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Fungal Quality and Molecular Characterization of Aflatoxin-Producing *Aspergillus species* in Irrigation Water and Fresh Vegetables in Southwest Nigeria

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ABSTRACT

Fungal species were isolated from irrigation water, cultivation soil, and fresh vegetables samples were obtained from a vegetable field in Southwest Nigeria. *Aspergillus* group, identified by amplification and sequencing of their internal transcribed spacer (ITS) region, were further characterized for aflatoxin production using standard spectrophotometry (365nm). The highest mean ± SD fungal counts were $3.23 \times 10^3 \pm 0.03$ CFU/g, $3.07 \times 10^4 \pm 0.01$ CFU/g and $8.67 \times 10^2 \pm 0.13$ CFU/ml for vegetable, soil, and water samples, respectively. A total of 32 fungal strains isolated were presumptively identified as *Aspergillus sp.* (50%), *Fusarium sp.* (6.25%), *Chrysonilia sitophila* (3.13%), *Acremonium sp.* (6.25%), *Mucor sp.* (12.5%), and *Rhizopus sp.* (21.87%). The molecular characterization of *Aspergillus* strains revealed *Aspergillus aculeatus*, *Aspergillus fijiensis*, *Aspergillus flavus*, *Aspergillus japonicas*, and *Aspergillus niger*. All the 16 *Aspergillus* species showed aflatoxin B1 production with *Aspergillus japonicus* (AWF27) isolated from the irrigation water having the highest concentration (106µg/kg) and *Aspergillus fijiensis* (ASF6) isolated from soil having the lowest concentration (93µg/kg). The work revealed the potential for human exposure to mycotoxin through contaminated fresh vegetables. Concerted effort is required, especially in developing countries for cultivation of fresh vegetables in hygienic environment with clean irrigation water.

Keywords: Fungi, *Aspergillus*, aflatoxin, vegetable, soil, irrigation water.

INTRODUCTION

Fresh leafy vegetables are categorized as vegetables and herbs of a leafy nature and of which the leaf (and core) is intended to be consumed raw (FAO/WHO, 2008). They are grown in large quantities and widely consumed for nutritional benefits and medicinal purposes. The tropical and sub-tropical countries of the world are blessed with varieties of vegetables, some of which are domesticated, while others grow wild and their prices are relatively affordable when compared with other food items in the areas (Chubike *et al*., 2013).

As long as they are prepared in a healthy way, leafy vegetables, like other non-starchy vegetables, are a great addition to diet and offer countless health benefits (National Research Council, 2014). Vegetables are known to contain vitamins, minerals, disease-fighting phytochemicals, fiber, water, and antioxidants in various

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proportions (Hung *et al*., 2004).

Based on the European Union (EU, 2007) report, the global production per annum (p.a.) of fruit and vegetables grew by 94% from 1980 to 2004. During that period, the average yearly production growth of vegetables (4.2% p.a.) was almost twice that of fruits (2.2% p.a.). About 15.43% of vegetables and 1.88% of fruits were consumed in Nigeria between 2009 and 2010 (NBS, 2012). The statistical figures of consumption pattern in Nigeria, therefore, revealed that vegetables as the second most consumed food commodity only exceeded tubers and plantain (22.6%) within the study period.

Fresh vegetable produce at harvest has a natural epiphytic microflora much of which is non-pathogenic (FAO/WHO, 2008). During any of the steps in the farm-to-table continuum (growth, irrigation, harvest, processing, packaging, transportation, handling, retail) further microbial contamination can occur from a variety of sources, e.g. environmental, animal, or human. There is a risk that this may include pathogenic or toxin-producing microorganisms.

Multiple outbreaks with high numbers of illnesses have been reported due to the consumption of contaminated vegetables. Most well-characterized outbreaks have been linked to bacteria, viruses, and parasites (Beuchat, 1996; WHO, 1998; Ponka et al., 1999; Johnston et al., 2005; FAO/WHO, 2008). However, little attention is paid to the associated public health risks that may emanate from the presence of pathogenic or toxin-producing fungi in fresh vegetables.

It is also a known fact that most vegetables consumed in Nigeria are usually cultivated by peasant farmers usually, without formal education or adequate knowledge of vegetable cultivation under hygienic conditions. Therefore, there is a need to pay close attention to the quality of the vegetables we eat.

Certain fungi, such as *Aspergillus*, *Fusarium*, and *Penicillium* as commonly occurring filamentous fungi grow in vegetables and their growth may result in the production of toxins known as mycotoxins, which can cause a variety of ill effects in humans from allergic responses to immunosuppression and cancer (Pitt *et al*., 1998). The most important mycotoxins associated with human and veterinary diseases include aflatoxin, citrinin, ergot akaloids, fumonisins, ochratoxin a, patulin, trichothecenes, and zearalenone.

The aflatoxins are a group of structurally-related toxic compounds produced by certain strains of the *Aspergillus* fungi, specifically *Aspergillus flavus* and *Aspergillus parasiticus* (Othman and Al-Delamiy, 2012). Under favorable conditions of temperature and humidity, these fungi grow on certain foodstuffs and feeds, resulting in the production of aflatoxins, which can enter into the human food chain directly through foods of plant origin, such as vegetables indirectly through foods of animal origin, such as kidney, liver, milk, and eggs (Hudler, 2000).

The technical term for poisoning by aflatoxin mycotoxins is *Aflatoxicosis*. The most pronounced contamination has been encountered in vegetables, peanuts, and oilseeds, including corn and cottonseed. The major aflatoxins of concern are designated Aflatoxin B1, Aflatoxin B2, Aflatoxin G1 and Aflatoxin G2. These toxins are usually found together in various foods and feeds in various proportions (Tangendjaja, 2002).

Three fungal species, namely *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius* produce aflatoxins as secondary metabolites (Pitt and Hocking, 1997). The toxins are known to be carcinogenic, hepatotoxic, and teratogenic in test animals. Moisture content is the most important factor affecting fungal growth in stored products. According to Sauer *et al.* (1992), some fungal species were observed to become dominant with as little as 0.2% change in moisture content.

The main four aflatoxins produced by *Aspergillus* species have been recognized as B1, B2, G1, and G2 (Othman and Al-Delamiy, 2012). They are defined as

toxigenic, mutagenic, and carcinogenic toxins. The poisoning which occurs as a result of ingesting foods containing aflatoxins has been identified either as acute severe intoxication, which results in direct liver damage or chronic subsymptomatic exposure. The latter could result in a range of consequences, such as acute illness, nutritional immunologic consequence, and an accumulative effect on the risk of liver cancer (William *et al*., 2004).

