

Assessment of toxigenicity of *Aspergillus flavus* isolated from ginger and turmeric in Port Harcourt

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ABSTRACT

An experiment was conducted to determine the occurrence of toxigenic isolates of *Aspergillus flavus* in ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*) commonly marketed in Port Harcourt, Nigeria. Seventy two (72) samples each of ginger and turmeric were procured from four major markets in Port Harcourt namely; Fruit Garden (D-Line), Mile One, Rumuokoro and Oil Mill markets between December 2017 and February 2018. Rhizomes of ginger and turmeric were sliced on a sterilized work table in the laboratory with a sterile scalpel into smaller particles (5 mm), surface sterilized with 2% sodium hypochlorite solution for 2 minutes and rinsed in three changes of sterile distilled water, placed on Malt Extract Agar and incubated for 5 days. A pure culture of each colony type on each plate was obtained and maintained. *Aspergillus flavus* isolates were identified microscopically and morphologically in line with standard methods. The isolates were further cultured on Coconut Agar Medium (CAM) to test for their toxigenicity. Results showed that 34 isolates of *A. flavus*, ginger (18) and turmeric (16) were recovered. On CAM, 14 (41%) of the isolates had yellow pigmentation and blue-green fluorescence under UV light thus confirming their toxigenicity. Toxigenicity of the *A. flavus* varied from market to market with Fruit Garden market having the highest incidence of 60% and 45%, in ginger and turmeric, respectively. Poor post-harvest handling and storage of ginger and turmeric in Port Harcourt markets could increase the risk of contamination with aflatoxins, thus management strategies for quality control of foods and food products should be maintained in all the markets.

Keywords: *Aspergillus flavus*, Coconut agar medium, *Curcuma longa*, isolate, toxigenic, *Zingiber officinale*.

INTRODUCTION

Aspergillus flavus is a large genus widely distributed in nature and is largely found in cereal and other grains. *A. flavus* grows on crops before harvest and during storage (Saini and Kaur, 2012). The growth of *A. flavus* is seriously affected by environmental condition such as relative humidity and temperature (Giorni *et al*, 2012). Some species *A. flavus* have potential to produce

mycotoxin such as aflatoxin. Aflatoxins are very slightly soluble in water, insoluble in non-polar solvents, and soluble in moderately polar organic solvents such as chloroform, methanol and extremely soluble in dimethyl sulfoxide (Bertuzzi *et al.*, 2012). The production of an orange-yellow pigmentation of the mycelium on plates prior to the appearance of blue fluorescence is an estimator of toxin producing isolates (Lin & Dianese 1976). Toxigenic

Aspergillus is produced only by a closely related group of Aspergilli: *A. flavus*, *A. parasiticus* and *A. nomius* strains (Moss, 2002). Other species such as *A. bombycis*, *A. pseudotamari* and *A. ochraceoroseus* are also toxigenic, but at less frequency (Ito et al., 2001). Consumption of contaminated products such as ginger and turmeric infected by toxigenic fungi are of serious concern worldwide. The effect of this can lead to serious threats to human and animal health by causing diverse complications such as teratogenicity, hepatotoxicity and immunotoxicity (Amaike and Keller, 2011; Roze et al., 2013).

Ginger, turmeric, coriander and red chilli are important spices widely used in cooking especially in Asia and most parts of Africa. In Nigeria for instance, ginger and turmeric are commonly used in most cookery. Nigeria ranks the fourth world producer of both spices, with northern parts such as; Gombe, Bauchi, Benue, Nassarawa and Kaduna States being the highest producers. In literature, it has been recorded that these spices contain a number of unique organic phytochemical ingredients that have medicinal values (Egbuchua and Enujeke, 2013) and are widely used in traditional medicine (Sherman and Billing, 1999).

