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Public Health Implication of Mycotoxin Contaminated Pawpaw (*Carica papaya* L) on Sale in Nigerian Markets

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Abstract

Purpose: To evaluate the mycotoxigenic potential of fungi associated with marketed pawpaw in South Southern Nigeria and the public health significance.
Methods: Pawpaw fruits with lesions or rots were obtained from three different markets in three different states in South Southern Nigeria. Five genera of moulds (Rhizopus, Aspergillus, Fusarium, Curvularia and Trichoderma) were isolated and used for inoculation of sound pawpaw samples at two wound depths of 5 and 7mm with either single or paired cultures. The resultant isolates were evaluated for mycotoxin production using thin layer chromatography and the mycotoxigenic potential of isolates was assessed by animal feeding trial using albino rats of the wistar strain fed orally with different concentrations of the mycotoxin extracts
Results: Mycotoxins were detected from pawpaw samples inoculated with Rhizopus, Aspergillus and Fusarium, before and after autoclaving for 15 min at 121 ^{o}C. Wistar rats fed on mycotoxin extracts developed symptoms of neurotoxicity characterized by ascending paralysis, convulsion and respiratory arrest.
Conclusion: Most of the fungal isolates in this study showed a great potential for mycotoxin production with associated neurotoxicity which is of concern in public health.

Keywords: Fungi, Pawpaw, Mycotoxins, Neurotoxicity, Public health

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Introduction

Pawpaw (*Carica papaya*) fruit is usually grown in the wild and is harvested by local farmers in Nigeria using long sticks into a large open basket or fiber bags. The baskets/fiber bags may be contaminated with spoilage fungi and are often piled on top of one another during transportation and retail, resulting to bruising and squeezing of fruit. Bruised fruits readily become colonized by propagules of the pathogens associated with the fruit surfaces and those in the fluid leaking from already rotten fruits. *Aspergillus niger, Aspergillus flavus, Rhizopus nigrican, Curvularia lunata, Rhizopus oryzae, Fusarium equiseti* and *Fusarium moniliforme* have been reported to be responsible for post harvested losses in pawpaw in southern Nigeria [1]. Invasion by pathogens either through natural openings or wounds is

considered the most critical factor in fruit rot [2, 3].

Beside the losses in income to the pawpaw fruit markets, the rotten or fungal spoilt pawpaw could also cause a health hazard to the consumers. Most microbes that infect plant tissues often produce secondary metabolite known as mycotoxins in their hosts which are known to be hazardous to animals including humans [3]. Mycotoxins are naturally occurring toxic chemical (often of aromatic structures) compounds which are capable of inducing mycotoxicoses (toxic syndromes especially cancer) in humans following ingestion or inhalation. However, they differ in their degree and manner of toxicity [2, 4]. Some of these metabolites include the ergot alkaloid by *Clavisep* species, zearalenone and vomitoxin by *Fusarium* species, ochratoxin A, produced by *Penicillium* species and aflatoxin produced by *Aspergillus* species [5]. Aflatoxins, which are a group of highly toxic, mutagenic and carcinogenic polyketide compounds, have been detected in pawpaw in South Western Nigeria [3].

This study therefore evaluated the mycotoxigenic potential of fungi associated with marketed pawpaw in South Southern Nigeria and the public health significance.

Experimental

Pawpaw Fruits

Pawpaw fruits (*Carica papaya* L) were obtained from three different markets located in three different states, Rivers, Bayelsa and Delta States in Southern Nigeria. Thirty fruits with lesions or rots and 60 standard (good) fruits were procured on two different occasions from each market for this work.

Fungal Evaluation of Rotting Pawpaw Samples

The fruits were first washed with clean water, dried and their surfaces were sterilized by exposing them for 1 min in 90% ethyl alcohol (BDH Chemicals Ltd. Poole England) and then for 3 min in 1% sodium hypochlorite. This was followed by rinsing (three times) in sterile distilled water. Fruits with rotten parts were selected and segments (3-5cm) of tissues from the margins of the rotted areas were cut out with a sterile scalpel and placed on previously prepared potato dextrose agar (PDA, Difco) in duplicate plates. The plates were incubated at 28 ± 1 ^{o}C and examined for microbial growth every 3days for 6 days [6].

Fungal colonies that developed on the plates were aseptically transferred onto PDA plates and incubated as above for 6days. The colony morphology and pigmentation of the isolates were recorded before sub-culturing for purification and storage under refrigeration (approximately 7^{0}C) until required. A portion of the fungal mycelium was teased out in a drop of lactophenol cotton blue on a grease-free microscope slide and examined microscopically. Cultural and morphological characteristics were observed for identification as earlier reported [7].

