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Detection of Fusarium Verticillioides in Maize Sold at Some Markets in Ibadan Metropolis, Nigeria

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Abstract
Fusarium is a prominent fungal genus connected with maize in Africa. In this genus, there are two most common species (F. verticillioides and F. proliferatum) that have many toxigenic features and produce fumonisins. Fumonisins are a collection of economically significant mycotoxins that are widely found in maize-based foods and feeds across the globe. This preliminary study was conducted to detect the occurrence of Fusarium verticilliodes in infected maize collected from four main markets in the Ibadan metropolis. A total of ninety-five (95) maize samples were collected from three major commercial markets in Ibadan. Maize seeds were prepared following standard sterilization procedure after which, ten (10) seeds each were then plated on PDA media in triplicates and incubated at 25 ^{o}C for six days. Identification of isolates was performed using conventional cultural and morphological characteristics. Results show that the maize samples obtained from Apata market had the highest occurrence of Fusarium verticilliodes with 28.2% while the least occurrence of 22.3% was found in maize samples obtained from Bodija market. Other species of fungi, Aspergillus flavus, Aspergillus niger, and Botryodiplodia Theobroma, were also detected with a negligible count. The study also revealed that there was a significant difference at p = 0.002 in the occurrence of Fusarium verticilliodes between the yellow and white maize seeds studied. These findings call for great concern as it detected that maize and maize-based feed consumed could be infected with Fusarium and fumonisin toxin.

Keywords: Maize, Fungal infection, *Fusarium verticilliodes*, Fumonisins, Occurrence,

INTRODUCTION
Maize (*Zea mays L.*) is a grain crop that is planted worldwide because of its high yields per hectare, ease of cultivation, resilience to a variety of agro-ecological zones, diverse food

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applications, and storage properties. It is a major part of the diet of millions of people in African countries (Saleem *et al*., 2022). However, this grain can be susceptible to spoilage organisms. Among these, fungi are considered to be ranked second in terms of maize destruction and loss (Penagos-Tabares *et al*., 2022). According to Fandohan, *et al.* (2003), fungi can cause 50–80% of damage to farmers' maize during preservation if conditions are suitable for their growth. In the tropical regions of the world, *Fusarium, Aspergillus*, and *Penicillium* are the most prevalent genera found in maize (Getachew *et al*., 2018). In soils, *Fusarium* species are common (Kamali-Sarvestani *et al*., 2022) They are widely thought to be field fungi that infest more than half of maize grains before harvest (Temesgen, 2019). Several phytopathogenic species of *Fusarium* that are commonly associated with maize are *Fusarium verticillioides* (Sacc.) Nirenberg, *F. proliferatum* (Matsushima) Nirenberg, *F. graminearum* Schwabe, and *F. anthophilum* (A. Braun) Wollenweber (Fandohan *et al*., 2003; Maheshwar & Janardhana, 2010; Okoth & Siameto, 2010). *Fusarium verticillioides* is likely to be the most prevalent species identified with infected maize in the world (Duan *et al*., 2016). There is a paucity of information on *F. verticillioides* incidence in maize in Africa. However, investigations into a fungal infection of maize detected *F. verticillioides* to be the most common fungus of maize in African countries (Fandohan *et al*., 2003; Koskei, 2022; Mukanga *et al*., 2010). *Fusarium verticillioides* is a maize endophyte that forms long-term relationships with the plant (Chowdhury *et al*., 2019). The existence of the fungus in leaves, stems, roots, and grains might be completely asymptomatic, and the occurrence of the fungus is often overlooked since it does not induce any evident damage to the plant (Fandohan *et al*., 2003). *Fusarium verticillioides* attack maize at all stages of growth, affecting grain rot for the period the pre-harvest and post-harvest, via contaminated seed, the silk channel, or wounds (Asran & Aboul-Nasr, 2011). *Fusarium* infection in maize is of great agricultural and economic importance causing the fungal disease of maize which has been reported to affect maize at all stages of plant development. Moreover, some species of *Fusarium* in infected maize produce fumonisins, recently discovered mycotoxins, which are of great health risks such as oesophageal and liver cancer in humans and leukoencephalomalacia in horses and pulmonary oedema in pigs (Fandohan *et al* 2003). Therefore, the study aimed to detect the occurrence of *Fusarium verticillioides* in maize sold in Ibadan main commercial markets.