These spices are exposed to contamination with aflatoxin producing fungi during growth and development, drying, storage, transport and processing (Elshafie et al., 2002) and in the market due to poor environmental conditions. Studies have shown that ginger and turmeric samples were found to be contaminated with toxigenic strains of *Aspergillus flavus* (Madhyastha and Bhat, 1985). A similar work using the UV light method on toxigenic *Aspergillus flavus* producing strains from poultry feeds showed that 52% of isolates from 180 samples were toxigenic, while 48% of isolates were non-toxigenic (Raed et al., 2016). Though many methods have been used to identify *Aspergillus* spp, morphological and microscopic examinations are still fundamental to their

identification. The production of blue fluorescence on coconut agar medium (CAM) under UV light has been used qualitatively for the identification of toxigenic *Aspergillus flavus* (Davis et al., 1987; Abbas et al., 2004; Atanda et al., 2011). The aims of this study were to isolate and identify *Aspergillus flavus* from ginger and turmeric commonly consumed in Port Harcourt, Nigeria and examine the isolated *Aspergillus flavus* for toxigenicity.

MATERIALS AND METHODS

Sample Collection

Ginger and turmeric samples were obtained from four open markets in Port Harcourt namely: Fruit gardens (D-Line), Mile One, Rumuokoro, and Oil Mill market. Sampling was done between December, 2017 and February, 2018, which correspond to the dry season. Seventy two (72) samples of each were thoroughly washed, dried and kept in sterile polythene bags at room temperature (26-28°C) in the laboratory of the Department of Crop and Soil Science, University of Port Harcourt.

Isolation and Identification of *A. flavus*

Rhizomes of ginger and turmeric were sliced on a sterilized work table with a sterile scalpel into smaller particles (5 mm) and were surface sterilized in 2% sodium hypochlorite solution for 2 mins and washed aseptically with 4 changes of sterilized water and plated on Malt Extract Agar (MEA) amended with 0.2g Chloramphenicol/litre to suppress bacterial growth and incubated for 5 days. Colonies of different *Aspergillus* spp were observed on plates. Pure cultures of each colony type was obtained and maintained according to Ibrahim and Rahma, (2009). The identification of *A. flavus* was done microscopically, based on the morphology of spores and conidial, colony growth and with reference to Pitt & Hocking (2009).

Detection of toxigenic *A. flavus*

A. flavus isolates were screened using Coconut Agar Media (CAM) (Davis et al. (1987). Two hundred and fifty (250) mL of

coconut cream (Sainsbury's, UK) were mixed with 250 mL of sterile distilled water using a heated stirrer at 75°C. 10 g of Technical Agar No.3 (Sigma Aldrich, USA) and 0.16 g of chloramphenicol was added to the solution. The medium was autoclaved and poured into 9 cm Petri plates. After cooling at room temperature, the plates were inoculated with a suspension of each isolate of *A. flavus* containing 1.3×10^5 spores/mL. The plates were then incubated at room temperature $27 \pm 2^\circ\text{C}$ for 7 days. At 3 – 5 days, they were examined for orange-yellow pigmentation and at the reverse side for the presence or absence of a blue fluorescent ring under UV light, characteristic of aflatoxin-producing strains (Lin and Dianese, 1976). For comparison, *Aspergillus*

flavus strain NRRL 3357 (a known toxin producing strain) was used as control.

RESULTS

Incidence of *A. flavus*

The result shows the presence of *A. flavus* in the samples. Turmeric samples from Fruit Gardens (D-Line) in December had the highest occurrence of 25% with the lowest observed in February (6.1%) (Table 1). Ginger samples from Fruit Garden (January), Mile One (December) and Rumuokoro (December) had 22% incidence each. The same was observed for turmeric samples in fruit garden (February), Mile one (February) and Rumuokoro (December) with 19% incidence each (Table 1). Oil Mill market had no incidence of *A. flavus* contamination in both crops.

Table 1. Incidence of *A. flavus* in ginger and turmeric across markets in Port Harcourt.