Inoculation of Pawpaw Samples for Induction of Rot

Washed (with water) good pawpaw fruits were surface-sterilized in 90% ethyl alcohol for 1min; 1% sodium hypochlorite for 3 min and rinsed three times in sterile distilled water. A sterile 5mm diameter cork borer was used to make two wounds (5 and 7mm deep) 3cm apart. A 3 mm disc cut from a 6-day-old fungal test isolate growing on PDA was introduced into the holes (4-5 holes per fruits depending on the size of the fruit) which were then sealed with sterile Vaseline [7]. Control samples were treated in the same manner except that uninoculated PDA was used. The treated samples and control were placed individually in sterile polyethylene bags moistened with wet balls of absorbent cotton wool to create a humid environment and incubated at 28 ± 1 ^{o}C for 6 days. At 3 days intervals, the samples were sectioned through the site of inoculation and examined for lesion development. Infected or decayed portions were aseptically transferred onto PDA to confirm that the infection was caused by the inoculants.

Mycotoxin Detection

Mycotoxin was detected essentially as previously described [3, 6]. Ten grams (10g) each of a set of sterilized (autoclaved at 121 ^{o}C for 15 min) inoculated pawpaw samples and another set of unsterilized inoculated pawpaw samples were extracted with chloroform (May and Baker Ltd. England) and concentrated. Of the extracted samples, 5, 10 and 15 L were spotted on three different points on a ruled line of the thin layer chromatography (TLC) coated with plates of silica gel (Merck TLC grade 7749). Also 5, 10 and 15 L of the mycotoxin standards were spotted on another three points near the previous samples extract spotted points. These were then developed in TLC tanks containing the solvents (toluene, isoamylalcohol and methanol) at a ratio of 3:3:2. When the solvent emigrated to about two-third of the plates, the plates were removed, air dried and examined under UV light at a distance of 365mm. The mycotoxin levels were semi-quantified based on comparisons with control levels, while the presence of mycotoxin in pawpaw samples was determined.

Animal Feeding Trial

Fifty four albino rats of the wistar strain (males and females, age 6-8weeks, weight 20-25 gram) were used and grouped into 6 groups of 2 (in duplicate), numbered and housed individually in wire-screen-plastic-bottom cages and fed with a conventional diet (Figure 1). Group 1, contained rats fed with sterile distilled water (0.1ml), this served as the control rats while groups 2, 3, 4, 5, and 6, contained rats fed with 10, 20, 30, 40 and 50 µg/100 µl of each extract from *Rhizopus, Aspergillus, Fusarium, Curvularia and Trichoderma* species. Animals were fed *per oral* with mycotoxin extracts using 0.1 ml graduated syringe and kept off food and water for 24 hours. The animals were observed over a period of 24 hours for onset of symptoms typical of neurotoxicity. Ethical approval was obtained from the Department of Animal and Environmental Biology, University of Port Harcourt Institutional Review Board, Port Harcourt.

Results

Five genera of moulds; *Rhizopus, Aspergillus, Fusarium, Curvularia* and *Trichoderma* were isolated from pawpaw samples showing rot. Colour, magnitude and texture of the symptoms varied with the organism. For example, the tissues infected by Rhizopus sp. were marked by brownish-black discoloration and softness. In contrast, *Aspergillus* specie-infected tissues were characterized by brownish-black coloration and were firm, rough and dry with some 'cracks'. *Fusarium* specie-infected tissues were pale-pinkish but smooth and dry. *Curvularia* specie-infected tissues were characterized by brownish-black coloration and were also firm, rough and dry while the tissues infected by *Trichoderma* sp. were characterized by greenish patches, rough and dry. Comparable symptoms (lesions/rots) were observed following inoculation except that the symptoms became more pronounced with time.

The thin layer chromatography (TLC) spot extracted from most of the inoculated pawpaw samples (sterilized and unsterilized) shows that *Aspergillus*, *Rhizopus* and *Fusarium* specie-inoculated fruits and the standard mycotoxin, fluoresces and produced bluish spots of equal intensities indicative of mycotoxin while *Curvularia* and *Trichoderma* species did not fluoresce, thus lack production of mycotoxin.

Wistar rats fed with different concentrations of mycotoxin extractss, showed that three of the fungal isolates (*Aspergillus*, *Rhizopus* and *Fusarium* species) produced mycotoxin with consequent neurotoxic symptomswith varying severity characterized by ascending paralysis, convulsion and respiratory arrest within 24 hours of ingestion of the extracts. At concentration of 10-20 µg/100 µl, only extract from *Aspergillus* specie produced paralysis in rats and as concentration increased (3040 µg/100 µl) paralysis occurred in rats fed with *Rhizopus* and *Fusarium* species extract. In contrast, *Aspergillus* sp. extract produced paralysis and convulsion at these concentrations, followed by a combination of

Table 1: Effect of different concentrations of mycotoxin extract on severity of symptoms

