FUNGAL CONTAMINATION AND ITS EFFECT ON THE PROXIMATE COMPOSITION OF STORED MAIZE (*Zea mays*)

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Abstract
Fungal contamination poses significant concerns for the nutritional composition of stored maize, a staple food crop consumed worldwide. This study aimed at investigating the effects of fungal contamination on the proximate composition of stored maize. Samples of maize grains were collected from different store houses in Makurdi, Benue State. Potato Dextrose Agar (PDA) was used for the fungal isolation. It was prepared according to the manufacturer's instruction. The fungi were isolated by plating the sterilized maize grains on the prepared culture medium, and kept at room temperature for three days for fungal growth. The fungi observed were identified macroscopically and microscopically. Six (6) fungal isolates were observed which include: *Aspergillus niger* 1(5.26%), *Aspergillus flavus* 9(47.37%), *Aspergillus fumigatus* 1(5.26%), *Alterneria sp* 4(21.05%), *Mucor sp* 3(15.79%) and *Fusarium sp* 1(5.26%). Proximate parameters such as moisture, crude protein, crude fat, crude fiber, ash and carbohydrate contents were determined for both the healthy and infected maize grains. A comparative analysis of the proximate composition of the healthy and infected seeds showed a significantly higher crude fibre content (P= 0.008), Crude protein content (P=0.004) and carbohydrate content (P=0.053) in the healthy seeds compared to the infected seeds. Fungal attack had negative effect on the proximate composition of stored maize as the crude fibre, crude protein and carbohydrate contents were observed to be higher in the healthy seeds than in the infected seeds. Proper storage techniques and management should be encouraged among farmers.

Keywords: Fungi, fungal contamination, maize, proximate composition

Introduction

Maize, scientifically known as *Zea mays*, is one of the most important cereal crops in the world (Olaniyan, 2015). It plays a vital role in global food security and serves as a staple food for millions of people, particularly in regions of Africa, the Americas, and parts of Asia. In Nigeria, it is the most important cereal crop after Sorghum (Olufunso and Oluwole, 2019). Maize has a wide range of uses, including human consumption, animal feed, and industrial applications (Murdia *et al.,* 2016). The importance of maize stems from its nutritional value and versatility. Maize kernels are rich in carbohydrates, providing a significant source of energy. They also contain essential vitamins, such as thiamine, niacin, and folate, along with minerals like phosphorus and magnesium. Additionally, maize is a good source of dietary fibre (Olaniyan, 2015).

Fungal contamination is one of the major concerns in the storage of grains in the tropical and sub-tropical regions of the world especially in Africa. Fungal attack on grains can lead to loss of viability, stale and mouldy smell and principally the production of mycotoxins which are the major cause of food poisoning in man and animals. The presence of fungi in foods has also been reported to alter their chemical and nutritional compositions (Sauer, 2019).

Fungi are inevitable to encounter in man's daily activities owing to their ubiquitous nature. They are also known to have a host-pathogen relationship which could either be beneficial or harmful to the host. Harmful fungi cause diseases in plants, animals, and humans, as well as spoilage in food and feed (Al-Shikli *et al*., 2010). Among the fungi classified as microorganisms, the filamentous fungi have the greatest economic significance. They do not only cause food spoilage during pre- and postharvest stages of production but may release various mycotoxins (Magan *et al*., 2014; Omotayo *et al*., 2019). Filamentous fungi pose serious health risks and have been implicated in the incidence of many diseases of public health concern. Fungal contamination of stored maize occurs due to various factors, including high moisture content, inadequate drying, poor storage facilities, and suboptimal temperature and humidity conditions (Atanda *et al*., 2011). Common fungi associated with maize contamination include *Aspergillus, Fusarium,* and *Penicillium species*. These fungi can thrive in the presence of moisture and can colonize maize kernels, leading to the production of mycotoxins such as aflatoxins, fumonisins, and ochratoxins (Atanda *et al*., 2011).

The study of fungal attacks on stored grains is imperative as such knowledge could guide farmers, storage facility operators, and policymakers in adopting appropriate pre- and post-harvest interventions to minimize fungal growth and mycotoxin contamination. It will also ensure the preservation of nutritional quality, and reduce economic losses. This study therefore investigated the effect of fungal contamination on the proximate composition of stored maize.

