AFLATOXIN- AND OCHRATOXIN- PRODUCING POTENTIALS OF MOULDS ASSOCIATED WITH SPOILAGE OF FRUITS MARKETED IN AKWA IBOM STATE

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ABSTRACT

The presence of fungal deteriogens on fresh fruits frequently leads to spoilage, financial losses to the farmers and possible illnesses to consumers owing to their production of mycotoxins. Enzyme Linked Immune Sorbent Assay kits and UV spectrophotometer was used to assay mycotoxins (aflatoxin and ochratoxin) in mouldy fruits fruits (*Persea americana*, *Annona muricata*, *Citrus sinensis*, *Carica papaya* and *Lycopersicon esculentum*) in Akwa Ibom Markets and the toxins producing potential of the biodeteriogens. Fruit samples analyzed contained variable levels of aflatoxin and ochratoxin. The results of the assay showed that the mycotoxins detected in fruits were produced mainly by species of *Aspergillus*, *Geotrichum*, *Lasiodiplodia*, *Penicillium* and *Talaromyces* associated with spoilage of fruits Of the thirty nine (39) fungal strains screened for aflatoxin production, only 24 (61.5 %) were aflatoxigenic, while 15 (38.5 %) were non-aflatoxigenic. *Aspergillus nomius* AFAUY4 produced the highest concentration (15.6 ppb) of aflatoxin, followed by *A. nomius* AFAIK1 with 13.9 ppb, while *Aspergillus aculeatus* AASUY7 and *Aspergillus carbonarius* ACTUYI elaborated low concentration (0.3 ppb) of aflatoxin. More than 41 (93. 2%) of the fungal isolates from the spoilt fruit samples produced ochratoxin with the highest concentration (10.5 ppb) detected in *Aspergillus niger* ANTUY1, followed by *A. niger* ANTEK2 and *A. niger* ANTEK1 with 9.7 ppb and 7.5 ppb, respectively. *A. sclerotiorum* ASOEK1, *Lasiodiplodia theobromae* LTAIK3 and *A. paraciticus* APOIK1 were non-ochratoxin producers. The occurrence of mycotoxigenic strains of fungi on the moldy fruits is pointer to the risks associated with consumption of spoilt fruits and should be avoided.

INTRODUCTION

The association between fruits, microorganisms and humans have been long, interesting and developed before recorded history. Nutrients needed for growth, repair and control of body processes are usually obtained from fruits for they contain mineral elements, vitamins and sugar (Nahar *et al.*, 1990). The fruits could be used for making of juices, wine, marmalades, jams and salads (Isitua and Ibeh, 2010; Awe, 2011). Medicinal properties of fruits such as *Carica papaya* and *Citrus sinensis* have been reported. *C. papaya* reduces the risk of some types of cancer and lowers blood pressure (Nakamura *et al*., 2007). The infusion of *C. sinensis* stops headaches, stabilizes heart palpitations, hastens removal of metabolic waste from the body and also boosts the body immune system, thus helping the body to fight infections (Etebu and Nwauzoma, 2014).

During the sequence of fruit handling from post-harvest handling, transport, storage, marketing to the final consumption, microorganisms can affect the fruit quality as well as human health (Adeoye, 2009; Victor, 2014). Fungi, agents of deterioration can invade and cause spoilage of fruits after the tissues of the fruits have been damaged by some physical or physiological causes. The changes in the fruits condition as a result of spoilage are often accompanied by alteration of taste, smell, appearance or texture (Adeoye, 2009; Victor, 2014). Since fruits are harvested locally, thus, fruits displayed in the market often have cuts and bruises which aid in the penetration of microorganisms. Owing to the high nutritional value, particularly sugar and low pH, fruits serve as good substrates for microorganisms whose activities constitute the most important causes of fruit rots. The fruits harbouring some of these fungal agents may cause food infection or food poisoning if consumed by humans. Some fungi associated with spoilages produce mycotoxins, secondary fungi metabolites, which have various health implications when consumed with the fruits (Pitt, 1996; Ismaiel and Papenbrock, 2015). Mycotoxins such as aflatoxin, citrinin, patulin, tenuazonic acid, ochratoxin A and fumonisin have been reported to be associated with plant produces (Ismaiel and Papenbrock, 2015). These mycotoxins are low molecular weight molecules produced as secondary metabolites by saprophytic fungi, especially *Fusarium, Penicillium* and *Aspergillus* species (Benneth and Klich, 2003).