Mycotoxin extracts	Mycotoxin concentration (µg/100 µl)				
	10	20	30	40	50
Rhizopus sp.	-	-	+	+	++
Aspergillus sp.	+	+	++	++	+++
Fusarium sp.	-	-	+	++	++
Curvularia sp.	-	-	-	-	-
Trichoderma sp.	-	-	-	-	-
Control	-	-	-	-	-

- = No symptom observed, + = Paralysis only, ++ = Paralysis and convulsion, +++ = Paralysis,convulsion and respiratory arrest

paralysis, convulsion and respiratory arrest for *Aspergillus* sp. extract at concentration of 50 µg/100 µl (Table 1), whereas, animals fed on extracts from *Curvularia* and *Trichoderma* species did not develop neurotoxic symptoms and remained healthy after 24 hours, thus lack production of mycotoxin.

Discussion

Fungi have been substantially reported to be responsible for the post harvest losses of pawpaw [2, 3, 6]. This is corroborated by the present study which showed five genera of moulds (*Rhizopus*, *Aspergillus, Fusarium, Curvularia* and *Trichoderma*) as the causal agents of lesions/rots of pawpaw (*Carica papaya*). The magnitude of the lesions/rots varied with the organisms responsible. This is probably due to their widespread occurrence in the environment, their ability to produce pectinolytic enzymes and the relative ease with which they penetrate pawpaw fruits [2, 8]. Furthermore, the indiscriminate post harvest handling of these commodities, such as placing pawpaw fruits on the ground during retail/marketing in Nigeria and other developing countries, further predispose them to storage fungi.

The detection of mycotoxins in inoculated (sterilized and unsterilized) pawpaw fruits suggests that *Rhizopus* sp., *Aspergillus* sp. and *Fusarium* sp. might be the main producer of mycotoxins in marketed pawpaw fruits. Heating of mycotoxins has little or no effect on reducing the toxicity [9]. This is corroborated in the present study; autoclaving at 121^{o}C for 15 minutes did not inactivate mycotoxins. This is of public health concern because pawpaw juice and jellies may be subjected to mild heating to eliminate fungal contaminants without inactivating mycotoxins. *Aspergillus flavus* has been reportedly isolated from pawpaw fruits in Nigeria and is probably the main producer of Aflatoxin in pawpaw [3]. Extracts from *Aspergillus* species showed the highest potency in this study. Previous studies reported that the most potent and best characterized mycotoxin is aflatoxin (B_1, B_2, $B2_a$, G_1, G_2, and $G2_a$) produced by certain strains of *Aspergillus flavus* and other fungi [9]. Aflatoxins have been reportedly detected in grapes, tomatoes, and oranges [10, 11]. Out of 342 samples of different fruits and spices obtained from the stores of commercial centers screened for aflatoxin, 95 of them were positive [12]. It is probable that chronic ingestion of aflatoxin at low levels in moldy foods is a cause of hepatic disease in man in some parts of the world including Nigeria, where studies have linked the presence of high levels of aflatoxin in most common foods stored or retailed in unhygienic environments to the high incidence of primary liver cancer in young people under the age of 40 [9]. Aflatoxins are high risk factors for neonatal jaundice [13]. *Fusarium verticilliodes* isolated from African star apple (*Chrysophyllum albidum*) has confirmed mycotoxigenic potential [2]. *Rhizopus oryzae* and *Rhizopus stolonifer* have also been reported to be mycotoxigenic [14]. Although the most apparent concern in mould-induced adverse quality changes in pawpaw is the economic loss due to consumer rejection or price reduction [6], an elusive and perhaps equally important issue is that of health safety, since many of the moulds isolated in this study have toxigenic effects. The development of neurotoxic symptoms such ascending paralysis, convulsion

and respiratory arrest in experimental rats in this study confirms the mycotoxigenic potential of the producing fungi, since these symptoms may be produced in humans if mycotoxins accumulate. The degree of toxicity is often influenced by the age, sex, nutritional and immunological status of the host [9]. The fact that most people have not been diagnosed as having hepatoma or mycotoxicosis does not mean that the toxic metabolite is absent in their body system [11]. Aflatoxin M1, for instance, has been reportedly detected in the urine of the Philippine woman that consumed peanut butter containing aflatoxin [10].

Conclusion

The majority of fungi isolated in this study produced potent mycotoxins evidenced by the associated neurotoxicity, which is of public health significance. Therefore, analysis of mycotoxin is essential to minimize the consumption of contaminated fruits. The amount of mycotoxin levels can be minimized by prevention of contaminated pawpaw fruits consumption and by reduction of fungal growth.

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Conflict of interest

No conflict of interest associated with this work'.

Contribution of authors

The authors declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. OOO conceived and designed the study and was involved in data analyses while CAI and OO collected and analysed the data. All authors approved the final paper.

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