Materials and Methods

Study area

The study was conducted in Makurdi, the capital of the food basket of Nigeria (Benue State). Located in the

North Central Nigeria within the lower river Benue trough, it lies between Latitudes 6.5' and 8.5' North and Longitudes 7.47'N and 10' East (Ode *et al*., 2023).

Sample collection

Ten samples of maize grains were obtained from four different store houses each in Makurdi, Benue State making a total of 40 grains. Precautions were taken to obtain random samples. The maize samples were placed in sterile paper bags, labeled, and transported to the laboratory for fungal and proximate assessments.

Preparation of culture medium

Potato Dextrose Agar (TM Media - MG3E4EW01) was used for this study. It was prepared according to the manufacturer's instructions in which 39 g was dissolved in 1000 mL of distilled water in a conical flask and homogenized using a sterile rod. The mixture was further dissolved by heating using the heating mantle. It was stirred continuously until the solution became clear. Afterwards, the mouth of the flask was covered with cotton wool, wrapped in foil paper and sterilized at 121°C for 15 minutes at 5 psi using an autoclave. The sterile medium was allowed to cool to about 40°C and some amount of lactic acid was added to inhibit bacteria growth. The medium was aseptically dispensed into sterile Petri dishes by flame sterilizing the mouth of the flask and was allowed to solidify.

Detection and isolation of fungi

The maize grains used for the study were sterilized in 1% sodium hypochlorite and rinsed thrice in sterile distilled water. The sterilized seeds were inoculated on Petri dishes containing PDA. This was done by first dispensing the medium into the sterilized petri dishes and then placing the maize seeds on the medium.

The plating of the maize grains was done at the rate of 10 maize grains per plate and an even spread was maintained in the plates. The plates were covered by their lids and fastened using parafilm. They were allowed to stay at room temperature for 3 to 5 days for fungal growth. Pure cultures were maintained by aseptic transfer of distinct colonies to a freshly prepared medium. The infected and healthy maize grains were separated and prepared for the determination of proximate composition.

Morphological identification of the fungi

The fungi were characterized and identified based on their colonial and microscopic characteristics. The fungal mycelia were picked from the sub-cultured plates in very small amounts with an inoculating needle and placed on a clean glass slide and lactophenol cotton blue was added, it was covered with a cover slip and heat-fixed by passing it through a naked flame thrice. The prepared glass slide was mounted on the microscope and observed under low (×10) and high power objectives (×40) using a microscope (TM Research Binocular Microscope - CH 1200). The observed characteristics of the fungi were compared with a standard identification chart by Li *et al*. (2023).

Proximate analysis

The moisture content, crude protein, crude fat, crude ash, and fibre contents of the infected and healthy seeds were determined according to the standard methods of the Association of Official Analytical Chemist (AOAC) (2012).

Statistical analysis

Data collected was computed on IBM SPSS Version 20.0. The percentage occurrence of each fungus was calculated. Chi-square was used to determine the significance of fungal occurrence. Independent T-test was also used to determine the statistical effect of fungal contamination on nutritional composition. Statistical significance was determined at 5 % level of significance ($P<0.05$).

Results

This study investigated the fungal pathogens associated with maize grains in storage, and their effect on nutritional composition. The rate of infection of maize grains with fungal pathogens in Makurdi metropolis is presented in Table 1. A total of 40 seeds in 4 replications containing 10 seeds each were examined. The result showed that out of the 40 seeds examined, 22 seeds representing 55 % were infected with fungi

Table 1: Rate of maize infection with fungal pathogens in Makurdi metropolis

Replication	No of Seeds Examined	No of Seeds infected (%)
R1	10	2(20.00)
R2	10	6(60.00)
R3	10	9(90.00)
R4	10	5(50.00)
Total	40	22(55.00)

The fungal pathogens associated with stored maize in the Makurdi metropolis are presented in Table 2. A total of 6 different fungi were observed which include: *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Alternaria sp, Mucor sp* and *Fusarium sp* respectively. *Aspergillus flavus* was observed to be the most occurring fungus; 9(47.37%) followed by *Alternaria sp* 4 (21.05 %) and *Mucor spp.*; 3 (15.79 %). The least occurring fungi were the *A. fumigates, A. niger* and *Fusarium sp.* with the occurrence of 1 (5.26 %) each. The result further revealed a highly significant fungal occurrence in relation to the respective isolates ($\chi2=57.554$; df= 5; P=0.000).