To the best of our knowledge, fungal studies have hitherto been limited to their involvement in plant diseases and microbial spoilage. A survey of the relevant literature also indicates that an insufficient attempt has been made in Nigeria to characterize fungi associated with apparently fresh vegetables, cultivation soil, and irrigation water using molecular method. This work, therefore, investigated the fungal quality of fresh vegetables, cultivation soil, and irrigation water obtained from a vegetable cultivation site in Southwestern Nigeria. Inasmuch as the presence of aflatoxin in food may constitute a health hazard. A study was also made to determine the aflatoxin production potential by associated *Aspergillus species*.

MATERIALS AND METHODS

Collection of samples

Akanran indigenous vegetable site of the NICANVEG Project 106511 was selected for this study. A total of 3 fresh vegetable species: *Telfairia occidentalis* (Ugu), *Solanum macrocarpon* (Igbagba) and *Amaranthus cruentus* (Tete) were aseptically obtained from the field and preserved in sterile Ziploc bags. The three vegetable species are commonly consumed vegetable species in Nigeria. Composite surface soil samples (0-15cm depth) were collected from 6 randomly selected vegetable beds using hand auger. A total of seven water samples were obtained down gradient from the river used for irrigation of vegetables. All samples were preserved in ice packs, transported to the laboratory and analyzed within 48 hours of collection.

Count and isolation of fungal isolates

Spread-plate technique was used for fungal enumeration, since this method avoids the risk of thermal inactivation of fungal propagules and facilitates the maximum exposure of cells to atmospheric oxygen, thus allowing sporulation to proceed unencumbered (Corry *et al*., 2003). Sub sample of 25g of each vegetable sample was diluted in 225ml of 1X phosphate buffered saline (PBS buffer) and manually homogenized for 5 minutes in sterile 1L Erlenmeyer flask (Abadias *et al*., 2008) while 1g of soil or 1ml of each water sample was suspended in 9ml of sterile Ringer's solution. All prepared samples were further diluted serially (10-fold) up to 10^{-4} in sterile Ringer's solution for enumeration of total heterotrophic fungi (THF).

Potato Dextrose Agar (PDA) medium supplemented with chloramphenicol was prepared as specified by the manufacturer, and all plates (9cm-size) were dried at 60^{o}C prior to use. Aliquot of 0.1ml from each dilution was deposited in triplicate on PDA plates and spread evenly using a sterile bent glass rod. All plates were incubated in an upright position at 25±2^{o}C for 5-7 days (Corry *et al*., 2003; Hoque and Shamsi, 2011).

Plates with 10-100 fungal colonies were selected for counting using Colony Counting Chamber where scanty growth was observed, and the number of viable cells was expressed as CFU per gram, ml. Frequency of occurrence of fungal isolates were calculated by adopting the formula of Hoque and Shamsi (2011), who followed sub-culture to obtain pure cultures that were stocked and maintained on PDA slants for characterization and identification.

Identification of fungal isolates

From the stock cultures, mycelia fragments were picked with sterilized inoculating needle and transferred onto sterile PDA plates. A 5mm square block was gently cut at the center of the PDA plate with a sterile forceps, and mycelia fragments were transferred to the squared block at the center of the PDA plate. The cultures were

incubated at room temperature for 3 to 5 days. This procedure was repeated until pure cultures were obtained.

Identification of fungal isolates was based on their colonial morphology and microscopic characteristics. On PDA plates, fungi morphological features including mycelia form, colony colour, and reverse colour were observed and recorded. For microscopic observations, fungal structures like mycelia, spore bearing structures, and spores were picked up with a needle and were mounted on a lactophenol cotton blue stain over a clean slide (Hoque and Shamsi, 2011). Observation was done under the microscope, using X40 objective lens. Identities of the isolates were determined following the standard literatures (Guy and Richard, 2011).

Molecular and sequencing of aspergillus isolates

Molecular characterization was carried out at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Pure cultures of all fungal isolates were cultivated in PDA broth in test tubes for 4 to 5 days for DNA extraction. DNA extraction and purification was done with QIAamp DNA Mini Kit (Qiagen with catalogue number 51304) following the manufacturer's instructions. The extracted nucleic acid was treated with RNase. Three microliter of the DNA was loaded on 1% agarose and run at 96 – 100 V using 1X TBE for 1 hour.

The universal primers used for fungal amplification were ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') as forward and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') as reverse. 25-µl PCR reaction mixture contained 2.5µl of 10X Bioline *Taq* Reaction Buffer, 2.0µl of dNTPs (2.5 mM), l µl of both Forward and Reverse Primer (5µM), 3µl of DNA template (10ng/ul), 0.4µl of *Taq* DNA Polymerase (5 unit/µl), and 15.1µl of nuclease free H_2O.

The reaction was mixed gently and all liquid was collected to the bottom of the tube by a quick spin. The thermocycler program for each PCR reaction began with an initial denaturation step at 94°C for 5min followed by 32 cycles of a 30 seconds denaturation step at 94°C, a 30 second annealing step at 54°C, and a 45 second elongation step at 72°C, then a final 5 minute elongation step at 72°C. Then, the PCR products were held at 10°C for sequence analysis.

Three microliter of the PCR product was loaded on 1.5% agarose. The PCR product was purified with absolute ethanol and 70% ethanol respectively. Finally, 2µl of the purified PCR product was used for sequencing. Sequencing was done with Bigdye v3.1 cycle sequencing kit (Applied Biosystems, Britain). All PCRs and sequencing reactions were performed on a GeneAmp PCR System 9700 (Applied Biosystems).

Gene sequences were assembled using BioEdit 7.2 Sequence Alignment Editor (USA), and aligned using ClustalW Multiple Alignment, after which the alignments were manually corrected where needed. Sequences were then blasted against known sequences in the NCBI database to provide species identification.