MATERIALS AND METHODS

Sample Collection

A total of 95 maize samples were randomly collected from four (4) different main markets in Ibadan, Oyo state Nigeria. These main markets included: Bodija, Ojoo, Apata and Oja Oba Market. The maize samples were yellow and white varieties. The distribution of which is explained in Table 1.

Sample Preparation

Maize seeds were prepared following the procedure employed by Alakonya *et al.* (2008) with slight modification. Seeds from each sample were dipped in 70% ethanol, and were further sterilized in 10% sodium hypochlorite for 60 seconds before being rinsed twice in sterile distilled water, followed by drying of the sample with sterilized filter paper. About ten seeds were plated in triplicate on a PDA medium and cultured at 25 °C for six days.

Isolation and Identification of fungal isolates

Fusarium verticilloides were isolated using a conventional approach developed by Guo *et al* (2022). Identification of fungal isolates was performed using cultural and morphological characteristics. The formula in equation (1) employed by Sebayang *et al.* (2021) was used to calculate the occurrence of *F. verticilloides.*

$$\text{Incidence} = \frac{\text{no of germinated seeds with infection}}{\text{total number of germinated seeds}} \text{x100}\ldots\ldots\ldots\ldots\ldots\ldots\text{equation (1)}$$

Table 1: Sample size of maize collected from different main markets in Ibadan, Oyo State

Sample Source	Maize Variety			
	Yellow	White	Sample Size	Sample Mean
Bodija Market	12	11	23	11.50
Ojoo Market	13	11	24	12.00
Apata Market	11	17	28	14.00
Oja Oba	12	8	20	10.00
Total	**48**	**47**	**95**	**47.50**

RESULTS AND DISCUSSION

The results in Table 2 reveal that more than 50% of the assessed seeds germinated with fungal infection. The highest fungal infection (28.2%) was observed in maize collected from Apata market while the least fungal infection (22.3%) was recorded from the maize samples collected from Bodija market. Moreover, the Table further depicts different identified fungal isolates which included *Fusarium verticillioides, Aspergillus flavus, Aspergillus niger* and *Botryodiplodia theobroma*. Figure 1 shows that from among all the fungal isolates identified, *Fusarium verticillioides* showed the highest percentage occurrence in both yellow and white maize with 98 % and 95.7 % respectively. The mean incidence of *Fusarium verticillioides* in maize varieties collected from different main markets in Ibadan is presented in Figure 2. The figure depicts the highest incidence of 69 % in yellow maize and 70 % in white maize varieties obtained from Apata market and the least incidence of 58 % in yellow and 50 % in white maize varieties obtained from Bodija market. Table 3 depicts the statistical analysis using regression. It shows that there was a strong positive correlation between the yellow and white maize varieties, although, there was a significant difference at p-value = 0.002 in terms of incidences of *Fusarium verticillioides* between yellow and white maize varieties.

Table 2. Mean number of seeds germinated with infection and identified causative agent of infection