Aflatoxins have four major subgroups: Aflatoxin B_1, B_2, G_1, and G_2 based on their fluorescence under UV light and relative chromatography (Rastogi *et al.*, 2001), while ochratoxin could be Ochratoxin A, B and C. This study aimed at determining the concentration of aflatoxin and ochratoxin produced by fungal isolates from spoilt or mouldy fruit samples.

MATERIALS AND METHODS

Study Area

Akwa Ibom State located in the Niger Delta regions of Nigeria is between latitudes $4^0 33^1$ and $5^0 33^1$ north and longitude $7^0 35^1$ and $8^0 25^1$ east. The state is rich in varieties of leafy vegetables and fruits (Udousoro and Ekanem, 2013).

Collection of Samples

The mouldy or spoilt fruits samples (*Carica papaya, Persea americana, Lycopersicon esculentum, Ammona muricata* and *Citrus sinensis*) investigated were randomly purchased from the markets in the three senatorial zones (Eket, Uyo and Ikot Ekpene) of Akwa Ibom State. The samples were transported to Microbiology Laboratory, University of Uyo, in sterile polythene bags for mycological analyses.

Isolation and Identification of Fungi Associated with the Spoilage of Fruits

The fruits were washed with distilled water and surface sterilized with 10% hypochlorite solution. A sterile scalpel was used to cut 3 mm × 3 mm sections of the fruits (from healthy portions to the diseased portions). The cut section was aseptically plated on Potato Dextrose Agar (PDA) and incubated at 28^0C ± 2^0C for 5 days. Each colony on the primary plates was subcultured onto fresh plates of PDA and incubated at 28^0C ± 2^0C for 5 days. Wet-mount of the fungal mycelium was prepared using cotton blue in lacto-phenol and observed under a microscope (× 40 objective). Identification was based on their cultural and morphological features as described by Samson *et al.* (1984); Barnett and Hunter (1987).

Detection of Mycotoxins in Diseased fruits and Fungal Isolates

The mycotoxin (aflatoxin and ochratoxin) in spoilt fruits were detected using Enzyme Linked Immune Sorbent Assay (ELISA) kits and UV spectrophotometer as described by Hassan (2017) with slight modifications. The extracted diseased fruit samples, aflatoxin / ochratoxin enzyme conjugate and aflatoxin / ochratoxin antibody working solution were mixed and added to micro wells. On removal of non-specific reactants, substrate (A and B) were added, then the micro wells were measured optically using microplate reader at 450 nm for yellow colour to determine the optical density (OD) values. The concentration of aflatoxin and ochratoxin in diseased fruits were calculated using aflatoxin and ochratoxin standard curves (Hassan, 2017).

RESULTS AND DISCUSSION

The common fungal isolates obtained from the *P. americana, C. sinensis, C. papaya, A. muricata* and *L. esculentum* fruit samples were *A. nomius, A. niger, A. carbonarius, L. theobromae* and *P. citrinum*) samples (Table 1).The research findings have shown that some mouldy fruits are contained mycotoxins. The concentrations of aflatoxin and ochratoxin in *P.*

americana, C. sinensis, C. papaya, A. muricata and *L. esculentum* fruit samples analyzed varied with fruits samples and market centres (Table 2).. . In Uyo markets, the highest and lowest concentration of aflatoxin of 13.8 ppb and 1.9 ppb were obtained in spoilt *P. americana* and *C. papaya* fruits respectively, while the concentrations of aflatoxin in other fruit samples were as follows: *C. sinensis* (7.2 ppb), *A. muricata* (3.0 ppb) and *L esculentum* (6.7 ppb). The concentrations of aflatoxin observed in spoilt fruit samples from Eke markets in increasing order were 1.5 ppb (*C. papaya*) > 2.7 ppb (*A. muricata*) > 6.0 ppb (*C. sinensis*) > 6.7 ppb (*L. esculentum*) > 11.6 ppb (*P. americana*). Of the five (5) spoilt fruits from Ikot Ekpene markets, *A. muricata* fruits had the highest aflatoxin concentration of 10.1 ppb, followed by *L. esculentum* with 6.0 ppb, while *C. papaya* had the lowest with 1.0 ppb (Table 2). Variable levels of ochratoxin were also detected on mouldy fruits. The concentrations (ppb) of ochratoxin in *P. americana, C. sinensis, C. papaya* and *L. esculentum* fruit samples ranged from 5.0 to 5.7, 6.5 to 7.7, 5.0 to 6.5 and 7.4 to 8.1, respectively. The least level (≤ 1.6 ppb) of ochratoxin was recorded for *A. muricata* fruits (Table 2).