Preliminary screening of Aspergillus sp. for aflatoxin production

Preparation of CAM and PDB for the screening Coconut Agar Medium (CAM) and Potato Dextrose Broth (PDB) served as screening media for the production of aflatixin by *Aspergillus* isolates. CAM was prepared using the method of Davis *et al*. (1987). Readily available tropical brand of shredded coconut was obtained locally. The procedure involved homogenization of 100g of shredded coconut with 200ml of hot distilled water for 5 minutes. Two hundred (200) ml of distilled water was measured into 500ml beaker and placed in water bath set at 100ºC. The water was allowed to boil and the beaker removed from the water bath. 100g of shredded coconut (using smaller cup of the blender) was added to the hot distilled water and the beaker was placed on a magnetic stirrer. The mixture was stirred to homogenize and immediately filtered with cheese cloth. The remaining volume was determined and the clear filtrate was adjusted to pH 7, using 2N NaOH. Agar No. 1 was added in an equivalent proportion of 20g/l and the mixture was heated

to boil on the magnetic stirrer and then cooled to about 50°C. The pH was checked and adjusted again to pH 7 when necessary. The mixture was thereafter autoclaved for 121°C for 18 minutes and cooled to about 40 – 45°C. Streptomycin antibiotic (50mg/l) was added to inhibit the growth of bacteria and about 15ml of the molten agar was poured into sterile Petri dishes under aseptic conditions and allowed to set.

PDB was prepared using sweet potato. The potato was cut into little chunks and 200g was weighed into 1 litre of distilled water in stainless pot. It was boiled for about 1 hour and the content was allowed to cool to room temperature. The fluid was sieved into clean conical flask using cheese cloth followed by the addition of 20g dextrose (glucose D). The medium was also adjusted to pH 7 and sterilized by autoclaving at 121°C for 15 minutes. After autoclaving, the mixture was allowed to cool to about 40 – 45°C, streptomycin antibiotic (50mg/l) was added to inhibit the growth of bacteria and about 15ml of the broth was poured into sterile MacCartney bottles under aseptic conditions.

Observation of fluorescence on CAM:

The 4 days old pure culture fragment of *Aspergillus* species on PDA plates were transferred, using an inoculating needle by mass conidial transfer to the center of the CAM plates and incubated in inverted position at 27°C for 4 days. After incubation, a single, large, central fungal colony was observed on each plate. The reverse side of the fungal growth on CAM plate was observed under long-wave (365nm) UV light (Analytik Jena AG, Germany) for blue fluorescence on days 4, 5, and 6. An un-inoculated plate was used for reference purposes. Observation was recorded on day 6 as either positive or negative for the presence or absence of blue fluorescence, respectively. The plates were then extracted for aflatoxin production as described below.

Observation of growth in PDB

Potato dextrose broth (PDB) in MacCartney bottles were inoculated with the 4 day old pure culture fragment of each *Aspergillus* species by mass conidial transfer using sterile inoculating needle. All MacCartney bottles were then incubated for 5 days at room temperature (27 ± 2°C). About 1.5ml of each broth culture was dispensed into separate eppendorf tubes in triplicates and stored at 4 ± 2°C prior to spectrophotometric analysis. For growth observation in PDB, a set each of eppendorf tubes were harvested on days 5, 6, and 7 and spin in refrigerated centrifuge (4°C) for 5 minutes at 10,000 revolution/min to separate the mixture. The absorbance of the supernatant was read at 365nm wavelength, using a UV spectrophotometer (Great medical, England, Model SS2209059). The remaining content in MacCartney bottles were also extracted for aflatoxin as described below.

Assay for aflatoxin production by *Aspergillus sp.* in CAM and PDB

Extraction of aflatoxin metabolite in CAM and PDB

The entire content of each plate (fungal growth and CAM agar) used for blue florescence observation was scraped into an explosion-proof stainless steel blender. 100ml of chloroform-acetone (85: 15) was added and blended for 3 minutes. The blended mixture was filtered through Whatman No. 4 filter paper and filtrate was evaporated to dryness in an oven at 50°C. The extract was then stored at room temperature for further analysis (Davis *et al*., 1987) The dried extract was re-suspended into chloroform: acetone mixture (90: 10), dispensed into an eppendorf tube, and spin in a refrigerated centrifuge (4°C) for 5min at 10000 rev/min to separate the solvent, which separates into two layers in the tube. The upper layer was gently pipetted into a beaker and evaporated to dryness at 50°C in the oven.

The metabolite produced by the *Aspergillus sp* in the remaining PDB broth culture in MacCartney bottles were extracted on day 5 with chloroform in ratio 1: 3. This was done by mixing 2.5ml of the broth culture with 7.5ml of coliform in a clean beaker. The content was dispensed into eppendorf tubes, one tube per isolate and allowed to

stand for 5 minutes. The mixture separated into three layers, with the chloroform at the lower layer, the broth at the middle layer, and metabolites at the upper layer. The metabolite layer was gently pipetted into a beaker and evaporated to dryness at 50°C in the oven.

The two sets of aflatoxin metabolite extracts were then preserved at room temperature for thin-layer chromatographic analysis.

Analysis of aflatoxin metabolite using thin-layer chromatography:

Five hundred microliter (500µl) of acetone: chloroform mixture (10: 90) was added to re-suspend the dried metabolite extract obtained from both CAM and PDB. Thin-layer chromatography plate (silica gel) was scored with vertical lines, 1cm apart, to result into 20 individual channels (Shannon *et al*., 1983). One hundred microliter (100µl) of the dissolved metabolite was spotted on the plate. The metabolite was applied slowly within approximately 2 min on the plate allowing to dry fast enough to reveal small spot sizes (Stroka *et al*., 1999).

All spots were applied equally resulting in the same size and as small as possible. A pre-run procedure was performed prior to the main separation to focus the spots to small horizontal bands. This was done with methanol which was allowed to run just over the applied analyte spots. The solvent front of the methanol was marked and the plate was dried with warm air to assure that no methanol residues remained on the TLC plate.

A TLC chamber was filled with the mobile phase (chloroform: acetone: water - 90: 10: 1.5) about 15min prior to running. The plate was run in the dark for approximately 2 hours up to a distance of 10 – 15cm for a full separation of all aflatoxin spots. The solvent front at the end of the chromatographic run was marked. The TLC plate was dried under air steam and viewed under UV light (254 and 365nm, Gallenkamp, U.K.) at 365nm in the absence of light (Stroka *et al*., 1999).

Statistical analysis

In calculating statistics for acquired fungal and aflatoxin data from vegetable, cultivation soil and irrigation water, all enumerations were converted to $\log_{10}$ values and the calculations of the mean and standard deviation (SD) were completed on the transformed data using excel spreadsheet.

