. A drop of the stain was placed on a clean grease-free slide. A small portion of the fungal culture was emulsified in the stain after which the slide was covered with a coverslip avoiding bubbles. The slide was thereafter viewed under the microscope (Leica cameras) using magnifications x40 in Biological Sciences laboratory, Kogi State University Anyigba.

2.5 Pathogenicity Test

Pathogenicity test was carried out to know if the isolated fungi were really responsible for the spoilage of citrus fruits. Healthy fruits were surface sterilized with 70% alcohol. Cylindrical plug tissues cut out from the fruits using a sterilized 2mm sized cork-borer. Agar disc containing one week old fungal culture was aseptically placed in these holes, then covered and sealed off by means of petroleum jelly. The procedure was repeated separately across each of the fungal isolates (Akintobi *et al.,* 2011 [3] and Tafinta *et*

al., 2013). The inoculated samples and the control were placed in sterile polythene bags and incubated at 28 ± 30°C for 14 days. The point of inoculation of each type of fungus was examined and recorded. The diameter of the rotten portion of the orange fruits was measured to know the rate of spoilage caused by individual organisms after the fifth (5) day. This process was carried out in triplicate for each fungus. The fungi was later re-isolated from the inoculated fruits and compared with the initial isolates to substantiate if the initial isolates were actually the cause of spoilage in the first place.

3. Results and Discusion

The fungi identified were *Aspergillus niger*, *Aspergillus fumigatus*, *Penicillum digitatum, Fusarium oxysporum, Aspergillus terreus* and *Fusarium solani*. Table 1**,** shows the occurrence and distribution of each fungal isolates from the sampling points. *Aspergillus niger, Aspergillus terreus, Fusarium oxysporum* and *Penicillium digitatum* were fungal species isolated from Anyigba market. *A. niger, P. digitatum, F. solani, A. fumigatus* and *A. terreus* were fungal isolates from Abocho market. Fungal isolates such as *A. niger, A. terreus* and *F. solani* were identified in Egume market.

Table 1: Occurrence and distribution of the fungal isolates from the sampled markets

Fungal Isolate	Anyigba	Abocho	Egume
Fusarium solani	-	+	+
Penicillium digitatum	+	+	-
Aspergillus fumigatus	-	+	-
Aspergillus niger	+	+	+
Aspergillus terreus	+	+	+
Fusarium oxysporum	+	-	-

+ = presence: - = absence

The distribution of occurrence for the fungi isolates after isolation and the decay rate when inoculated into healthy oranges shows that *F. solani* had 17 % of occurrence and decay rate of 74 mm*, P. digitatum* had 11 % and 62mm, *A. fumigatus* had 6% and 52mm*, A. niger* had 27% and 55mm, *A. terreus* had 22% and 96mm and *F. oxysporum* had 17% and 77mm as presented on Table 2 below

Table 2: Percentage of occurrence, distribution and decay rate of the fungal isolates from sampled markets

Fungal Isolates	Percentage of occurrence (%)	Decay rate (mm)
Fusarium solani	17	74
Penicillium digitatum	11	62
Aspergillus fumigatus	06	52
Aspergillus niger	27	55
Aspergillus terreus	22	96
Fusarium oxysporum	17	77
	100	

P > 0.05

Table: 3: Colonial and microscopic characteristics of fungi in spoilt oranges identity colonial characteristics microscopic characteristics

Identity	Colonial characteristics	Microscopic characteristics
Aspergillus niger	Black colonies with reverse as yellow	Conidiophores were unbranched with round ends that bore conidia
P. digitatum	Greenish colonies with yellowish dots.	Brush-like conidiophores bearing conidia
F. oxysporum	Blackish wall colonies.	Colonies were spindle shaped.
A. fumigatus	Greenish colonies.	Conidial head typically columnar Conidiophores short, smooth-walled.
A. terreus	Whitish colonies at first, later turn brown and gets darker with age.	Conidial head radiate tending and splits in loose columns with age.
F. solani	Whitish woolly colonies	Conidiophores are smooth-walled.