Table 1: Occurrence of Fungal Isolates in Spoilt Fruits

Fungal Isolates	Occurrence in Spoilt Fruits				
	P. americana	*A. muricata*	*C. sinensis*	*C. papaya*	*L. esculentum*
Aspergillus nomius	+	+	+	+	+
Aspergillus niger	+	+	+	+	+
Aspergillus carbonarius	+	+	+	+	+
Aspergillus aculeatus	+	+	+	+	-
Talaromyces verruculosus	+	-	+	-	-
Lasiodiplodia theobromae	+	+	+	+	+
Aspergillus paraciticus	-	+	+	-	-
Aspergillus sclerotiorum	-	-	+	-	-
Penicillium citrinum	+	+	+	+	+
Geotrichum candidum	+	-	+	-	-
Talaromyces koningiopsis	+	-	-	-	-

Key: +: Isolated; - : Not Isolated

Table 2: Concentration of Aflatoxin and Ochratoxin in Spoilt Fruit Samples

Location//Market	Fruits	Concentration (ppb)	
		Aflatoxin	Ochratoxin
Uyo	*P. americana*	13.8	5.7
	C. sinensis	7.2	7.7
	C. papaya	1.9	6.5
	A. muricata	3.0	1.6
	L. esculentum	6.7	8.1
Eket	*P. americana*	11.6	5.5
	C. sinensis	6.0	6.5
	C. papaya	1.5	5.0
	A. muricata	2.7	1.0
	L. esculentum	6.7	7.4
Ikot Ekpene	*P. americana*	10.1	5.0
	C. sinensis	5.5	7.0
	C. papaya	1.0	6.0
	A. muricata	2.5	1.2
	L. esculentum	6.0	7.5

Key: ppb = part per billion

Assay of the potential of the spoilage agents to produce toxins revealed that of the seven fungal isolates from *P. americana* fruit samples for aflatoxin analysis, *A. nomius* AFAUY4 produced the highest amount of aflatoxin (15.6ppb), followed by *A. nomius* AFAIK1 with 13.9 ppb, while *A niger* ANAEK2, *A. aculeatus* AAAUY3 and A. *carbonarius* ACAIK2 produced < 2.5 ppb of aflatoxin each. The results of *T. verruculosus*, *L. theobromae* and eight (8) Aspergilli from the *C. sinensis* fruits subjected to aflatoxin analysis are presented in Table 3. Of the eight species of *Aspergillus* tested, only 4 (50.0%) produced aflatoxin. Among the four aflatoxigenic Aspergilli, *A. parasiticus* APOIK1 and *A. aculeatus* AAOIK produced the highest (7.9 ppb) and least (0.5 ppb) concentrations of aflatoxin, respectively. The concentrations (ppb) of aflatoxin produced by the fungi from the *C. papaya* fruits were as follows: *A. niger* ANPEK5 (0.5), A. *nomius* AFPUY3 (3.0), *A. nomius* AFP EK2 (3.6) and *T. verruculosus* TVAUY1 (0.5). The results also showed that out of 9 fungal isolates from the *A. muricata* fruits analysed for aflatoxin production, only 5 (55.6 %) were aflatoxin producers, while 4 (44.4 %) were non-aflatoxin producers and the concentrations (ppb) of aflatoxin among the producers were as follows: *A. parasiticus* APSUY1 (5.1), *A.nomius* AFSUY4 (4.8), *A. nomius* AFSIK3 (3.9), *A. niger* ANSUY2 (0.5) and *A. aculeatus* AASUY7 (0.3). The concentrations (ppb) of aflatoxin in fungal isolates from the *L. esculentum* fruits ranged from 0.3 in *A. carbonarius* ACTUY1 to 7.5 in *A. nomius* AFTUY2. $\leq$ 0.5 ppb and $\leq$ 7.0 ppb of aflatoxin were produced by *A. niger* ANTUY2 and *A. nomius* AFTEK1, respectively (Table 3). Aflatoxins pose a serious health risk to humans and livestock. Large doses of aflatoxins lead to acute food poisoning (aflatoxicosis) that can be life threatening and are hepatocarcinogenic in humans. Consumption of food containing aflatoxin concentrations of 1 mg/kg or higher has been suspected to cause aflatoxicosis. Based on past outbreaks it has been estimated that when consumed over a period of 1 - 3 wks, an AFBI (Acute Food Bolus Impaction) dose of 20 - 120ug/kg bw per day is acutely toxic and potentially lethal. and concentration as low as 20 ug/kg can be of a great risk.

