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# Diversity Index of Fungi Isolated from Six Maize (*Zea mays L*) Varieties in Rivers State, Nigeria

**Orikoha, E. C[1*]., Osakwe, J. A[1]., Nnodim, L. C.[2]**
[1]Department of Crop Science, Rivers State University, Nkpolu-Oroworukwo, Rivers State, Nigeria
[2]Department of Microbiology, Rivers State University, Nkpolu-Oroworukwo, Rivers State, Nigeria.
***Corresponding Author:** *ucharia@hotmail.com*

## ABSTRACT

The species diversity of fungi on cultivated maize varieties was carried out at the Teaching and Research Farm of the Rivers State University to identify mycotoxin-producing fungi in 6 maize (*Zea mays L*) varieties **(**Land Race (Local variety), Premier Oba Super 6, Premier Oba Super 2G, Premier Oba Super 13, SAMAZ 60, and SC719 Seedco**)**. The experiment was laid out in Randomized Complete Block Design (RCBD) having 4 replications. Isolation and identification of fungi was done following standard microbiological procedures. The total heterotrophic fungal (THF) count of fresh and dry maize ranged from $4.58x10^4$CFU/g – $12.70x10^4$CFU/g and $10.37x10^4$CFU/g – $29.83x10^4$CFU/g respectively. Results on the species diversity of fungi associated with fresh maize, *Alternaria* sp. revealed greater diversity of fungi community with Simpson_1-D of (0.89) and reflects greater diversity for both abundance and evenness of the species present with a Shannon_H value of 1.97. Fungi Dry maize storage *Aspergillus flavus* (24) with the highest value was the most abundant in diversity. *Aspergillus parasiticus* revealed greater diversity of fungi community with Simpson_1-D of (0.91) and reflected greater diversity for both abundance and evenness of the species present with a Shannon_H value of 2.07. This research study recommends that Farmers in Rivers State should apply good agronomic practices during cultivation and proper management during harvest and storage to ensure mycotoxin-free grains. To prevent mycotoxin contamination in pre-harvest, farmers should carefully study weather conditions and only practice when it is most favourable.

**Keywords:** Maize, *Zea mays*, diversity, mycoflora, mycotoxins, *Aspergillus flavus*, *A. parasiticus*.

## Introduction

Maize is one of the most important food crops in the world. The grains are susceptible to being contaminated by mycotoxin-producing fungi and maize growers are frequently challenged by fungi both in the field and storage posing a risk to food safety. Mycotoxins are toxic secondary metabolites, produced by several fungi that frequently contaminate maize in the field and/or during storage. According to Miller (1995), four types of toxigenic fungi can be distinguished: (1) Plant pathogens such as *Fusarium graminearum* and *Alternaria alternaria*; (2) Fungi that grow and produce mycotoxins on senescent or stressed plants, e.g., *F. moniliforme* and *Aspergillus flavus*; (3) Fungi that initially colonize the plant and increase the feedstock's susceptibility to contamination after harvesting, e.g., *A. flavus*; (4) Fungi that are found on the soil or decaying plant material that occurs on the developing kernels in the field and later proliferate in storage if conditions permit, e.g., *P. verrucosum* and *A. ochraceus*.

*Aspergillus flavus* is an opportunistic fungal pathogen that infects developing maize kernels, attacking plants that are weakened by environmental stresses such as drought and heat. The disease reduces grain quality and contaminates the kernel with the carcinogenic mycotoxin aflatoxin (Scheidegger, 2003; Payne, 2010; Dolezal *et al.*, 2013; Hruska *et al*., 2013; Kew, 2013).



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The consumption of corn contaminated by mycotoxins may cause several severe toxic effects in both animals and humans.