3.1 Photomicrographs

Photomicrographs of fungal isolates and fungi colonies of citrus fruits were taken and are represented in Plates 4.1-4.6 below respectively. Microscopic analysis supported morphological identification. The photomicrographs in Plates 4.1 to 4.6 visually confirm the structural features used to differentiate each fungal isolate: Plate 4.1 (*P. digitatum*): Displayed brush-like conidiophores bearing round conidia. The greenish appearance of colonies with yellow dots aligns with typical *Penicillium* morphology, confirming its identity as the causative agent of green mold. Plate 4.2 (*F. oxysporum*): Showed characteristic spindle shaped macroconidia and darkened mycelial masses. This confirms its classification among high decay agents causing soft rot in oranges. Plate 4.3 (*A. niger*): Revealed unbranched conidiophores with globose heads bearing black conidia, corresponding to the black colony morphology observed in cultures. Plate 4.4 (*A. terreus*): Exhibited radiating conidial heads that split with age. This matched its morphological description and explains its aggressive spoilage pattern with 96 mm decay the highest among the isolates. Plate 4.5 (*F. solani*): Presented woolly, white colonies with cylindrical microconidia typical of *Fusarium* species. It ranked second in decay severity (74 mm) and was found in Egume and Abocho and Plate 4.6 (*A. fumigatus*): Showed short, smooth walled conidiophores with columnar conidial heads.