RESULTS

Enumeration, isolation and identification of total heterotrophic fungi (THF)

Log_{10} of the mean viable Total Heterotrophic Fungal (THF) count of vegetable, cultivation soil and irrigation water samples are shown in Figure 1. The three selected vegetable samples were characterized with mean ± SD fungal counts of $9.67 \times 10^2 \pm 0.02$ CFU/g for *Telfairia occidentalis* (Ugu), $3.23 \times 10^3 \pm 0.03$ CFU/g for *Solanum macrocarpon* (Igbagba) and $1.40 \times 10^3 \pm 0.03$ CFU/g for *Amaranthus cruentus* (Tete). THF count of the cultivation soil samples ranged between $1.47 \times 10^3 \pm 0.03$ CFU/g and $3.07 \times 10^4 \pm 0.01$ CFU/g, while that of irrigation water samples was in the range of $7.67 \times 10^1 \pm 0.07$ CFU/g and $8.67 \times 10^2 \pm 0.13$ CFU/g. THF was isolated in all the vegetable, cultivation soil and irrigation water samples analyzed. The highest fungal count was obtained for the cultivation soil followed by irrigation water and vegetable samples.

From the fresh vegetable, cultivation soil and irrigation water samples, 32 strains of fungi were isolated. After isolation and purification, the analysis of the morphology revealed six (6) morphologically distinct groups based on their appearance, density, colour, size, and the mycelium. The 32 fungal strains isolated were presumptively identified as *Aspergillus sp.* (50%), *Acremonium sp.* (6.25%), *Chrysonilia sitophila* (3.13%), *Fusarium sp.* (6.25%), *Mucor sp.* (12.5%), and *Rhizopus sp.* (21.87%).

Molecular characterization of aspergillus isolates

DNA from all the *Aspergillus* fungal isolates was successfully extracted and amplified using the ITS primer pair. The banding pattern produced by the species of

Aspergillus on a 1.5% agarose gel is shown in Figure 2. All the *Aspergillus* fungal strains tested were PCR positive as reflected by the presence of band on the agarose gel.

Sequence analysis of the ITS regions of the nuclear encoded rDNA showed significant alignments as shown in Table 1. The blast results as presented in Table 2 revealed the species identities of the *Aspergillus* isolates to be *Aspergillus flavus*, 99% (ASF6), 85% (ASF21) and 100% (AVD4), *Aspergillus niger*, 98% (ASF9), 100% (ASF11) and 98% (ASF20), *Aspergillus fijiensis*, 96% (ASF18), and *Aspergillus japonicus*, 97% (AVD12). Molecular characterization of *Aspergillus* strains revealed *Aspergillus aculeatus*, *Aspergillus fijiensis*, *Aspergillus flavus*, *Aspergillus japonicus* and *Aspergillus niger*.

Frequency of occurrence of fungal isolates in the samples

Figure 3 showed the frequency of occurrence of fungal isolates in fresh vegetable, cultivation soil and irrigation water samples. A total of six (6) fungal species were apparently isolated from the three studied vegetable species. *Telfairia occidentalis* (Ugu), *Solanum macrocarpon* (Igbagba) and *Amaranthus cruentus* (Tete) vegetable samples from the study site were predominantly *Aspergillus flavus* (28.57%), followed by equal occurrence of 14.28% for *Aspergillus japonicus*, *Chrysonilia sitophila*, *Fusarium sporotrichoides*, *Mucor sp* and *Rhizopus stolonifer*. The cultivation soil harbored five (5) fungal species with *Aspergillus niger* having the highest occurrence of 33.33%. This was followed by *Acremonium sp* and *Aspergillus flavus* with 22.22% occurrence as well as *Aspergillus fijiensis* and *Rhizopus stolonifer* with 11.11% occurrence. Congruently, six (6) fungal species were also isolated from the flowing river which serves as the only irrigation water source in the studied vegetable farm and other adjoining farms. *Aspergillus niger* and *Rhizopus stolonifer* were the predominant fungal species with 25.00% occurrence followed by *Aspergillus aculeatus* and *Mucor sp.* (18.75%). *Fusarium oxysporum* had the least occurrence of 6.25%.

Preliminary Screening for aflatoxin production by Aspergillus isolates

Preliminary screening for the production of aflatoxin by the *Aspergillus* group was carried out by the observation of blue fluorescence on the reverse growth of the fungi under UV light and growth confirmation with UV visible spectrophotometer delete (Table 3). Blue fluorescence observed on all CAM plates under UV light is an evidence of detectable levels of aflatoxin production in day 7 by all the *Aspergillus* species isolated from vegetable, cultivation soil, and irrigation water samples. Spectrophotometric readings at 365nm wavelength in the range of 0.151 and 0.720nm depict fungal growth in PDB.

Production of aflatoxin by Aspergillus isolates

Figure 4 presents the mean concentration of aflatoxin produced by all the *Aspergillus* isolates on CAM and in PDB media. All the 16 *Aspergillus* species used for this study showed evidence of aflatoxin production on both coconut agar medium (CAM) and potato dextrose broth (PDB). However, the highest aflatoxin production for all the *Aspergillus* isolates was observed when CAM was used. Levels of aflatoxin production by *Aspergillus* isolates on CAM ranged from 93.402µg/kg to 106.386µg/kg compared to the range of 83.157µg/kg and 99.673µg/kg obtained with PDB at room temperature. *Aspergillus japonicus* (AWF27) isolated from the irrigation water had the highest mean aflatoxin production values, while *Aspergillus fijiensis* (ASF6) isolated from soil recorded the lowest mean aflatoxin production values in both CAM and PDB media. However, the concentration of data obtained did not reflect any significant difference in the aflatoxin produced by all the *Aspergillus* isolates.

DISCUSSION

Determination of fungal populations in the samples

Heterotrophic fungi were isolated from all the vegetable, cultivation soil and irrigation water samples

obtained from Akanran vegetable farm used for this study. The highest fungal count was obtained for the cultivation soil followed by irrigation water and vegetable samples (Figure 1). Soil environment naturally harbours more microbial load compared to other environmental matrices. This may be as a result of their direct and indirect involvement in many of the key processes required for ecosystem functioning (Anderson *et al*., 2003). They are important as pathogens of plants and animals, as mycorrhizal symbionts of plants and as the main agents for the decomposition of organic material. Fungi, therefore, possess the ability to control nutrient fluxes in natural ecosystems that may be aided through extensive below-ground mycelial networks. Other anthropogenic activities around the study site may contribute to the observed fungal counts.