The International Agency for Research on Cancer (IARC) has classified AFB1 as a carcinogen to humans (group 1) and fumonisin B1 (FB1) and OTA as possible human carcinogens (group 2B), IARC (2002). The most relevant fungal genera affecting maize are *Aspergillus and Fusarium* (Nyangi *et al.*, 2016). Sobel (2017) is often confused with the closely related species, *A. flavus*, *A parasiticus* that has defined morphological and molecular differences. Hockin (1999) reported that *Aspergillus parasiticus* is one of three fungi able to produce the mycotoxin aflatoxin. Exposure to *A. parasiticus* toxins can cause delayed development in children and produce serious liver diseases and/or hepatic carcinoma in adults. Among the mycotoxin-producing species, the most relevant being *Fusarium verticillioides* and *F. proliferatum*, the main Fumonisins (FUM) producing species and *F. graminearum*, which produces Trichothecenes **(**TCTs) and Zearalenone (ZEA). It damages the host plant severely causing a decrease in quality and productivity. *Fusarium* sp are ubiquitous mostly soil-borne pathogens which affect plant development throughout the cultivation period (Mansfied, 2005).

## Materials and Methods

### Study Area

The experiment was conducted in Rivers State University Teaching and Research farm. The farm lies between latitude 4.5°N and longitude 7.0°E on an elevation of 18m above sea level in the humid rainforest region of Southern Nigeria. The climate of the area is tropical with two prominent seasons, the wet (rainy) and dry seasons. The dry season is short, usually lasting for 4 months, from November to March, with little rains during this period, while the longer wet season prevails during the remaining months. The mean annual rainfall in Port Harcourt ranges from about 3,000mm to 4,500mm (FAO, 1984). Annual maximum temperature ranges from 22°C to 29°C while relative humidity varies between 75% and 95%. Port Harcourt represents soils of coastal plain sands which have brown to dark brown colour.

### Sample collection

A total of six (6) maize varieties were used for the experiment. Five 5 varieties of maize (Premier Oba Super 6, Premier Oba Super 2g, Premier Oba Super 13, SAMAZ60, SC719 Seedco) were collected from the National Agricultural Seeds Council Abuja Nigeria (NASC), and a local variety was bought from a market in Port Harcourt, Rivers State.

### Experimental Design

The experimental design used was laid out Randomized Complete Block Design (RCBD. The six (6) maize varieties were replicated four (4) times on the design having a total of twenty-four (24) treatments. Treatment 1: Landrace (Local variety), Treatment 2: Premier Oba Super 6, Treatment 3: Premier Oba Super 2g, Treatment 4: Premier Oba Super 13, Treatment 5: SAMAZ 60, Treatment 6: SC719 Seedco.

### Isolation and identification of fungi

Isolation and identification of fungi from maize samples were done according to the method of Okioma *et al*. (2021). For two minutes, 70% ethanol was used to surface sterilize the maize samples. To get rid of the ethanol on the surface, the samples were rinsed with distilled water. Blot drying was done on the maize samples. One kilograms of each sample of maize was ground in a dry mill household blender.
An orbital shaker was used to shake one gramme of each ground maize sample in nine millilitres of sterile distilled water, and the samples were serially diluted up to $10^{-2}$. Potato Dextrose Agar (PDA) was plated using 0.1 mL aliquots, and the plates were incubated for 7 days at 28°C. The fungal isolates were sub-cultured on PDA. The fungal isolates were enumerated using a formula;

$$\frac{\text{CFU}}{\text{g}} = \frac{\text{Number of colonies of a fungal species}}{\text{Amount plated x Dilution factor}}$$

Macroscopic and microscopic characteristics were used in identifying the fungal isolates, using fungal identification keys.

### Statistical analysis

The fungi species diversity indices analysis was determined using Paleontological Statistics (PAST) version 4.17c.



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## Results

Table 1 shows the Total Heterotrophic Fungal (THF) count of fresh and dry maize on the six maize varieties. The THF count of fresh and dry maize ranged from 4.58 – 12.70 ($x10^4$ CFU/g) and 10.37 – 29.83 ($x10^4$ CFU/g), respectively. In fresh maize samples, the Local variety recorded the highest mean value of 12.70 ($x10^4$ CFU/g) compared to other maize varieties. There were no significant differences between THF count of SC719 (8.37), Premier Oba Super 2g (7.87) and SAMAZ 60 (6.66).