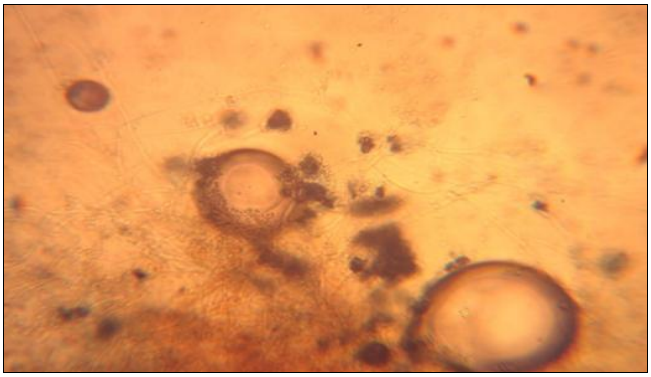

Plate 1: Photomicrograph of *P. digitatum*

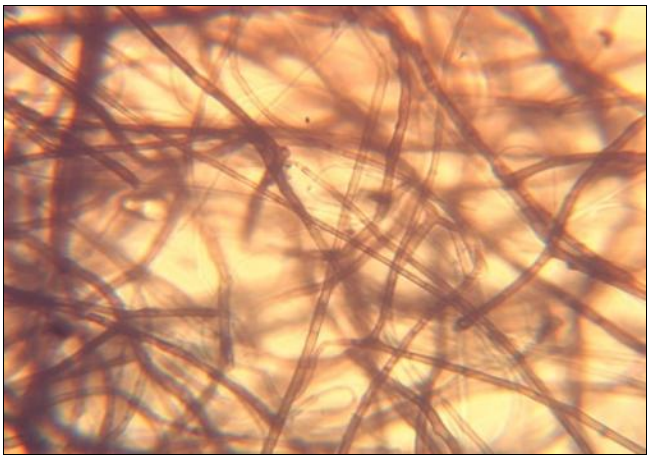

Plate 2: Photomicrograph of *F. oxysporum*

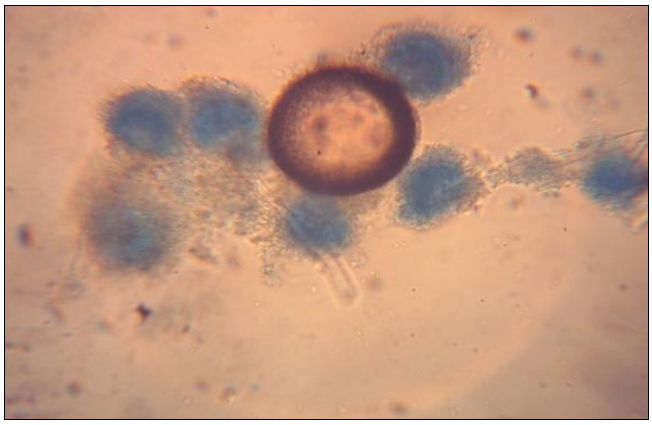

Plate 3: Photomicrograph of *Aspergillus niger*

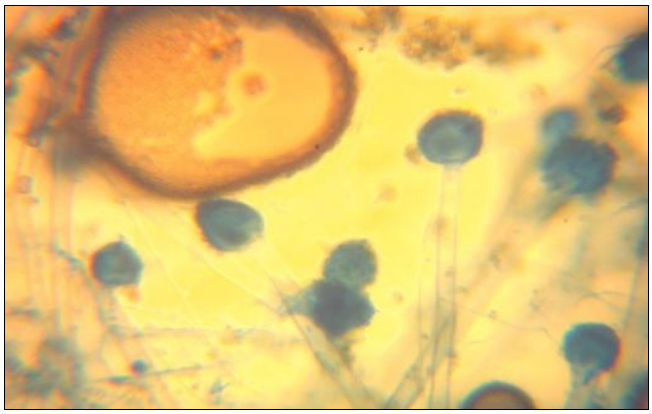

Plate 4: Photomicrograph of *A. terreus*

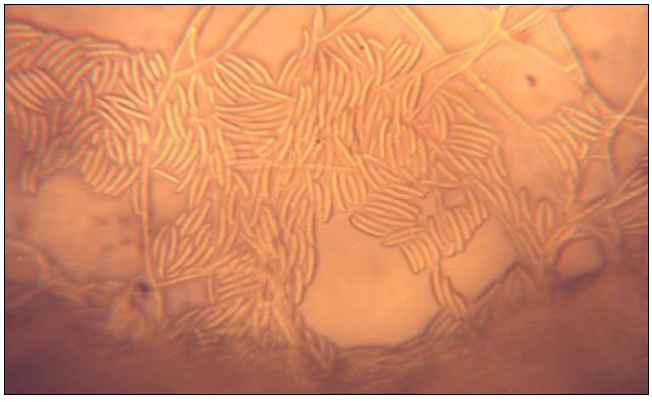

Plate 5: Photomicrograph of *Fusarium solani*

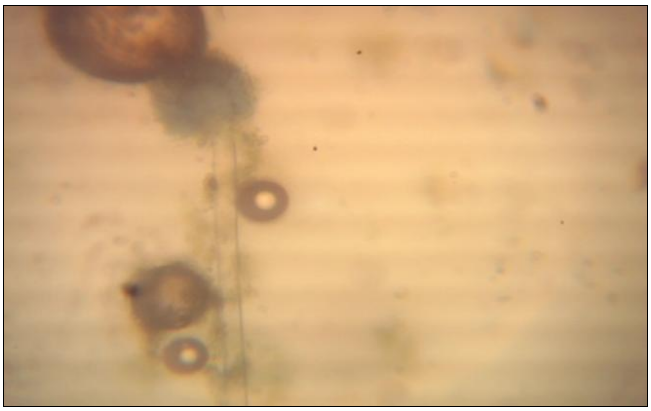

Plate 6: Photomicrograph of *A. fumigat*

3.2 Pathogenicity test

All data collected during the pathogenicity test was subjected to One way Analysis of Variance (ANOVA) to establish which organism is most pathogenic in the various markets and to determine the significant differences. *F. solani* and *F. oxysporum* also caused large decay indicating high pathogenic potential**,** differences across markets may reflect storage conditions**,** fruit handling, or fungal adaptation**.**

Table 4: Mean values of sampled marked

Isolates	Abocho	Anyigba	Egume	Mean
P. digitatum	15.33	60.33		37.83
F.solani	72.00	-	65.67	68.84
A. niger	41.33	22.67	53.00	39.00
A.fumigatus	50.33	-	-	50.33
F. oxysporum -		75.00	-	75.33
A. terreus	94.67	17.67	31.67	48.00