Telfairia occidentalis (*ugu*), *Solanum macrocarpon* (*igbagba*), and *Amaranthus cruentus* (*tete*) are among the well cherished, mostly cultivated and widely consumed indigenous vegetables in the Southwestern and Southeastern parts of Nigeria. The mean THF counts recorded for these vegetable species are similar to those reported for fresh vegetables obtained from similar environment in Nigeria. Akinyele *et al*. (2013) reported fungal counts in the range of $3.0 \times 10^2 – 5.0 \times 10^2$ sfu/ml for *Senecio biafrae* (worowo) and $2.52 \times 10^2 – 7.4 \times 10^2$ sfu/ml for *Amaranthus cruentus* (tete) samples obtained from Akure, another city in Southwestern Nigeria. Unlike aerobic colony count for bacteria, the limit of aerobic colony count for fungi (THF) in fresh vegetables is not common in the literature.

In the field, environmental stressors, such as repeated moist/dry cycles, temperature fluctuations, competition with or stimulation by other microorganisms and nutrient fluctuation as well as anthropogenic influence can control the composition of fungal population dynamics and species diversity. All of these could also influence the selection or stimulation of toxin producing strains in the given environment (Faraj, 1991; Rao *et al*., 1997).

Identification of fungal isolates

Aspergillus sp., *Acremonium sp.*, *Chrysonilia sp.*, *Fusarium sp*., *Mucor sp.* and *Rhizopus sp*. constituted the predominant fungal genera in the vegetable, cultivation soil, and irrigation water samples. They are among the important fungi of the environment responsible for various biogeochemical cycling of materials in nature. Aside from their roles in bioconversion and deterioration of materials, some of these fungal genera are also potential or opportunistic plant pathogens (Narayanasamy, 2011).

The predominant fungal genera isolated from the vegetable samples were *Aspergillus, Chrysolilia, Fusarium*, *Mucor,* and *Rhizopus*. Tournas (2005) isolated various species of *Aspergillus*, *Fusarium* and *Rhizopus* amongst others from vegetable samples. He attributed the spoilage of fresh vegetables to the presence of these fungi.

The fungal isolates obtained from soil samples were *Acremonium sp.*, *Aspergillus flavus*, *Aspergillus fijiensis*, *Aspergillus niger* and *Rhizopus stolonifer*. Their role in soil is in bioconversion of organic materials. The predominant *Aspergillus aculeatus*, *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporum*, *Mucor sp.*, and *Rhizopus stolonifer* in irrigation water samples have been reported for similar environment. Fungal presence in flowing river can be attributed to the presence of high organic content and an enabling condition for their proliferation.

The present study revealed that *Aspergillus flavus*, *Mucor sp* and *Rhizopus stolonifer* isolated from cultivation soil and irrigation water samples were also present in the vegetable samples. Consequently, cultivation soil and irrigation water could be major sources of fungal contamination to the vegetables. Influx of wastewater from animal farms and dumping of refuse were observed during sampling at the upstream end of the river used for irrigation in the studied vegetable farm. Investigators had suggested that the presence of many pathogens in cultivation soils and irrigation maybe the

major sources of microorganisms present on many vegetables (Beuchat and Ryu, 1997; Mapanda *et al*., 2005; Tsado *et al*., 2013).

Molecular identity of the Aspergillus isolates from vegetable, irrigation water, and cultivation soil samples

The use of rRNA genes for the identification of fungal species is based on the detection of conserved sequences in the rDNA genes. This method is rapid, sensitive, and precise for the identification of fungi to species level (Ferrer *et al*., 2001; Ehrlich *et al*., 2007). Our results showed high fungal specificity with the selected primers.

The sequence analysis of the ITS regions of the nuclear encoded rDNA of the *Aspergillus* isolates showed significant alignments for *Aspergillus aculeatus*, *Aspergillus fijiensis*, *Aspergillus flavus*, *Aspergillus japonicas*, and *Aspergillus niger*. Ehrlich *et al*. (2007) used a similar molecular method to identify aflatoxin-producing *Aspergillus* species isolated from soil samples in Thailand. Alwakeel (2013) also used the molecular method for identification of various species of *Aspergillus* and *Penicillium* isolated from stored apples.

Apart from Aspergillosis caused in man, *Aspergillus* species are known to produce several toxic metabolites, such as malformins, naphthopyrones, ochratoxins (Frisvad and Samson, 1991 and Al-Hindi *et al*., 2011).They can also produce aflatoxins, a mycotoxin which is a very important toxin worldwide because of the hazard it poses to human and animal health (Peraica *et al*., 1999).

Aflatoxin production by Aspergillus isolates

Krogh (1992) had earlier reported that most microbes infecting plant tissues often produce secondary metabolites in their hosts, which are known to be hazardous to animals including man. Some of these metabolites include the ergot alkaloids on cereals by *Clavisep sp*., fumonisin on maize by *Fusarium sp*. and Aflatoxins on grains by *Aspergillus sp*.

One of the primary objectives of this study is to verify the potentials of fungi that might associate with fresh vegetables during cultivation for their ability to produce toxins of public health implication. The concentrations of aflatoxin produced by the *Aspergillus* isolates in the course of this study, however, confirm this result. Observation of blue fluorescence under UV light and spectrophotometric determination revealed preliminary positive production of aflatoxin by *Aspergillus* isolates.

The US Food Safety Regulations safe level limit for aflatoxins is 20µg/kg for all products. Aflatoxin production by all *Aspergillus* isolates in the range of 93.402µg/kg and 106.386µg/kg on CAM as well as 83.157µg/kg and 99.673µg/kg for PDB exceeded this limit. The vegetable products from the study field may, therefore, be regarded as unsafe for human consumption without proper processing.

The production of aflatoxin by species of *Aspergillus* most especially *Aspergillus flavus* isolated from various sources is well documented (Lillard *et al*., 1970; Rao *et al*., 1997; Othman and Al-Delamiy, 2012). Aflatoxin production is believed to be conditional and dependent upon the combination of several environmental factors which include the amount and wavelength of available light, pH, growth substrate, relative humidity, temperature, substrate, and the presence or absence of other microorganisms (Pitt and Hocking, 1985; Rao *et al*., 1997). Our study confirmed the ability of *Aspergillus flavus* associated with fresh vegetable cultivation to produce aflatoxin *Aspergillus fijiensis*, *Aspergillus japonicas*, and *Aspergillus aculeatus*. It also showed detectable amounts of aflatoxin production when grown on certain media (CAM and PDB).

