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ORIGINAL RESEARCH

Mycological Quality of Powdered Pepper (*Capsicum annuum*) Retailed in Lokoja Markets, Kogi State, Nigeria

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Abstract

Fungal contamination is a significant concern in agricultural commodities such as powdered pepper as it can compromise product safety and quality. This study aimed at assessing the mycological quality of powdered pepper sold in Lokoja markets, Kogi State, Nigeria. A total of forty powdered pepper samples were collected from five different markets and their fungal load were evaluated using culturing and enumeration techniques. The overall prevalence of fungal contamination of the samples was 93.3 % (42/45). Kpata market samples had the highest mean fungal load (4.8×10^3 CFU/g), followed by the Old market samples (4.0×10^3 CFU/g), Adankolo market samples (1.6×10^3 CFU/g), New market samples (1.1×10^3 CFU/g) while samples from Lokongoma market had the least fungal load of 0.7×10^3 CFU/g. There was no statistical significant difference between the mean fungal load obtained from the five markets when compared at $P < 0.05$. Four fungal species: *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus* and *Rhizopus arrhizus* were isolated and identified from the samples. *Aspergillus niger* (32.5 %) and *Rhizopus arrhizus* (17.5 %) were the most and least prevalent fungi across the markets respectively. The presence of fungal contaminants especially mycotoxigenic fungi in the powdered pepper samples is a public health concern hence it is recommended that powdered pepper are processed, packed, stored and sold under hygienic condition.

Keywords: *Aspergillus* species, powdered pepper, food safety, fungal contamination, mycological quality

Introduction

Globally, pepper (*Capsicum* spp.) is widely consumed and recognized for its nutritional and medicinal properties (Dhamodharan et al., 2023; Salehi et al., 2018). *Capsicum annuum* is one of the major pepper species cultivated in Nigeria and other African countries such as Kenya, Uganda, Sudan and Ethiopia (Afolabi et al., 2023; Fern, 2023). It is rich in bioactive compounds including capsaicinoids which possess antimicrobial and anti-inflammatory properties (Batiha et al., 2020; Jolayemi and Ojewole 2013). Capsaicin, a primary capsaicinoid has been extensively studied for its pharmacological and health benefits such as pain relief,

weight management and cardiovascular health improvement (Jolayemi and Ojewole, 2013; Sanati et al., 2018). In Nigeria, powdered pepper is a popular spice used in various culinary preparations because it is ready to use and convenient to store. Pepper is a commonly used spice that not only add flavor and enhance the taste of food but also provide various health benefits. Despite the beneficial properties of pepper, the presence of fungal contaminants in pepper can compromise its safety and potentially diminish its medicinal value (Frimpong et al., 2019; Hadil et al., 2022).

Fungal contamination is a common issue affecting processed and stored agricultural products (Ham et al., 2016; Garuba et al., 2022). Fungal contamination of spices can occur at all stages of spice cultivation, processing, storage and distribution (Ham et al., 2016). The preparation (drying or/and grinding) and storage stages of pepper are critical stages of contamination and spoilage during which pepper are susceptibility to fungal infections (Costa et al., 2019). As a result, the contamination of powdered pepper can persist for several months from the time of production to consumption. When powdered pepper are displayed for sale in open bowls or buckets in open markets, they can become contaminated with fungal spores and absorb moisture from the environment which create favourable environment for the growth and proliferation of fungi. Among these biological contaminants, fungi have been identified as major contributors to the contamination of processed (Ogu et al., 2017) and stored dry food products (Ham et al., 2016). A study conducted in Nigeria reported that fungal count and aflatoxin level of pepper increased during the drying process, emphasizing the importance of proper drying techniques (Adegoke et al., 1996). Fungi not only reduce the quality and market value of pepper but can also produce mycotoxins such as aflatoxins which are hazardous to human health (Yogendrarajah et al., 2014; Frimpong et al., 2019). The presence of fungi in commercial powdered pepper pose potential risks to consumers due to the production of mycotoxins (Frimpong et al., 2019). Previous studies have reported the occurrence of various

fungal species including toxigenic fungi in different species of peppers (Lema et al., 2018; Hadil et al., 2022). However, there is dearth of information on the fungal composition of powdered pepper sold in Lokoja markets, Kogi State, Nigeria. Therefore, this research aimed at assessing the mycological quality of powdered pepper sold in the five major markets in Lokoja, kogi State, Nigeria.