- = absent

Table 5: Mean values and Standard error of sampled markets

Isolates	Abocho	Anyigba	Egume
P. digitatum	15.33±1.45^{a}	60.33±0.88^{d}	-
F.solani	72.00±1.15^{e}	-	65.67±1.76^{d}
A. niger	41.33±0.88^{c}	22.67±1.45^{b}	53.00±1.15^{c}
A.fumigatus	50.33±0.88^{d}	-	-
F. oxysporum	-	75.00±1.16^{e}	-
A. terreus	94.67±0.88^{f}	17.67±0.88^{a}	31.67±1.20^{b}

(P: 5 %), - = absent

Analysis of variance (ANOVA) for the various markets showed that there were significant differences of 5% (P> 0.05) in the samples represented. Abocho market had the highest sum of square of (13819) fungi isolates which is 50% of the total sum, followed by Anyigba with the total sum of square of (9096) fungi isolates taking 33% and Egume had the least sum of (4860) isolates. Practically, the isolates from Abocho were the highest. The statistical analysis revealed that the total isolates across markets varied greatly.

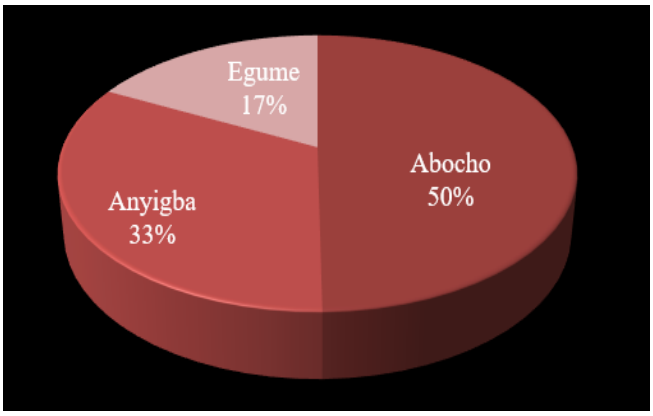

Fig 1: Pie Chart showing sampling sites isolates in Percentage

Key:
Red Bar-**Abocho**
Green Bar- **Egume**
Blue Bar- **Anyigba**

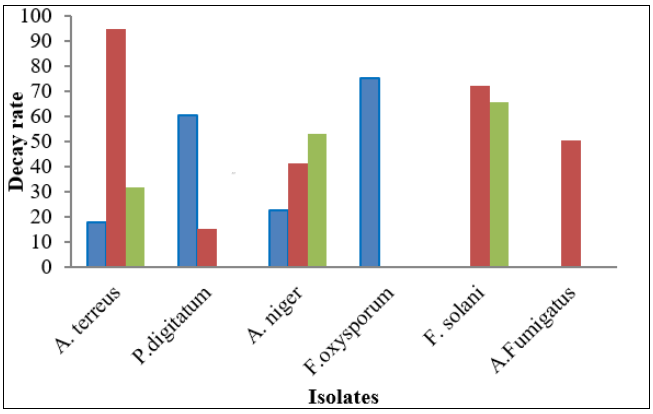

Fig 2: Graphical representation of isolates from all sampled markets

4. Discussion

The present study identified six fungal species associated with post-harvest spoilage of sweet oranges (*Citrus sinensis*) collected from markets in Dekina Local Government Area (LGA), Kogi State, Nigeria. These fungal species include *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus terreus*, *Penicillium digitatum*, *Fusarium solani*, and *Fusarium oxysporum*. Among these, *A. niger* was the most prevalent, with a 27% occurrence rate across all markets, while *A. terreus* exhibited the highest pathogenicity, with a decay diameter of 96 mm, particularly in the Abocho market. The use of photomicrographs strengthened the identification process and added visual confirmation to the microscopic data (Plate 4.1-4.6). This multi-step identification approach (photomicrography) adds robustness to the study and sets a foundation for future molecular analyses. In addition, Yusuf *et al.* (2022) [19] conducted a marketing point survey in Kano and identified a variety of fungal species including *Aspergillus niger* and *Fusarium* spp. as the leading agents of orange spoilage at distribution depots. This finding aligns with the present study, especially the identification of *A. niger* and *F. solani* as dominant spoilage organisms. Their study emphasized the influence of supply chain handling and environmental exposure on the proliferation of fungi, which correlates with the significant differences observed across the markets in Dekina LGA. Like Abocho market in the current study, Yusuf *et al.* (2022) [19] reported higher fungal presence in locations with minimal preservation practices.