CONCLUSION

We have reported that fungi with the potential for toxin production are associated with a fresh vegetable cultivation site in Southwestern Nigeria. It is clear from our data that *Aspergillus flavus*, *Aspergillus fijiensis*, *Aspergillus japonicus* and *Aspergillus aculeatus* isolated

from fresh vegetables, cultivation soil, and irrigation water samples possessed the capability to produce toxigenic material suspected to be aflatoxin.

Although efforts have been put in this study to establish the possibility of fungal contamination of vegetable products during cultivation, the information provided is, however, meager compared to the huge number of similar sites that are available in this region. There is a need to extend this study to various fresh vegetable cultivation sites for more site-specific microbiological quality determination in order to proactively proffer a more holistic and sustainable solution.

Additional research is essential to document the toxin class produced by these groups of fungi and to document toxin-specific health risks. Future studies would seek to determine the intergenic region for the aflatoxin biosynthesis genes aflJ and aflR in addition to establishing the relatedness and phylogeny of the common fungal species that are present in the fresh vegetables, cultivation soil, and irrigation water. ELISA assay and HPLC measurement should also be considered for the qualitative and quantitative determination of aflatoxin production.

In view of the public health risks associated with the consumption of raw vegetables that are contaminated with pathogenic/ toxin-producing fungi and the carcinogenic properties of aflatoxin, adequate measures should be put in place to cultivate vegetables in soils with minimal fungal contaminants. Internationally accepted methodologies such as good agricultural practices (GAP) as well as water safety plan (WSP) can be domesticated for use in vegetable cultivation by local farmers.

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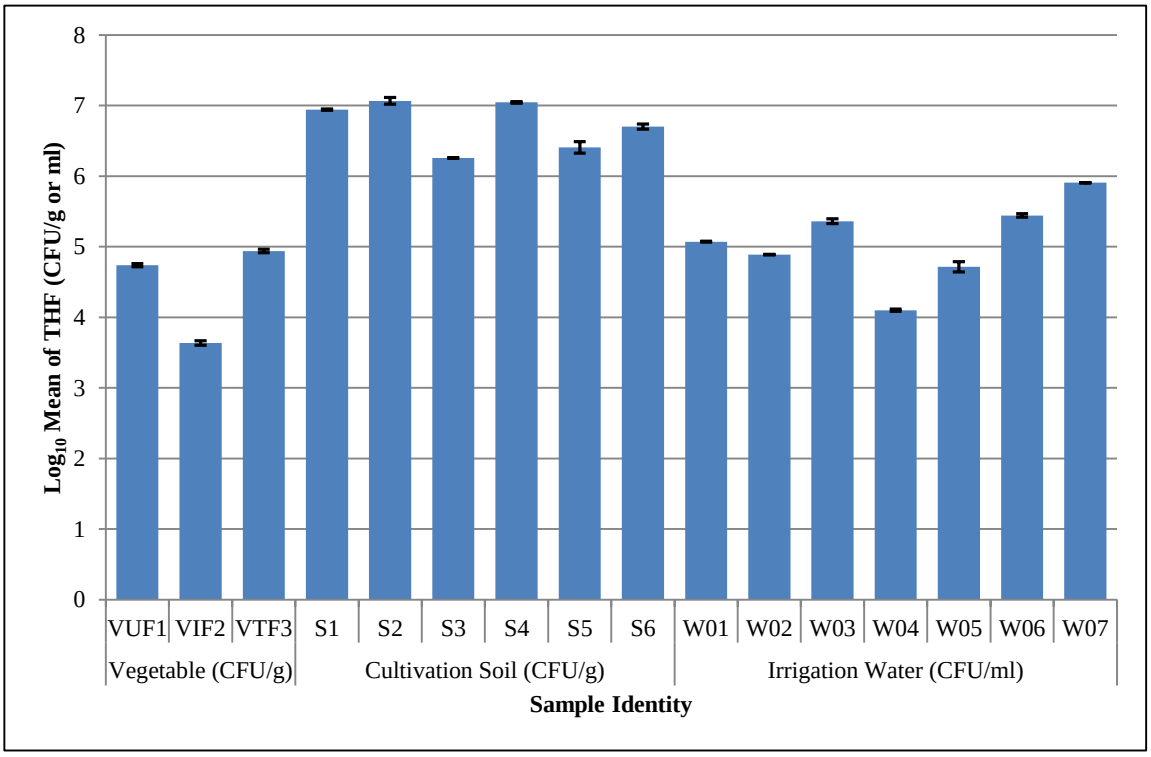

Figure 1: Total Fungal Counts in Fresh Vegetable, Cultivation Soil and Irrigation Water.