Materials and Methods

Study Area

This study was conducted in Lokoja, the capital city of Kogi State, Nigeria. Lokoja lies between latitude 7.8023^0 N and longitude 6.7333^{0}E at 55m above the sea level. Lokoja is strategically located at the confluence of the Niger and Benue rivers making it a significant commercial center in the region (Figure 1). The city hosts several markets including those specialized in the sale of spices such as powdered pepper. The city's bustling trade activities make it an ideal location to investigate the fungal composition of commercial dry ground pepper. The samples for this research were obtained from five markets (Old market, New market, Kpata market, Adankolo market and Lokogoma market) in Lokoja.

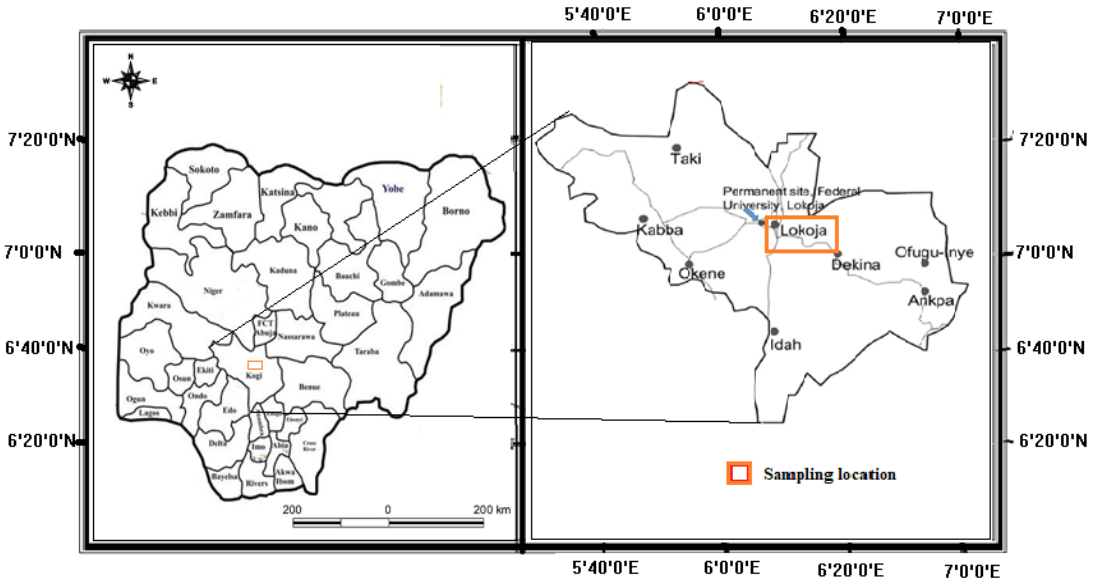

Figure 1: **Map showing location of Lokoja, Kogi State, Nigeria (Ojiego et al., 2022)**

Sample collections

A total of 45 samples of powdered pepper was collected for this study. Nine (9) samples were collected from each of the five selected markets: Old market, New market, Kpata market, Adankolo market and Lokogoma market using sterile containers. The collected samples were labeled, properly sealed and transported to the laboratory for further analysis.