Table 2: Fungal pathogens of stored maize in Makurdi metropolis

S/N	Fungal Isolate	Occurrence	% Occurrence
1	*Aspergillus niger*	1	5.26
2	*Aspergillus flavus*	9	47.37
3	*Aspergillus fumigatus*	1	5.26
4	*Alterneria sp*	4	21.05
5	*Mucor sp*	3	15.79
6	*Fusarium sp.*	1	5.26
	Total	**19**	**100**

$\chi2=57.554$; df=5; P=0.000

The effect of fungal contamination on the proximate composition of stored maize grains in Makurdi is presented in Table 3. It was observed that the healthy seeds had slightly higher ash content (P=0.375), and significantly higher in crude fibre (P=0.008), crude protein (P=0.004) and carbohydrate (P=0.053) than the infected seeds. The infected seeds on the other hand, had significantly higher moisture content (P=0.001) and crude fat (P=0.045) than the healthy seeds respectively.

Discussion

This study assessed fungal contamination of stored maize (*Zea mays*) and its effect on the nutritional composition. A total of 6 different fungi were observed which include: *Aspergillus niger, Aspergillus flavus,*

Table 3: Effect of fungal contamination on the proximate composition of stored maize

	Proximate Composition (%)					
Maize	**Moisture**	**Ash**	**Fibre**	**Fat**	**Protein**	**Carbohydrate**
Healthy	10.21±1.90	0.62±0.27	7.85±0.64	0.76±0.06	8.11±0.01	31.75±0.35
Infected	16.00±1.41	0.41±0.01	2.26±0.34	1.05±0.07	4.89±0.30	27.39±1.43
P-Value (0.05)	**0.001**	**0.375**	**0.008**	**0.045**	**0.004**	**0.053**

Aspergillus fumigatus, Alternaria sp, Mucor sp and Fusarium sp. The presence of the fungi contaminants in this study may be due to the high moisture content of the seeds. In this study, significantly higher moisture content was observed in the infected samples compared to the healthy ones which may account for their colonization by fungi than the healthy seeds. Moisture content has been considered a very important storage index in dried seeds/grains. Very high levels of moisture content favour microbial growth while low levels on the other hand reduce microbial activities and allow relatively longer shelf life (Ibrahim *et al.,* 2018; Ijeoma *et al*., 2012). Several authors including Kortei *et al*. (2020), Mohammed and Kumsa, (2021), Curr *et al*. (2021), Aminu and Keta, (2021) and Shehu *et al*. (2020) reported fungal species that are similar to the ones observed in this study in different parts of Nigeria and Africa; moisture content was also reported as a key factor associated with the fungal presence in their studies. In addition to the moisture content, postharvest handling conditions such as transport conditions and facilities (Shehu *et al*., 2020), open drying in farms (Aklatu *et al*., 2020), as well as storage conditions and facilities (Suleiman *et al.,* 2013) can also expose maize and other grains to attack by fungi. In this study also, *Aspergillus flavus* was observed as the most occurring fungus in the study. According to Onyimba *et al*. (2014) the abundance of the *Aspergillus sp* in the environment, their high reproductive capacity and their resistance characteristics could be reasons for their competitive advantage over the other fungal species.