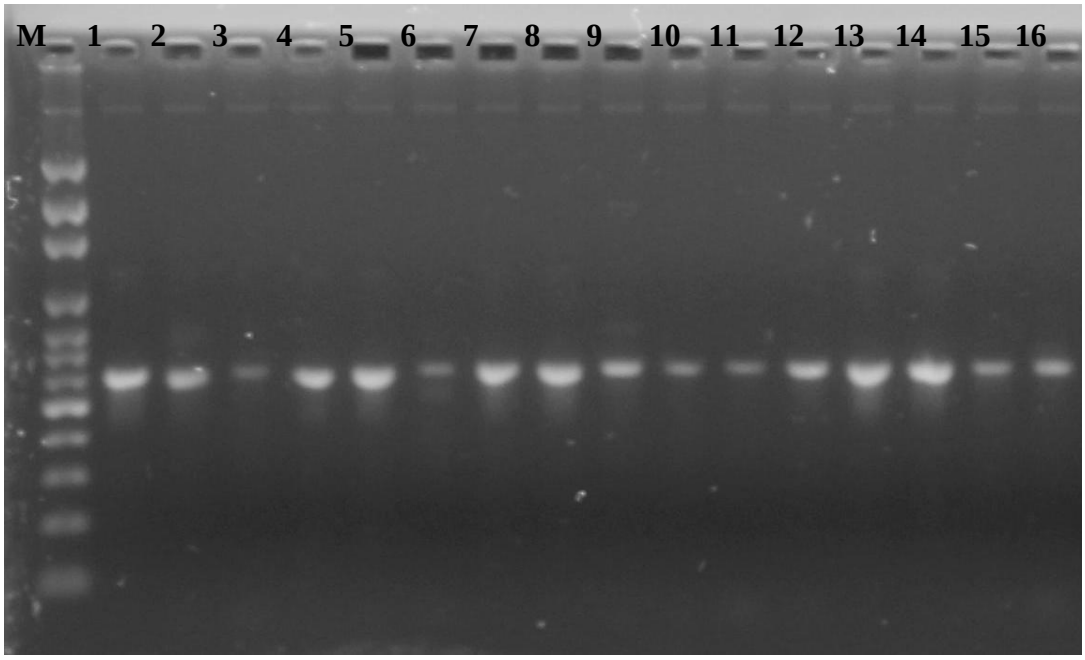

Figure 2: Gel of PCR of Fungi DNA Verification. M (1kb Marker), 1 (ASF6), 2 (ASF11), 3 (ASF21), 4 (AVD4), 5 (AWF9), 6 (ASF9), 7 (ASF20), 8 (AVD12), 9 (AWF4), 10 (AWF7), 11 (AWF12), 12 (AWF13), 13 (AWF24), 14 (AWF27), 15 (AWF31), 16 (ASF 18).

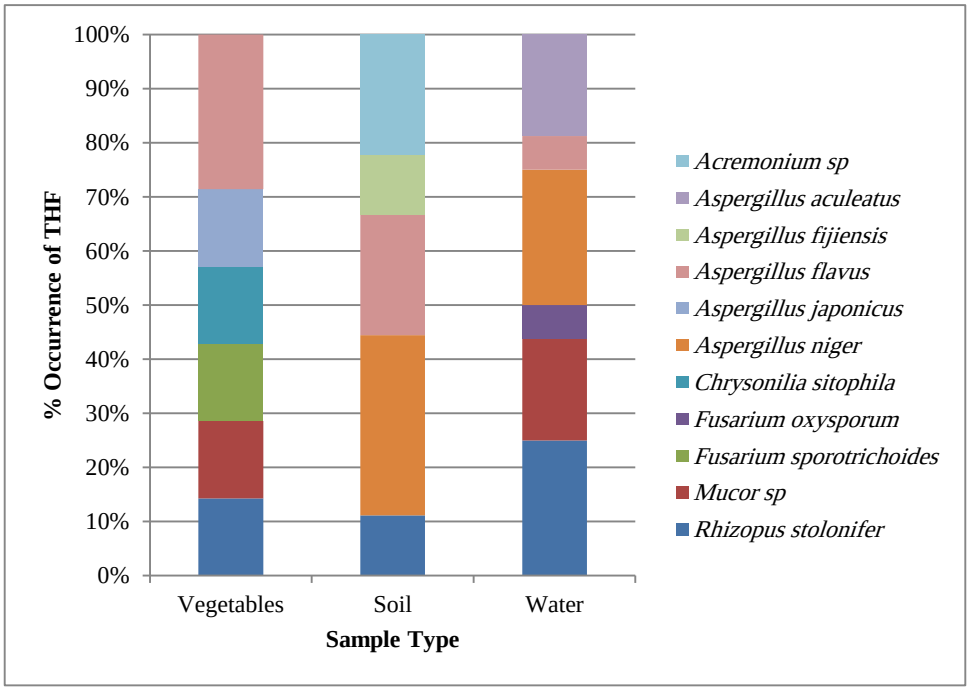

Figure 3: Percentage Occurrence of THF in Fresh Vegetable, Cultivation Soil and Irrigation Water Samples.

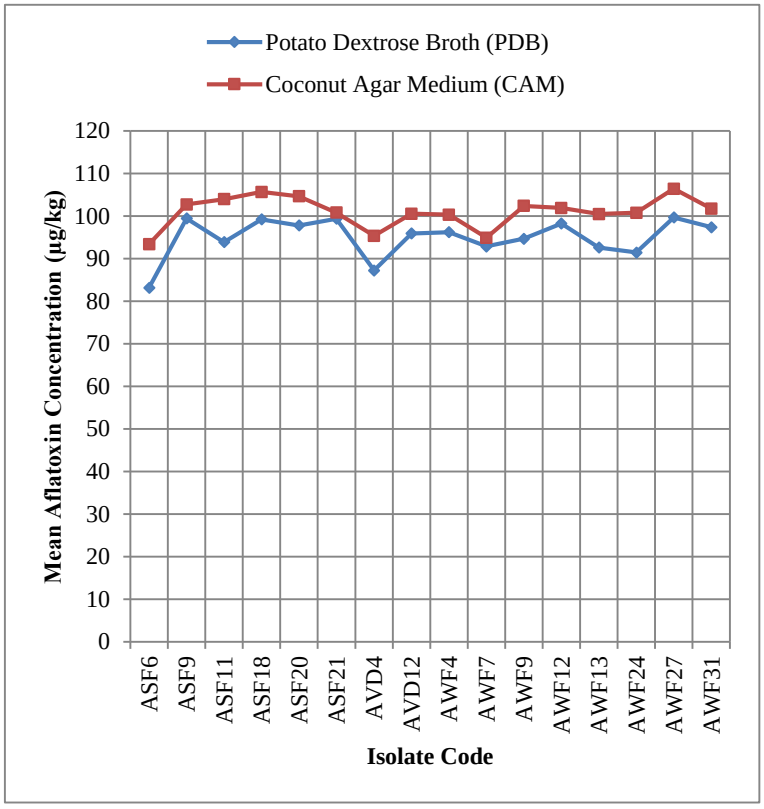

Figure 4: Mean Concentration of Aflatoxin Production by the *Aspergillus sp*

Table 1: Partial Nucleotide Sequences of the Fungal Isolates.