The dominance of *A. niger* in this study aligns with the findings of Abdul Rahaman *et al*. (2023) [1], who identified it as the most frequent fungal isolate during the storage of sweet oranges, using both morphological and molecular techniques. Similarly, Mailafia *et al*. (2017) [10] observed that *A. niger* was the most prevalent fungus on various spoiled fruits, including oranges, in Gwagwalada market, Abuja, with a frequency of 38%. The persistent prevalence of *A. niger* across different studies suggests its strong adaptation to fruit environments and highlights its potential to produce mycotoxins, such as ochratoxins, which pose significant health risks to consumers. This aligns with the known nephrotoxic and carcinogenic properties of *A. niger*, which emphasize the importance of controlling its presence in postharvest fruit storage environments.

Aspergillus terreus, although less frequently reported in previous studies, was identified in this study as the most aggressive pathogen, with a decay rate of 96 mm. This species was present in all sampled markets, indicating its widespread distribution. Despite its limited documentation in earlier studies of citrus spoilage, *A. terreus* demonstrated considerable pathogenicity, suggesting that it could be an emerging postharvest threat in humid environments such as Dekina. The high pathogenicity of *A. terreus* warrants further investigation, particularly in relation to its role in accelerating spoilage and its ability to thrive in tropical climates. Its aggressive nature suggests it should be prioritized in future postharvest disease management strategies. *Penicillium digitatum*, the well-known citrus pathogen responsible for green mold, was identified in Anyigba and Abocho markets, where it exhibited moderate pathogenicity with a decay rate of 62 mm. These findings are consistent with those of Bukar *et al*. (2009) [4], who identified *P. digitatum* as a significant spoilage organism in sweet oranges from Kano Metropolis. Similarly, Ezeonuegbu *et al*. (2024) [6] noted its crucial role in spoilage among Port Harcourt fruit markets. *Penicillium digitatum* is a major cause of economic loss in the citrus industry, and its ability to thrive in suboptimal storage conditions underscores the need for targeted interventions, particularly in regions with inadequate storage infrastructure. The identification of *Fusarium oxysporum* and *F. solani* further supports previous findings by Muhammad *et al*. (2018) [11], who isolated these species in Sokoto and linked them to advanced decay stages in both citrus and banana. These species were also reported by Mukhtar *et al*. (2019) [12] as common pathogens in various fruit crops, including sweet oranges, cucumber, and lettuce. Their broad host range and aggressive nature in tropical climates make them particularly problematic for postharvest management. Both *Fusarium* species can cause soft rot and wilting, further emphasizing the need for comprehensive control measures to prevent their spread in postharvest settings.

The findings from this study also match those of Onuorah *et al.* (2015) [14] in Awka, where *A. niger, F. solani*, and *P. digitatum* were major spoilage agents. They attributed their spread to poor hygiene in marketplaces and high fruit turnover. This aligns with the high fungal load seen in the Abocho market in the present study, where poor storage and handling conditions were observed.

Moreover, Oviasogie *et al.* (2015) [15] assessed fungal spoilage of oranges and reported a dominance of *A. niger, P. digitatum,* and *F. solani* all of which featured in this study. Their observation that spoilage intensity varied with environmental exposure and packaging confirms that market specific differences significantly influence fungal prevalence.