The Premier Oba Super 6 (7.87) THF count was significantly higher than Premier Oba Super 2g (4.58) ($P< 0.05$) at 95% confidence level. The THF count of dry maize showed that the Premier Oba Super 6 recorded the highest mean value of 29.83 ($x10^4$ CFU/g) which was followed by Premier Oba Super 2g (26.00), SC719 (22.79) and Premier Oba Super 13(21.38) showed no significant difference, but the THF count of SAMMAZ 60 (17.54) was significantly different from Local variety (10.37) ($P< 0.05$) at 95% confidence level.

**Table 1: Total Heterotrophic Fungi (THF) Count on the Fresh Maize of the Six (6) Maize Varieties**

| ***Zea mays* Variety** | **Total Heterotrophic Fungi (THF) Count ($x10^4$ CFU/g)** | |
|---|---|---|
| | **Mean fresh maize** | **Mean dry maize** |
| Local variety | $12.70\pm2.56^{a}$ | $10.37\pm2.24^{d}$ |
| Premier Oba Super 13 | $8.37\pm2.48^{b}$ | $21.38\pm5.04^{bc}$ |
| Premier Oba Super 2g | $7.87\pm1.96^{b}$ | $26.00\pm8.98^{ab}$ |
| SAMMAZ 60 | $6.66\pm1.46^{bc}$ | $17.54\pm5.88^{c}$ |
| Premier Oba Super 6 | $5.41\pm2.66^{cd}$ | $29.83\pm4.77^{a}$ |
| SC719 (Seedco) | $4.58\pm1.88^{d}$ | $22.79\pm5.48^{b}$ |
| P-value | < 0.05 | < 0.05 |

*Means that do not share a letter are significantly different (Turkey Pairwise Comparisons 95% confidence level)

The genera of fungi associated with fresh and dry maize are as following; *Alternaria* sp., *Fusarium* sp., *Penicillium* sp., *Aspergillus* sp., *Rhizopus stolonifera*, Yeast, *Penicillium citrinum*, *Aspergillus niger*, *Aspergillus flavus* and *Fusarium graminearum*. The dry had more fungi genera presence than in the flesh. *Rhizopus stolonifer* and *Fusarium* sp. were commonly found in fresh and dry maize as shown in Table 2**.**

The diversity index of fungi species associated with fresh maize is represented in Table 3. The Taxa_S (species richness) for fresh maize fungi recorded ranged from 2 – 6; and the fungal species *Aspergillus flavus*, *Rhizopus* sp., Yeast, *Penicillium* sp. and *Alternaria* sp. recorded species richness value of 6, respectively while *Fusarium* sp. recorded species richness of 2. Fungi species individuals' diversity indices values range from 8 – 42; *Aspergillus* sp. (42) while *Fusarium* sp. (8), respectively.

*Aspergillus* sp. recorded the highest individual diversity index. *Alternaria* sp. revealed a greater diversity community (Simpson_1-D) of (0.89). The Shannon_H value of the fungi species ranged from 0.43 - 1.97.

The result in Table 4 represents the diversity index of fungi associated with fresh maize, obtained using the following biodiversity indices. Fungi maize storage Taxa_S (species richness) recorded ranged from 5 – 6; and the fungal species *Yeast*; *Aspergillus flavus*, Yeast, *Penicillium citirnum*; *Alternaria* sp. *Aspergillus nige*; *Fusarium graminearum*; *Aspergillus parasiticus*; *Rhizopus stolonifer* and *Fusarium oxysporum* recorded 6, respectively. Species individuals' diversity indices range 11 – 24; *Aspergillus* sp. (24). *Aspergillus parasiticus* revealed a greater diversity community (Simpson_1-D) of (0.91) The Shannon_H value of the fungi species ranged from 1.73 - 2.07.