The effect of fungal contamination on the proximate composition of stored maize grains was also investigated in this study. The nutritional compositions of maize have been established in many studies including those of Nuss and Tanumihardjo (2010), Suleiman *et al. (*2013), Alvi *et al*. (2013) Bathla *et al*. (2019) and Curr *et al*. (2021). All the proximate components reported in previous studies were also observed in this study however, the healthy seeds had slightly higher ash content and significantly higher crude fibre, crude protein and carbohydrate contents than the infected seeds. Apart from the common effects of fungal deterioration such as decreased seed viability, visible mouldiness, stale and musty odour, discolouration, caking and mycotoxin production, Chuck-Hernández *et al*. (2012) and Sauer (2019), observed that fungal attack can also lead to chemical and nutritional changes in stored grains and seeds. Furthermore, many of the fungal species observed in this study are known to be mycotoxins producing species especially members of the *Aspergillus sp* and *Fusarium sp.* The detrimental effects of mycotoxins on the nutritional compositions have been reported. Fumonisins produced by members of the *Fusarium sp* have been associated with reduced levels of certain B vitamins, including niacin and folate (Thielecke and Nugent, 2018). It can also interfere with protein synthesis, leading to reduced protein content and altered amino acid profiles (Berthiller*et al*., 2013). Aflatoxins produced by the members of *Aspergillus sp* can lead to the degradation of vitamins A and E, which are crucial for maintaining healthy vision and immune function (Omotayo *et al*., 2019).

Conclusion

Stored maize in Makurdi were observed to be contaminated with fungi such as *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Alterneria sp, Mucor sp* and *Fusarium sp*. Fungal attack was observed to have a significant negative effect on the proximate composition of stored maize.

Recommendation

Based on the findings of the study, the following recommendations were made:

i. Further studies should be conducted to determine the mechanism of fungal deterioration on the proximate composition of grains and foods.
ii. More sensitization and awareness programs should be created for farmers and marketers on proper storage techniques/conditions as well as management

Declaration of interest

The authors declare that no conflict of interest was perceived as prejudicing the impartiality of this research.

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References

1. Aklaku, E. K., Sowley, E. N. K. and Ofosu, M. (2020). Incidence of fungi and aflatoxin contamination of maize in Tolon-Kumbungu district of Ghana. *African Crop Science Journal*, *28*(2), 195-202.
2. Al-Shikli, R. A., Abdulrasool, A. A. and Al-Hiti, M. M. (2010). Effect of some storage conditions upon the survival of some fungal spores. *Iraqi Journal of Pharmaceutical Sciences,* 19 (2):1-10.
3. Alvi, M.B., Rafique, M., Tariq, M.S., Hussain, A. and Mahmood, T. (2013). Hybrid vigour of some quantitative characters in maize (*Zea mays* L). *Pakistan Journal of Biological Sciences,* 6: 139-141.
4. Aminu, M. and Keta, J.N. (2021). Study of Fungi on Stored Maize (*Zea mays* L.) in Kebbi State, Nigeria. *Journal of Current Opinion in Crop Science,* 2(1): 55-59.
5. AOAC, (2012). Official method of Analysis Association of Analytical Chemists. 19th Edition, Washington DC, 121-130.
6. Atanda, S. A., Pessu, P. O., Agoda, S., Isong, I. U., Adekalu, O. A., Echendu, M. A. and Falade, T. C. (2011). Fungi and mycotoxins in stored foods. *African Journal of Microbiology Research*, *5*(25), 4373-4382.