Although the occurrence of *Aspergillus fumigatus* in the Abocho market was lower (6%), its presence should not be underestimated. *A. fumigatus* is known for its opportunistic pathogenicity, particularly in immunocompromised individuals. Its ability to survive under diverse environmental conditions makes it a potential concern for both public health and food safety (Chinyerum *et al.,* 2023) [5]. Given its opportunistic nature and association with airborne spores, the risk posed by *A. fumigatus* to vulnerable populations further underscores the importance of improving hygienic practices in fruit markets. The variation in fungal distribution and decay severity across markets can likely be attributed to differences in handling, hygiene, and storage conditions. Abocho market recorded the highest fungal load and decay severity, which may be linked to inadequate postharvest management practices. This is supported by the statistical analysis, which revealed Abocho as having the highest sum of squares (13819) in ANOVA tests, indicating significantly poorer conditions compared to Anyigba and Egume markets. This further suggests that improving the handling and storage conditions in Abocho could result in a marked reduction in fungal contamination and spoilage rates.

In the study by Olahan *et al.* (2023) [13], *P. digitatum, F. oxysporum*, and *A. niger* were the dominant fungi associated with sweet orange spoilage. This supports the present findings and reinforces the fact that these fungi are consistent spoilage agents in different Nigerian ecological zones. The presence of *F. oxysporum* in both studies highlights its potential as a cross-regional postharvest pathogen. These findings are consistent with the reports of Ezeonuegbu *et al.* (2024) [6], who isolated similar fungal species *A. niger, P. digitatum*, and *F. solani* from spoiled orange and tomato fruits in Port Harcourt markets. Like the current study, *A. niger* was also the most prevalent, confirming its widespread distribution and strong ecological adaptability to fruit environments. In both cases, the authors emphasized the role of poor handling and high humidity in enhancing fungal colonization.

Similarly, Tafinta *et al.* (2013) in Sokoto isolated *F. oxysporum, A. niger*, and *P. digitatum*, which further strengthens the claim of their wide geographic occurrence. Sokoto's drier climate compared to Dekina implies that these pathogens are not only favored by humidity but also by factors like mechanical injuries and poor sanitation. The high decay rate observed for *F. oxysporum* (77 mm) in the current study aligns with its classification as highly pathogenic.

In conclusion, the findings from this study highlight the critical need for improved postharvest handling practices, including better hygiene standards, fruit packaging, and storage conditions. Clean market environments and proper storage under controlled conditions are essential for reducing spoilage and ensuring the safety of consumers. Public education on the risks of fungal contamination and basic hygiene practices is necessary for stakeholders across the orange supply chain. Moreover, the incorporation of fungicidal treatments and the exploration of biological control measures, particularly against highly pathogenic fungi such as *A. terreus*, should be prioritized. The findings also suggest that ongoing research into the development of resistant orange varieties, coupled with effective fungicides and postharvest treatments, is critical to reducing fungal spoilage and enhancing the sustainability of the citrus industry in Nigeria.

5. Conclusion and Recommendations

This study identified and assessed the prevalence and pathogenicity of various fungal species responsible for the spoilage of sweet oranges (*Citrus sinensis*) in Dekina, Kogi State, Nigeria. *Aspergillus niger* was the most prevalent species, while *Aspergillus terreus* emerged as the most pathogenic. These fungi pose significant threats to orange quality, resulting in economic losses and potential health risks due to their toxigenic nature. The findings underscore the urgent need for improved post-harvest handling and storage practices to minimize spoilage and ensure consumer safety. To address these issues, it is recommended that farmers and marketers adopt better handling and storage practices, such as using well ventilated containers and avoiding overcrowding during transportation. Additionally, educating stakeholders in the supply chain about hygiene and fungal contamination is crucial. Investment in proper transportation and storage facilities, including refrigerated trucks and cool storage, is essential to extend the shelf life of oranges. Furthermore, agricultural extension services should help farmers implement practices that reduce post-harvest losses. Finally, further research into effective fungicides, treatments, and the development of resistant orange varieties will contribute to long-term solutions for fungal spoilage.

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