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**Table 2: Morphological and cultural characteristics of the fungal isolates**

| Maize | Macroscopic Characteristics | Microscopic Characteristics | Identified Organism |
|---|---|---|---|
| Dry maize | Green dusty growth with brown reverse | Highly branched septate hyphae with conidiophores and phialides with paired branches | *Aspergillus flavus* |
| | Green dusty growth with brown reverse | Poreling hyphae with round conidia head | *Mucor* sp |
| | Green lawns growth with redial periphery and brown reverse | Septate branching hyphae with chair like conidia spores present | *Penicillium* sp |
| | Gray colour dusty growth | Septate branching hyphae with chain-like conidia pores present | *Aspergillus* sp. |
| | Gray and black fluffy growth with brown reverse. | Aseptate hyphae with round conidia head | *Rhizopus stolonifer* |
| | White fluffy growth with yellow reverse | Canoe shaped conidia, with septate hyphae | *Fusarium oxysporium* |
| | Orange color lawny growth with brown reverse | Septate branching hyphae with chain-like conidia spores present | *Penicillium* sp |
| | Brown (light) fairy growth with wrinkled surface and brown reverse | Septate branching hyphae with chain-like conidia pores present | *Aspergillus flavus* |
| | Black cottony growth with white radial periphery and brown reverse | Septate hyphae with round conidia head spores present | *Rhizopus stolonifer* |
| | Brown fluffy growth with pink reverse | Canoe shaped conidia, with septate hyphae | *Fusarium* sp |
| | Dark grey lawny elevated growth with wrinkled surface and black reverse | Ellipsoidal conidial with septate branching hyphae | *Alternaria* sp |
| | Green lawns growth with white wrinkled periphery. | Septate branching hyphae with chain like conidia | *Penicillium* sp |
| Fresh maize | White fluffy growth with yellow reverse | Canoe shaped conidia, with septate hyphae | *Fusarium oxysporium* |
| | Gray and black fluffy growth with brown reverse | Aseptate hyphae with round conidia head | *Rhizopus stolonifer* |
| | Dark grey lawny elevated growth with wrinkled surface and black reverse | Ellipsoidal conidial with septate branching hyphae | *Alternaria* sp. |
| | Green lawns growth with white wrinkled periphery | Septate branching hyphae with chain like conidia | *Penicillium* sp. |



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**Plate 1:** ***Aspergillus flavus***

**Plate 2:** ***Rhizopus stolonifer***

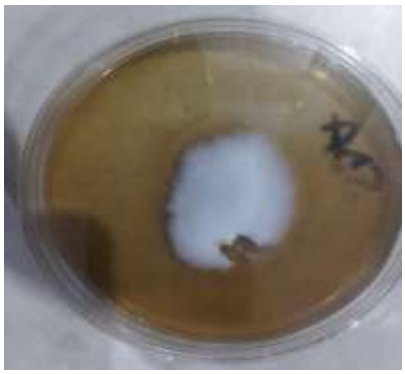

**Plate 3:** ***Fusarium oxysporum***

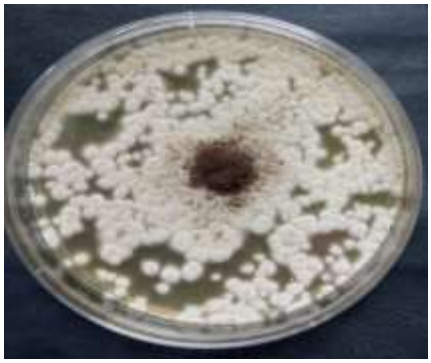

**Plate 4:** ***Aspergillus niger***

**Plate 5:** ***Fusarium graminearum***

**Plate 6:** ***Fusarium*** **sp.**

**Plate 7: Yeast**

**Plates 1 – 7: Macroscopic morphology of fungi isolated from maize**

**Table 3: Diversity indices of fresh maize infected with fungi**

| Fungi species | Taxa_s | Individual | Simpson 1-D | Shannon_H |
|---|---|---|---|---|
| *Aspergillus* sp. | 6 | 42 | 0.84 | 1.83 |
| Yeast | 6 | 20 | 0.86 | 1.89 |
| *Penicillium* sp. | 6 | 18 | 0.87 | 1.91 |
| *Alternaria* sp. | 6 | 13 | 0.89 | 1.97 |
| *Fusarium* sp. | 2 | 8 | 0.25 | 0.43 |
| *Rhizopus stolonifer* | 6 | 21 | 0.87 | 1.90 |