The results also showed that 9 (75.0 %) of the fungi isolated from the *P. americana* fruit samples produced ochratoxin. The nine (9) fungal isolates and their respective ochratoxin concentrations were *T. verruculosus* TVAUY1 (2.4 ppb), *P citrinum* PCAEK2 (6.1 ppb), *A. niger* ANAEK1 (7.5 ppb), *A. niger* ANAIK2 (7.0 ppb), *A carbonarius* ACAIK3 (6.1 ppb), *A carbonarius* ACAUY1 (5.7 ppb), *A. aculeatus* AAAUY2 (4.0 ppb), *A. nomius* AFAEK2 (4.6 ppb) and *A. nomius* AFAUY4 (6.3 ppb) (Table 4). The results obtained when fungal isolates from the *C. sinensis* fruits were subjected to ochratoxin analysis showed *A. aculeatus* AAOEK3 as the highest ochratoxin producer with concentration of 6.4 ppb, while *T. verruculosus* TVOUY1 produced the lowest concentration of ochratoxin (2.1 ppb). Table 4 shows the concentrations of the ochratoxin produced by fungal isolates from the *C. papaya* fruits. The results indicated that all the seven fungal isolates tested were ochratoxin producers. Their ability to elaborate ochratoxin ranged from 1.4 ppb in *A. nomius* AFPUY2 to 6.2 ppb in *P. citrinum* PCPEK2. The concentrations of the ochratoxin produced by fungal isolates from the *A. muricata* fruit samples ranged from 0.1 to 2.7 ppb in *A. nomius* AFSIK2, *A. nomius* AFSUY2, *A carbonarius* ACSEK3, *A. niger* AFSUY2 and *A. parasiticus* APSUY2. $\geq$ 6.0 ppb ochratoxin concentration was obtained in *P. citrinum* and *A. aculeatus*. The concentrations of ochratoxin in fungal isolates from *L. esculentum* fruits ranged from 2.1 ppb in *A. nomius* AFTEK1 to 10.5 ppb in *A. niger* ANTUY1. The results showed that 3.4 ppb, 6.5 ppb and 9.7 ppb ochratoxin were produced by *P. citrinum* PCTIK2, *A carbonarius* ACTUY1 and *A. niger* ANTEK2, respectively (Table 4). Ochratoxin is a known nephrotoxic, immunotoxic and carcinogenic mycotoxin. It is more deadly than aflatoxin, and chronic interstitial nephropathy has been reported at mean values of 25 - 29 mg/ml (Hope and Hope, 2012). Many of the fungi isolated in this study have previously been associated with spoilage of fruits and vegetables (Singh and Sharma, 2007, Chukwuka *et al*. 2010 and Oviasogie *et al*., 2015). These fungi have been reported to produce secondary metabolites such as aflatoxin and ochratoxin which are potentially harmful to humans and animals (Eaton and Groopman, 1994; Shephard, 2003).

Aspergillus spp have been reported to produce ochratoxins and this study confirms the findings of Petzinger and Weidenbach (2002).