7. Barnett, H.L. and Hunter, B.B. (1972). Illustrated genera of imperfect fungi. 4th edition, APS Press, Minnesota, USA pp 1-218.
8. Bathla, S., Jaidka, M. and Kaur, R. (2019). Nitritive Value. Maize-Production and Use. *Interchopen,* 88963.
9. Berthiller, F., Crews, C., Dall'Asta, C., Saeger, S. D., Haesaert, G., Karlovsky, P. and Stroka, J. (2013). Masked mycotoxins: A review. *Molecular nutrition & food research*, *57*(1), 165-186.
10. Chuck-Hernández, C., García-Lara, S. and Serna-Saldívar, S.O. (2012). Conversion into bioethanol of insect (*Sitophilus zeamais* Motschulsky), mould (*Aspergillus flavus* Link) and sprout-damaged maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L. Moench). *Journal of Cereal Science,* 55(3): 285-292.
11. Curr, J., Aminu, M. and Naka, K. J. (2021). Study of Fungi on Stored Maize (*Zea mays L.*) in Kebbi State. *Journal of Current Opinion in Crop Science*, 2(1), 60-79.
12. Ibrahim, A. G., Baba, J., Omosimua, R. O., Abdulrahman, A. A., Sani, A.R. Dauda, D. and Bala, A. (2021). Isolation and Identification of Fungi Associated with *Abelmoshus esculentus* (Okra) Sold in Abuja, Nigeria. *Nigerian Journal of Microbiology*, 35(2): -5759 - 5765.
13. Ijeomah, A. U., Ugwuona, F. U. and Ibrahim, Y. (2012). Nutrient composition of three commonly consumed indigenous vegetables of North-Central Nigeria. *Nigerian Journal of Agriculture, Food and Environment*, **8**(1):17-21.
14. Kortei, N. K., Amanor, D., Wiafe-Kwagyan, M., Annan, T., Boakye, A. A., Essuman, E. K. and Tettey. C. O. (2022). Profile of Fungal Contaminants of Maize *(Zea Mays)* Intended for Consumption and their Potential Health Implications in the Ho Municipality of Ghana. *African Journal of Food Agriculture and Natural Development,* 22(10): 21890-21918.
15. Li, D-W., Magyar, D. and Kendrick, B. (2023). Color Atlas of Fungal Spores: a Laboratory Identification Guide. American Conference of Governmental Industrial Hygienists (ACGIH), 852.
16. Magan, N., Sanchis, V. and Aldred, D. (2014). Role of spoilage fungi in seed deterioration. *Fungal biotechnology in agricultural, food and environmental applications*, 311-323.
17. Mohammed, K. and Kumsa, F. (2021). Fungal Spoilage and Its Economic Impact on Maize (*Zea mays* L.) and Wheat (*Triticuma estivum* L) In West Shoa, Ethiopia. *Journal of Agricultural Science,* 1: 1-7.
18. Murdia, L. K., Wadhwani, R., Wadhawan, N., Bajpai, P. and Shekhawat, S. (2016). Maize utilization in India: an overview. *American Journal of Food and Nutrition*, *4*(6), 169-176.
19. Nuss, E.T. and Tanumihardjo, S.A. (2010). Maize: a paramount staple crop in the context of global nutrition. *Food Science,* 9:417-436.
20. Ode, P.1., Ogwuche, S., Adoga, S.O., Onoja, J.F. and Utume L.N. (2023). Comparative Study of Bioethanol Yield from Selected Agro-waste Feedstocks UsingFermentative *Saccharomyces Cerevisiae - Nigerian Annals of Pure and Applied* Sciences, 6 (1): 121-128.
21. Olaniyan, A. B. (2015). Maize: Panacea for hunger in Nigeria. *African Journal of Plant Science*, *9*(3), 155-174.
22. Olufunso, O. A., and Oluwole, S. A. (2019). Comparative Proximate Composition of Maize (*Zea mays L.*) Varieties Grown in South-western Nigeria. *International Annals of Science*, 7(1): 1-5.
23. Omotayo, O. P., Omotayo, A. O., Mwanza, M. and Babalola, O. O. (2019). Prevalence of mycotoxins and their consequences on human health. *Toxicological research*, *35*: 1-7.
24. Onyimba, I.A., Ogbonna, I.C., Chukwu, O.O. and Collins, C.E. (2014). Occurrence of potentially hazardous fungi in exposed spent Sorghum grains. *European Scientific Journal*, 10(9): 1857-7881.
25. Sauer, D. B. (2019). Effects of fungal deterioration on grain: Nutritional value, Toxicity, Germination. *International Journal of Food Microbiology*, 7 (3): 267-275.
26. Shehu, K., Salau, I. A. and Salisu, N. (2020). Fungal and mycotoxin contamination of stored maize grains in Kebbi state, North-western Nigeria. *Journal of Advanced Botany and Zoology*, 8: 4-8.
27. Suleiman, R. A., Rosentrater, K. A., and Bern, C. J. (2013). Effects of Deterioration Parameters on Storage of Maize in Abia. *An ASABE Meeting Presentation.* Paper Number: 131593351.
28. Thielecke, F. and Nugent, A. P. (2018). Contaminants in grain–a major risk for whole grain safety? *Nutrients*, *10*(9): 1213.