Isolation of Fungi and Enumeration of the Fungal load of the Powdered pepper Samples

Isolation of fungi from the powdered pepper samples was carried out by using serial dilution and culturing method on Potato Dextrose Agar (PDA) supplemented with the antibiotic, chloramphenicol (0.05%) (Mailafia et al., 2017). One gramme (1 g) of each powdered pepper sample was aseptically transferred into 9 mL sterile distilled water in test tubes. Serial dilutions were carried out by transferring 1 mL of each stock solution into sterile test tube containing 9 mL of sterile distilled water. The dilutions were repeated until a suitable dilution of 10^{-6} was reached. Pour plate technique was used to culture the fungi in the powdered Pepper samples according to the method of Okolo et al. (2023). The plates were incubated between 25 - 27 °C for 3-7 days. The fungal isolates were counted using electronic colony counter and the colony forming units per gramme of the samples were calculated and recorded. The colony forming units per gramme (CFU/g) were determined by multiplying the number of colonies isolated by the dilution factor used and dividing by the volume of inoculum plated. The results were expressed as colony forming units per gramme (CFU/g) of the original powdered pepper sample.

Sub-culturing and Identification of Fungal Isolates

Representative isolates from the cultured plates were selected and sub-cultured onto fresh Potato Dextrose Agar (PDA) plates to obtain pure cultures. The sub-culture plates were

incubated between 25 - 27°C. The colonial morphology and microscopic characteristics of the fungal isolates were observed and used to identify them by comparing their colonial morphology and microscopic characteristics with those described in standard fungi atlas (De Hoog et al., 2000; Watanabe, 2010; Kidd et al., 2016).

Data analysis

Analysis of variance (ANOVA) was used to compare the mean of the fungal loads obtained from the various powdered pepper samples using SPSS version 20 at $p<0.05$ level of significance.

Results

A total of 45 samples of powdered pepper were investigated for fungal contamination and the prevalence of fungal contamination was 93.3 % (42/45) as presented in Figure 2. Fungal load was obtained for the powdered Pepper samples from the five markets and the results are presented in Table 1.

Table 1: Fungal Load of the Powdered Pepper Samples from Selected Lokoja Markets

Samples	Fungal loads of DGP (CFU/g)*				
	Lokongoma	**Kpata**	**New**	**Adankolo**	**Old**
1	2.0×10^3	1.0×10^3	1.0×10^3	2.0×10^3	5.0×10^3
2	1.0×10^3	0	1.0×10^3	2.0×10^3	3.0×10^3
3	1.0×10^1	3.3×10^4	2.0×10^3	2.0×10^3	4.0×10^3
4	1.0×10^3	2.0×10^3	1.0×10^3	2.0×10^3	1.0×10^3
5	1.0×10^1	2.0×10^3	1.0×10^3	2.0×10^3	1.9×10^4
6	1.0×10^3	2.0×10^3	1.0×10^3	2.0×10^3	1.0×10^3
7	0	1.0×10^3	1.1×10^2	1.0×10^3	1.0×10^3
8	1.0×10^3	1.0×10^3	1.0×10^3	1.0×10^3	1.0×10^3
9	1.0×10^1	1.0×10^3	1.0×10^3	0	1.0×10^3
Maximum	2.0×10^3	3.3×10^4	2.0×10^3	2.0×10^3	1.9×10^4
Minimum	0	0	1.0×10^3	0	1.0×10^3
Mean ± SD ($\times 10^3$)	0.7 ± 0.70	4.8 ± 10.6	1.1± 0.33	1.6 ± 0.73	4.0 ± 5.83

Key: **CFU**: Colony forming units, **SD**: Standard deviation *p = 0.4022

Table 2: Fungal Isolates from Powdered Pepper from Lokoja markets

Colonial morphology	Microscopic characteristics	Probable isolates
Black powdery colonies	Simple upright conidiophore terminating in a globose conidia head	*Aspergillus niger*
Yellow-green colony	Hyaline conidiophore with a radiate conidial head	*Aspergillus flavus*
Blue-green colony with suede-like surface	Smooth-walled conidiophore stipe with columnar conidial head	*Aspergillus fumigatus*
Fast growing white cottony colony that turn brownish-grey	Smooth-walled sporangiophore with flattened base globose sporangium	*Rhizopus arrhizus*