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**Table 4: Diversity indices of dry maize storage fungi**

| Fungi species | Taxa_s | Individual | Simpson 1-D | Shannon_H |
|---|---|---|---|---|
| *A. flavus* | 6 | 24 | 0.86 | 1.89 |
| Yeast | 5 | 12 | 0.84 | 1.73 |
| *P. citrinum* | 6 | 23 | 0.86 | 1.89 |
| *Alternaria* sp | 6 | 15 | 0.87 | 1.91 |
| *A. niger* | 6 | 17 | 0.84 | 1.83 |
| *F. graminearum* | 6 | 15 | 0.85 | 1.87 |
| *A. paraciticus* | 6 | 10 | 0.91 | 2.07 |
| *R. stolonifer* | 6 | 20 | 0.85 | 1.84 |
| *F. oxysporum* | 6 | 11 | 0.85 | 2.00 |

## Discussion

The high fungal load of $10^4$ was associated with fresh and dry maize. This indicates that maize either fresh or dry are conducive environment for fungal isolates. This finding is in collaboration with the report of Omonigho & Obatusin (2017), recording fungal count that ranged from $10^3$ -$10^4$ (CFU/g). This research study observed the fungi genera associated with maize in this research study are *Aspergillus flavus, Fusarium oxysporum, Yeast, Mucor* sp., *Aspergillus parasiticus, Peniciluim citrinm , Alternaria* sp., and *Rhizopus* sp were among those isolated in the findings of (Dawlal *et al*., 2012; Fisher *et al*., 1992; Okioma *et al*., 2021; Mansfied, 2005).

This study reported association of *Aspergillus flavus,* and *Rhizopus stolonifer* in maize storage. The findings collaborate with the report of fungal contamination in Ebonyi State, Nigeria Egwurochi *et al.* (2015). The isolation of *Rhizopus* sp., *Mucor* sp., *Penicillium citrinum, Fusarium oxysporum, fusarium graminearum* and *Aspergillus flavus, Aspergillus nigea* in this study agrees with the work of Onyeze *et al*. (2013) in their study of "isolation and characterization of fungi associated with the spoilage of corn (*Zea mays*) in Enugu.

Also, the presence of these fungi is in line with the works of Amadi and Adeniyi (2009) and (Okioma, *et al*., 2021) isolated *Rhizopus sp. Penicillium* sp, *Fusarium* sp and *Aspergillus* sp in their study. The presence of these isolates will not be unconnected with the fact that this important food stuff is poorly stored leading to its contamination either from the environment or handlers.

This position is in line with the works of Onyeze *et al*. (2013) when they opined that the conditions to which corn is exposed in the field and store, as well as the storage method used to preserve it have effects on the type, rate, and extent of infection of the corn by fungi. However, the rate and degree of spoilage has been shown to be higher under moist or high humidity conditions (Onyeze*et al*., 2013).

The most abundant were *Aspergillus flavus, Fusarium oxysporum, Fusarium graminearum* and *penicillium citrinum* in this study. The study collaborates with the reported of *A. flavus* as traditionally considered storage fungus that can infect maize both pre-harvest and during storage, and an increase in aflatoxin content is likely if the drying and storage conditions are not appropriate (Qi *et al*., 2023; Victor *et al*., 2013; Chulze, 2010).

In conclusion, this study has given insight into identification of fungi diversity in cultivated maize varieties. *Aspergillus flavus, Aspergillus parasiticus, Aspergillus* nigea, *Fusarium oxysporum, Fusarium graminearum, Penicillium citrinum, Mucor, Yeast, Alternaria* and *Rhizopus* species were detected in harvest and storage.

The presence of *Aspergillus flavus* was much more frequent and occurred at all stages with comparison to *Fusarium oxysporum, Fusarium graminearum* and *Penicillium citrinum*. Farmers in Rivers State should apply good agronomic practices during cultivation and proper management during harvest and storage. To prevent or reduce fungal contamination in pre-harvest and post-harvest practice when it is most favourable and conscious of fungi striving and survival in maize.



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