Table 3: The Occurrence and Concentration of Aflatoxin Produced by Fungal Isolates from Spoilt Fruits

Fruits	Fungal Isolates	Aflatoxin Level (ppb)
P. americana	*A. nomius* AFAUY4	15.6
	A. nomius AFAIK1	13.9
	A. niger ANAEK2	1.1
	A. carbonarius ACAIK2	2.3
	A. aculeatus AAAUY3	1.0
	T. verruculosus TVAUY1	1.0
	L. theobromae LTAEK3	0.0
C. sinensis	*A. niger* ANOUY2	0.9
	A. carbonarius ACOUY3	0.0
	A. carbonarius ACOEK1	0.0
	A. paraciticus APOIK1	7.9
	A. sclerotiorum ASOIK1	0.0
	A. aculeatus AAOIK3	0.5
	A. aculeatus AAOEK2	0.0
	A. nomius AFOEK1	6.6
	T. verruculosus TVOUY1	0.5
	L. theobromae LTOIK2	0.0
C. papaya	*A. niger* ANPUY4	0.0
	A. niger ANPEK5	0.5
	A. carbonarius ACPIK1	0.0
	A. aculeatus AAPUY2	0.0
	A. nomius AFPUY3	3.0
	A. nomius AFPEK2	3.6
	L. theobromae LTPIK1	0.0
A. muricata	*A. niger* ANSUY2	0.5
	A. carbonarius ACSEK1	0.0
	A. carbonarius ACSIK2	0.0
	A. paraciticus APSUY1	5.1
	A. aculeatus AASUY7	0.3
	A. aculeatus AASIK3	0.0
	A. nomius AFSUY4	4.8
	A. nomius AFSIK3	3.9
	L. theobromae LTSEK5	0.0
L. esculentum	*A. niger* ANTUY2	0.5
	A. niger ANTEK1	0.0
	A. carbonarius ACTUY1	0.3
	A. nomius AFTUY2	7.5
	A. nomius AFTEK1	7.0
	L. theobromae LTTUY1	0.0

Table 4: The Occurrence and Concentration of Ochratoxin Produced by Fungal Isolates from Spoilt Fruits

Fruits	Fungal Isolates	Ochratoxin Level (ppb)
P. americana	*T. verruculosus* TVAUY1	2.4
	P. citrinum PCAEK 2	6.1
	A. niger ANAEK 1	7.5
	A. niger ANAIK 2	7.0
	A. carbonarius ACAIK 3	6.1
	A. carbonarius ACAUY 1	5.7
	A. aculeatus AAAUY 2	4.0
	A. nomius AFAEK 1	4.6
	A. nomius AFAUY 4	6.3
	L. theobromae LTAIK3	0.0
	G. candidum GCAEK4	2.1
	T. koningiopsis TKAUY1	5.3
C. sinensis	*T. verruculosus* TVOUY 1	2.1
	P. citrinum PCOEK2	5.3
	A. niger ANOUY1	5.6
	A. carbonarius ACOUY3	2.6
	A. carbonarius ACOEK2	3.0
	A. sclerotiorum ASOEK1	0.0
	A. aculeatus AAOIK3	5.7
	A. aculeatus AAOEK2	6.4
	A. nomius AFOIK2	3.4
	A. paraciticus APOIK1	0.0
	G. candidum GCOEK1	6.0
C. papaya	*P. citrinum* PCPEK2	5.4
	P. citrinum PCPIK6	6.2
	A. niger ANPUY3	5.7
	A. niger ANPEK5	4.0
	A. aculeatus AAPUY1	6.1
	A. nomius AFPEK3	2.6
	A. nomius AFPUY2	1.4
A. muricata	*P. citrinum* PCSUY1	4.8
	P. citrinum PCSIK2	5.6
	A. niger AFSUY2	2.7
	A. carbonarius ACSEK3	1.5
	A. paraciticus APSUY2	0.1
	A. aculeatus AASUY3	6.0
	A. aculeatus AASIK1	5.8
	A. nomius AFSUY2	1.1
	A. nomius AFSIK2	0.9
L. esculentum	*P. citrinum* PCTIK 2	6.5
	A. niger ANTUY 1	10.5
	A. niger ANTEK 2	9.7
	A. carbonarius ACTUY 1	3.4
	A. nomius AFTEK 1	2.1

CONCLUSION AND RECOMMENDATION

The present study has shown that many fungi associated with spoilage of fruits in Akwa Ibom markets have strong potentials to elaborate mycotoxins which are harmful to human health. The secondary metabolites of these fungi are readily detected in fruits in which they grow to cause moldiness. Consumption of these diseased or moldy fruits is risky and may result in food infection / poisoning and should be avoided

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