The minimum and maximum fungal load for each market were as follows: 0 - 2.0 × 10^3 CFU/g (Adankolo and Lokongoma market samples), 1.0 × 10^3 - 2.0 × 10^3 CFU/g (New market samples), 0 - 3.3 × 10^4 CFU/g (Kpata market samples) and 1.0 × 10^3 - 1.9 × 10^4 CFU/g (Old market samples). The mean fungal load ranged from 0.7 ± 0.70 × 10^3 (Lokongoma market samples) to 4.8 ± 10.6 × 10^3 (Kpata market samples). One-factor ANOVA comparison at $p<0.05$ indicated no significant difference ($p=0.4022$) between the mean fungal loads.

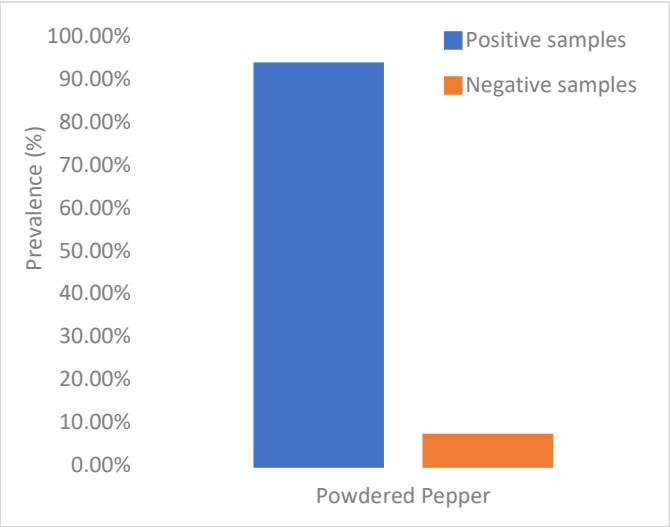

Figure 2: Prevalence of Fungi in powdered pepper samples

Fungal isolates recovered from the powdered pepper belong to two main genera: *Aspergillus* and *Rhizopus*, totaling 40 isolates (Table 3). The prevalence of each fungal isolate showed *A. niger* (32.5 %) as the most frequent, followed by *A. fumigatus* (27.5 %), *A. flavus* (22.5 %) and R. *arrhizus* (17.5 %) as the least frequent (Table 3). Kpata market samples yielded the most fungal isolates [12 (30 %)], followed by Old market samples [11 (27.5 %)], Adankolo market samples [10 (25%)], New market samples (4(10%)) and Lokongoma market samples with the least fungal isolates [3 (7.5%)] (Table 4).

Table 3: Occurrence of Fungus Isolated from the DGP samples

Fungal isolates	Number of isolates	Occurrence (%)
Aspergillus niger	13	32.5
Aspergillus flavus	9	22.5
Aspergillus fumigatus	11	27.5
Rhizopus arrhizus	7	17.5
Total	40	100

Table 4: Occurrence of Fungi isolated from the Sampled Markets

Fungal isolate	New Market	Lokongoma	Kapta	Adankolo	Old Market
A. niger	1	2	4	3	3
A. flavus	0	0	2	3	4
A. fumigatus	3	0	3	3	2
R. arrhizus	0	1	3	1	2
Total (%)	4 (10%)	3 (7.5%)	12 (30%)	10 (25%)	11 (27.5%)