Source of Seed	Mean seeds planted per variety	Mean seeds germinated		Mean seeds germinated with infection		The causative agent of infection identified (n)		*F. verticilloides* (%)
		Yellow maize	White maize	Yellow maize	White maize	Yellow maize	White maize	
Bodija Market	10	10	10	5.8	5.0	*Fusarium verticillioides* (22), *Aspergillus flavus* (1)	*Fusarium verticillioides* (20)	42 (22.3%)
Ojoo Market	10	10	9.8	6.3	5.8	*Fusarium verticillioides* (24), *Aspergillus niger* (1)	*Fusarium verticillioides* (22), *Aspergillus flavus* (2)	46 (24.5%)
Apata Market	10	9.8	10	6.8	7.0	*Fusarium verticillioides* (27)	*Fusarium verticillioides* (26), *Botryodiplodia theobroma* (2)	53 (28.2%)
Oja Oba	10	10	10	6.3	5.8	*Fusarium verticillioides* (25)	*Fusarium verticillioides* (22)	47 (25.0%)
Total of *F. verticilloides* (%)								188 (100%)

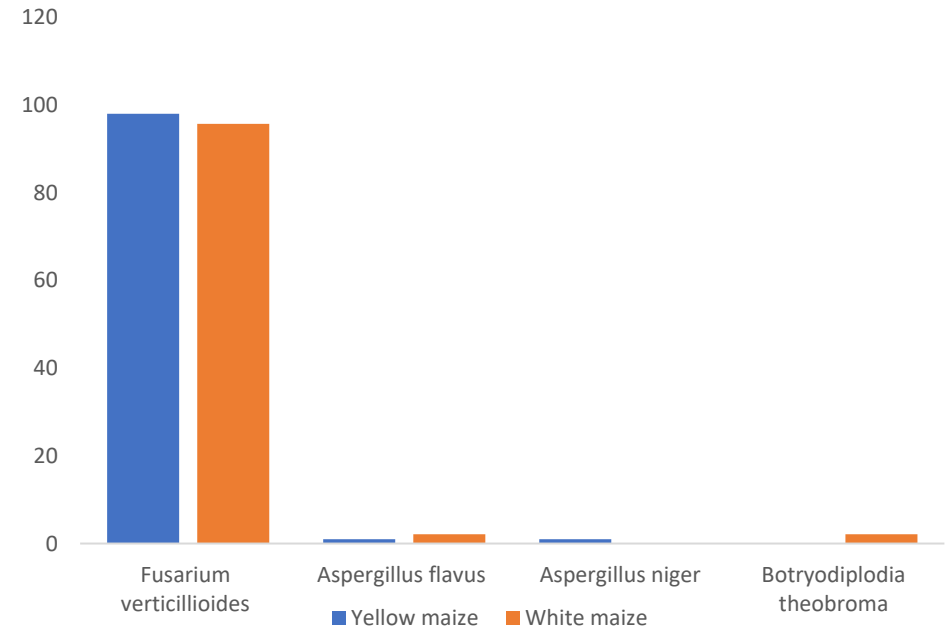

Figure 1. Percentage distribution of fungal isolates in infected maize purchased from Ibadan main markets

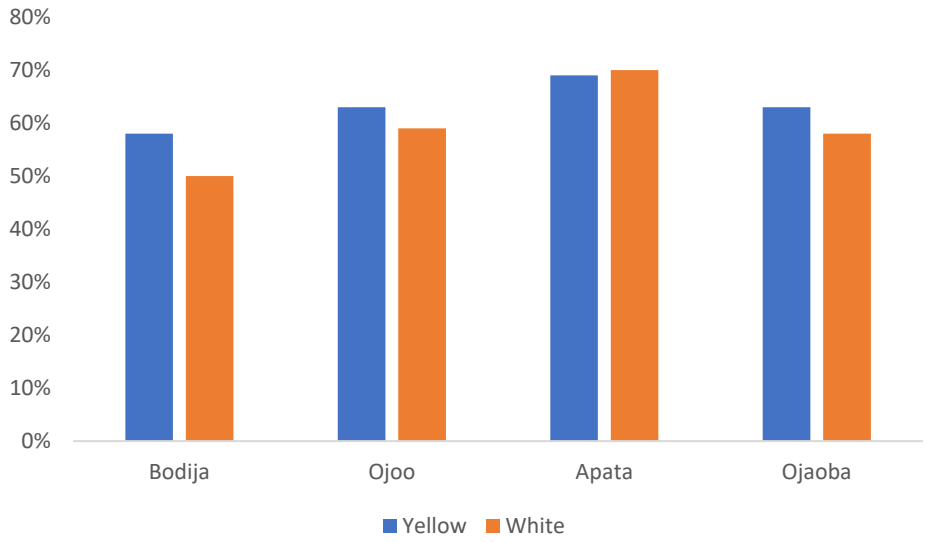

Figure 2: Mean incidence of *Fusarium verticillioides* in maize varieties collected from main markets in Ibadan Metropolis