Market	Month	% Incidence	
		Ginger	Turmeric
Fruit Garden	December	-	25.0
	January	22.2	12.5
	February	11.1	18.8
Oil Mill	December	-	-
	January	-	-
	February	-	-
Mile One	December	22.2	-
	January	5.6	-
	February	16.7	18.8
Rumuokoro	December	22.2	18.8
	January	-	-
	February	-	6.1

Production of orange-yellow pigmentation and blue fluorescence by toxigenic *A. flavus* isolates on coconut agar medium (CAM)

A total of 34 isolates of *A. flavus* (16 and 18 from ginger and turmeric, respectively) were obtained from the 72 samples across the four markets in Port Harcourt. Toxigenic and non-toxigenic *A. flavus* isolates were

detected using UV light at 365nm. *A. flavus* NRRL 3357 a relatively potent aflatoxin producer had orange-yellow pigmentation and blue fluorescence produced on the reverse side of the colonies in 2 days. The isolates of the experiment took 4–5 days to produce a blue fluorescent. At incubation longer than 7 days, it was difficult to evaluate because mycelial growth had reached the margin of the plate and rendered

the blue fluorescence invisible. For ginger, 5 of the isolates were toxigenic and 13 of the isolates were non-toxigenic representing 28% and 72%, respectively (Table 2). While in turmeric, out of the 16 isolates, 9 were

toxigenic while 7 were non-toxigenic representing 56% and 44%, respectively (Table 3).

Table 2. Production of orange-yellow pigmentation and blue fluorescence by *Aspergillus flavus* isolates from ginger on coconut agar medium (CAM)

<i>A. flavus</i> isolate	Orange-yellow Pigmentation	Blue fluorescence
AFg ₁	-	-
AFg ₂	-	-
AFg ₃	-	-
AFg ₄	+	+
AFg ₅	-	-
AFg ₆	-	-
AFg ₇	-	-
AFg ₈	+	+
AFg ₉	-	-
AFg ₁₀	+	+
AFg ₁₁	-	-
AFg ₁₂	-	-
AFg ₁₃	+	+
AFg ₁₄	+	+
AFg ₁₅	-	-
AFg ₁₆	-	-
AFg ₁₇	-	-
AFg ₁₈	-	-
NRRL 3357	+	+

+ = presence; - = absence; AFg = *A. flavus* isolate from ginger.

Table 3. Production of orange-yellow pigmentation and blue fluorescence by *Aspergillus flavus* isolates from turmeric on coconut agar medium (CAM)

<i>A. flavus</i> isolate	Orange-yellow Pigmentation	Blue fluorescence
AFt ₁	+	+
AFt ₂	+	+
AFt ₃	-	-
AFt ₅	+	+
AFt ₆	-	-
AFt ₈	+	+
AFt ₉	-	-
AFt ₁₁	+	+
AFt ₁₂	+	+
AFt ₁₃	-	-
AFt ₁₄	-	-
AFt ₁₅	+	+
AFt ₁₆	+	+
AFt ₁₉	-	-
AFt ₂₁	-	-
AFt ₂₂	+	+
NRRL 3357	+	+

+ = presence; - = absence; AFt = *A. flavus* isolate from turmeric

A summary of the incidence of toxigenic isolates of *A. flavus* across major markets in Port Harcourt shows that Fruit Garden market had the highest incidence of 60% and 44.5% for ginger and turmeric, respectively (Table 4). The lowest was

recorded in Oil Mill market with no incidence of the pathogen at all. However, Mile One and Rumuokoro markets had toxigenic isolates of between 20 – 33% in both crops.

Table 4. Incidence of toxigenic isolates of *Aspergillus flavus* from ginger and turmeric across markets in Port Harcourt.

Market	% Incidence	
	Ginger	Turmeric
Fruit Garden	60.0	44.5
Oil Mill	0	0
Mile One	20.0	33.3
Rumuokoro	20.0	22.2

DISCUSSION

The current study has shown that there was contamination of ginger and turmeric with toxigenic *A. flavus* across markets in Port Harcourt. The high occurrence of *A. flavus* in Fruit Garden, Mile One and Rumuokoro markets as observed in this study could be ascribed to the reality that these markets are

major and daily markets that receive bulk consignments from the production centres directly. They are depots of fruits and vegetables in Port Harcourt. The contamination was very high especially in Fruit Garden market as the bulk of the fungi may have been introduced by the influx of

foreign and local merchants who converge on daily basis.