Discussion

Spices constitute an important portion of humans' daily diet and therapeutic product. Among these spices, pepper particularly the *Capsicum* spp. has been widely relied upon for its nutritional and medicinal property (Dhamodharan et al., 2023). One of the major challenge facing the dietary and economic value of spices is persistent attacks by microorganisms in the field and also during processing and retailing/ storage.The fungi (*Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus and Rhizopus arrhizus*) isolated from powdered pepper samples in this study are consistent with the findings of a study conducted by Adebayo et al. (2021). However, they differ slightly from the isolates obtained from other states in Nigeria (Frimpong

et al., 2019; Darsana and Chandrascher, 2021; Garuba et al., 2022). This suggests that there may be regional variations in fungal contamination of powdered pepper. Among the markets examined, Adankolo, Old and Kpata market samples exhibited the highest percentage of fungal occurrence of 25 – 30 %. This may be attributed to prolonged storage after contamination during processing and before sale. Such prolonged storage can promote the proliferation of fungal contaminants in the powdered pepper leading to an increase in the fungal load. On the other hand, Lokongoma and New market samples recorded the lowest fungal occurrence of 7.5 – 10 %, possibly due to better drainage and the absence of nearby dumpsites. Also, the variation in the observed fungal occurrence in the samples from the five markets could be attributed to the variation in post-production handling practice employed by the traders. *Aspergillus niger* was found to have the highest occurrence percentage in this research, which aligns with previous reports (Saidi et al., 2021; Nordin et al., 2022). This is not surprising because *Aspergillus niger* is known to be one of the most abundant microorganisms on earth according to Klich (2009). The presence of *Aspergillus niger* and *Aspergillus flavus* raises concern because their mycotoxins can cause asthma, otomycosis, and immunosuppression (Kortei et al., 2022; Pandey et al., 2023). It is important to note that the production of mycotoxins in food or raw material is influenced by certain factors such as humidity levels, temperature and substrate content (Daou et al., 2021). Hence, understanding the environmental factors that enhance mycotoxigenesis is essential in developing effective strategies to mitigate the risk of contamination in dried pepper and its products. Furthermore, *Rhizopus arrhizus* isolated from dry ground pepper in this research, is an opportunistic pathogen that can cause mucormycosis in humans with weakened immune systems (Tabarsi et al., 2021). Fungi have been recognized as common contaminants of dried and stored spices (Rani and Saxena, 2022). Although, previous studies have highlighted that spices are poor medium for fungal proliferation (Madhyastha and Bhat, 1984; Kneifel and Berger, 1994), high incidences of fungal

contamination of powdered pepper and other spices have been reported over the years (Kahn et al., 2013; Rotsisen et al., 2016; Gambacorta et al., 2018; Costa et al., 2019; Nordin, et al., 2022).

Conclusion

In conclusion, this study revealed a substantial occurrence of fungal contamination of commercially sold powdered samples from five major markets in Lokoja. The fungal isolates belong to the genera, *Aspergillus* and *Rhizopus* with *Aspergillus niger* being the most dominant. The identified fungal isolates though consistent with previous studies, stresses the need for improvement in post-processing hygiene practice and proper storage of powdered pepper to curtail the risk of mycotoxigenic fungi proliferation. The high occurrence of *Aspergillus niger*, *Aspergillus fumigatus* and *Aspergillus flavus* raises health concerns due to their potential mycotoxin production. Furthermore, variation in the fungal occurrence among the markets is mostly likely an indication that handling practice and storage conditions may vary from time to time across the markets.

Recommendations

To reduce the risk of contamination of the powdered pepper, it is recommended that proper packaging and covering of powdered pepper containers with lid is always carried out after processing and during retailing in the open market. Additionally, strategies that will focus on environmental factors such as humidity levels, temperature and substrate content should be considered to develop effective measures for mycotoxin risk reduction in powdered pepper and related products. Awareness programmes and training for traders on best practice for handling and storage could further contribute to ensuring the safety and quality of powdered pepper in the market.

Declarations of Authors Contribution

O.E. and S.O.S. conceptualized and designed the study. They both participated in the laboratory work and data collection. They both participated in analysing and interpreting the data. Both authours prepared the first draft of the manuscript, reviewed it, contributed to the development of the final manuscript and approved its submission.

Conflict of Interest
None

Ethics Approval and Informed Consent
This study did not involve human or animal subjects. Therefore, ethical consideration was not applicable.

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