Table 3: Determination of relationship in occurrences of *Fusarium* in yellow and white maize varieties

Regression Statistics	
Multiple R	0.99791
R Square	0.99581
Adjusted R Square	0.99372
Standard Error	0.00356
Observations	4

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.00605	0.00605	476.3325	0.002093
Residual	2	2.54E-05	1.27E-05		
Total	3	0.006075			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0.30885	0.014936	20.67	0.002	0.24459	0.37312	0.24459	0.373117
X Variable 1	0.54624	0.025028	21.83	0.002	0.438552	0.65393	0.438552	0.653926

The main objective of this study was to determine the occurrence of *Fusarium verticillioides* in maize samples collected from main markets in Ibadan. Most consumers of maize usually buy in retail from the markets, and storage conditions have been identified as one of the elements that may impact fungal infection and subsequent production of fumonisin. Changes in rainfall patterns and stress during the final stages of maize grown in the field before harvest, temperature and moisture conditions during the growing season, maize cultivar and grain characteristics such as color, endosperm type, chemical composition, and stage of maize development are among other environmental factors (Kimmelshue *et al.*, 2022). The high incidence of 69 % and 70 % observed in yellow and white maize obtained from Apata market could pose a high health risk to consumers who will unknowingly purchase these seeds for different purposes. Contaminated maize seeds by fungal genera have been associated with several diseases in animals including pulmonary Oedema in pigs, and leukoencephalomalacia in horses (Fandohan *et al*., 2003).

Both the white and yellow maize samples purchased and examined for the possible presence of fungi in this study showed infection with *Fusarium verticillioides* after germination in Petri-dishes. A higher incidence of *Fusarium* was detected in yellow maize than in white maize, and the observed difference between the two maize varieties was statistically significant (p-value = 0.002). This negates the findings of Pilu *et al.* (2011) who reported that the accumulation of flavonoid pigments in the maize seeds, in particular phlobaphenes, has the potential to reduce the level of fumonisin B1. Also, a study by Landoni *et al*. (2020) suggested that the *P1* gene plays a critical role in regulating phlobaphenes accumulation in maize kernels, and indirectly, also tackles mycotoxins accumulation.

The influence of biotic factors on seeds in storage cannot be overlooked. Insects tend to cause injury to the seeds thereby increasing the ability of fungal species to bypass the protective

outer layer of the maize seeds, and gain entrance as well as establishing infections in the vulnerable interior (van Loon *et al.,* 2006). This may be a possible explanation for the high incidence of *F. verticillioides* observed in this study. A similar report by Warfield and Gilchrist (1999) indicated that Grain age may also be a factor that can influence *Fusarium* infection, and subsequently fumonisin production in maize. Although, other species of fungi were also seen their incidence was negligible. The study, therefore indicates that many of the maize sold in the market are infected with a fungal infection which is of a great health risk as maize is a major food in human nutrition and animal feeds. Therefore, control measures must be put in place to eradicate these occurrences of pathogenic fungi in the maize grains sold for human consumption.

CONCLUSION

The study has revealed the incidences of *Fusarium verticillioides* as over 50% of the maize samples collected from main markets in Ibadan are infected. Also, the study showed that there was a significant difference in the infectivity of white and yellow maize. It is therefore concluded that the maize samples implicated with fungal infection are of public health importance. Hence the need for effective management and control of *Fusarium* infection in maize production

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