There may have been less build-up of fungi in Oil mill market because it is a weekly market where traders come to sell their goods and go without leaving their products behind and the quantities handled are minimal. This situation has been reported by Okereke *et al.* (2017) who observed high incidence of *A. flavus* across markets in Port Harcourt. The authors observed incidence as high as 63% in commonly consumed groundnut. Akintobi *et al.* (2011) also reported high occurrence of *A. flavus* in fruits sold in major markets in south western Nigeria. Fapohunda *et al.*, (2012) in their studies reported that 35% of the fungal spp. isolated from food and organic matter in south western Nigeria was confirmed to be *A. flavus*. Jeswal and Kumar (2015) also reported a wide range of fungal contamination in Indian spices with *A. flavus* having the highest incidence. Our result is also in agreement with Akinmusire, (2011) who observed high incidence of *A. flavus* from *Ananas comosus*. In the works of Ewekeye, *et al.*, (2013), *A. flavus* was identified as one of the fungal species causing deterioration in Irish potato. In a similar work by Essawet *et al.*, (2017), *A. flavus* was reported to be present in spices collected from local markets in Tripoli.

The ability of *A. flavus* to produce resistant spores could account for their high incidence in food products worldwide (Hocking 2006). This may imply high level of aflatoxin contamination, thus a risk to human health.

Similarly, the production of orange-yellow pigmentation and blue fluorescent ring under UV light on CAM has been reported by many authors (Davis *et al.*, 1987; Abbas *et al.*, 2004 and Atanda *et al.*, 2011). According to Lin & Dianese (1976), the production of an orange-yellow pigmentation of the mycelium prior to the appearance of blue fluorescence is an estimator of toxin producing isolates. Raed

et al., (2016) observed that 52% of isolates from 180 samples of poultry feeds using the UV light method were toxigenic. Fazekas *et al* (2005) also identified different toxins in common spices, of which aflatoxin was the commonest contaminant. Their results and ours are in line with those obtained by Zrari, (2013), who studied detection of aflatoxin from some *Aspergillus* spp. isolated from wheat seeds using CAM. Nair *et al.*, (2014) while working with spices also observed fluorescence colonies of aflatoxigenic isolates of *A. flavus* upon exposure to UV light at 365nm. Yazdani *et al.* (2010) also detected toxigenic *A. flavus* isolates on CAM under UV light after 7 days of incubation.

However, the higher occurrence of toxigenic isolates of *A. flavus* in turmeric compared to ginger in our study contradicts the works of Punam and Kumar, (2015). In their work on mycobiota and natural incidence of aflatoxin in nine Indian spices, they observed that toxigenic *A. flavus* was higher in ginger than turmeric. The result in our findings could be attributed to the fact that farmers in Rivers State grow more turmeric than ginger to complement the ones coming from the northern part of Nigeria. Moreover, the environmental condition in this area favours the growth of the fungus and these products could serve as a source of inoculum to infect other products. Also the nature and location of Fruit Garden market where different kinds of fruits and vegetable from different diverse locations are sold is also a factor. More importantly, the way these products are displayed in Port Harcourt markets where products from different parts of the country are packed together encourages the growth of the fungus, thus leading to inoculum build up. Toxigenic *A. flavus* on agricultural commodities has been identified to pose a significant menace and thus mycotoxin contamination which is hazardous to human health.

CONCLUSION

This study has shown that *Aspergillus flavus* is one of the major fungal species contaminating ginger and turmeric in Port Harcourt. This was more evident in Fruit Garden (D-Line) with high incidence of toxigenic isolates of *A. flavus*. This could increase the possibility of contamination with aflatoxins above Legislative limits. Toxigenicity of the *A. flavus* varied from market to market, thus poor post-harvest handling and storage of ginger and turmeric in Port Harcourt markets could increase the risk of contamination with aflatoxins. Proper sanitary measures should be maintained and government should enforce management strategies for quality control of foods and food products in all markets. The low incidence of *A. flavus* in samples from Oil Mill market should not be taken for granted; proper handling and good sanitation in the market should still be maintained at all times.

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