Isolate Code	Sequences
ASF6	GATCATTACCGAGTGTAGGGTTCCTAGCGAGCCCAACCTCCCACCCGTGTTTACTGTACC TTAGTTGCTTCGGCGGGCCCGCCATTCATGGCCGCCGGGGGCTCTCAGCCCCGGGCCCG CGCCCGCCGGAGACACCA-CGAACT- CTGTCTGATCTAGTGAAGTCTGAGTTGATTGTATCGCAATCAGTTAAAACTTTCAACAAT GGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAGTGTGAAT TGCAGAATTCCGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGG GGGCATGCCTGTCCGAGCGTCATTGCTGCCCATCAAGCACGGCTTGTGTGTTGGGTCGTC GTCCCCTCTCCGGGGGGGACGGGCCCCAAAGGCAGCGGCGGCACCGCGTCCGATCCTCG AGCGTATGGGGCTTTGTCACCCGCTCTGTAGGCCCGGCCGGCGCTTGC- CGAACGCAAATCAATCTTCCAGGTGACCTCG-ATCATG

Isolate Code	Sequences
ASF9	CGAGTGCTGGGTCCTTCGGGGCCCAACCTCTCCAACCCGTGCTTACCGTACCCTGTTGCT TCGGCGGGCC-CGCCTTCGGGCGGCCCGGGGCC-TGCCCCCG- GGACCGCGCCCGCCGGAGACCCCAATGGAACACTGTCTGAAAGCGTGCAGTCTGAGTCG ATTGATACCAATCAGTCAAAACTTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAG AACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTT TGAACGCACATTGCGCCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTTCT CCCCCTCCAGCCCCGCTGGGTTGTTGGGCCGCGCCCC--- CGGGGGCGGGCCTCGAGAGAAACGGCGGCACCGTCCGGTCCTCGAGCG- TATGGGGCTCTGTCA-CCCGCTCTATGGGCC-GGCCGGG- CTAGACTCTACGGTAAGCACGGGTGGGAGGT- GGCCCGAAGACACAGACCGATGCCCCCACCAGC
ASF11	ACCCGTGCTTACCGTACCCTGTTGCTTCGGCGGGCCCGCCTTCGGGCGGCCCGGGGCCTG CCCCCGGGACCGCGCCCGCCGGAGACCCCAATGGAACACTGTCTGAAAGCGTGCAGTCT GAGTCGATTGATACCAATCAGTCAAAACTTTCAACAATGGATCTCTTGGTTCCGGCATCG ATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATTCAGTGAATCATCG AGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCA TTTCTCCCCTCCAGCCCCGCTGGTTGTTGGGCCGCGCCCCCCCGGGGGCGGGCCTCGAGA GAAACGGCGGCACCGTCCGGTCCTCGAGCGTATGGGGCTCTGTCACCCGCTCTATGGGC CCGGCCGGGGCTTGCCTCGACCCCCAATCTTCTCAGATT
ASF18	ATCATCGAGTCTTTGGACGCACATTGGCCCCTCTGGTATTCCGGGGGGCATGCCTGTCCG AGCGTCATTTCTCCCCTTCAGCCCGCTGGTTGTTGGGCCGCGCCCCCCGGGGGCGGGCCT TCGAGAGAAACGGCGGCACCGTCCGGTCCTCGAGCGTATGGGGCTCTGTCACCCGCTCT ATGGGCCGGCCGGGGCTTGACTCGACGGTAAGCACGGGTGGAGGCTGCACTCGATGCG ACGCGTATAAGCCCCTCATAAAG
ASF20	GCTACAAGCTAGGAGATCTAGGCCGAGATATATAGGGGGGAATTAGCGGGCTTGGGAA CCTCCACCCGTGCTTACCGTACCCTGTTGCTTCGGCGGGCCCGCCTACGGGCGGCCCGGG GCCTGCCCCCGGGACCGCGCCCGCCGGAGACCCCAATGGAACACTGTCTGAAAGCGTGC AGTCTGAGTCAATTGATACCAATCAGTCAAAACTTTCATCAATGGATCTCTTGGTTCCCG CATCGATGAAGAACGCACCGAAATGCGATAACTAATGTGAATTGCAGAATTCAGTGAAT CATCGAGTCTTTGAACGCACATTGGGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGA GCGTCATTTCTCCCCTCCAGCCCCGCTGGTTGTTGGGCCGAGCCCCCCCGGGGGCAGGCC TCGAGAGAAACGGCGGCACCGTCCGGTCCTCCAGCGTATGGGGCTCTGTCACCCGCTCT ATGGGCCCGGCCGGGGCTTGCCTCCACCCCCAATCTTCTCAGATTGACCTCGGATCAGGT AGGGATACCCGCTGAACTTA
ASF21	TTCCCGAGTGTAGGGTTCCTAGC- GAGCCCAACCTCCCACCGGTGTTTACTGTCCCTTAGTTGATTCGGCGGGCCCCCCATTCA TGGCCCCCGGGGGTTTTCAGCCCCGGGCCCGCGCCCCCCGGAGCCACCAGAAATTCTTT TTGATCTAGGGAATTTTGAGTTGATTGTATGCCAATCAGTTAAAACTTTCACCAATGGTT TTTTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAATTAGTGTAAATTGCA GAATTCCGTAATTCATGGAGTCTTTGAACGCCCATGGCGCCC--- CCGGGTATTCCGGGGGGCATCCCGGTCCAAGGTTCATTGCTGCCCATCAACCCCGGCTGT TGTGTGGGGTGTTCTTCCCTTTTCCGGGGGGGCCGGCCCCAAAGGGCGGGGCGGCCCCG GCTTCCGTCCTTGGGGCTAAGGGGGTTTGTCCACCCCTTTGGAAGCCCGGCCGGGCCCTC CCAAACCCAAATAAACTTTCTGCTATGATAGCGCACTA---ACAGGCAAT--TAATAC

Isolate Code	Sequences
AVD4	AGTGTAGGGTTCCTAGCGAGCCCAACCTCCCACCCGTGTTTACTGTACCTTAGTTGCTTC GGCGGGCCCGCCATTCATGGCCGCCGGGGGCTCTCAGCCCCGGGCCCGCGCCCGCCGGA GACACCACGAACTCTGTCTGATCTAGTGAAGTCTGAGTTGATTGTATCGCAATCAGTTAA AACTTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGAT AACTAGTGTGAATTGCAGAATTCCGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCC CTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCATCAAGCACGGCTTGT GTGTTGGGTCGTCGTCCCCTCTCCGGGGGGGACGGGCCCCAAAGGCAGCGGCGGCACCG CGTCCGATCCTCGAGCGTATGGGGCTTTGTCACCCGCTCTGTAGGCCCGGCCGGCGCTTG CCGAACGCAAAGC
AVD12	GGATCGGAAGGATCATTACCGAGTGCTGGGTCCTTCGGGGCCCAACCTTCCCCACCCGT GCTTACCGTACCCTGTTGCTTCGGCGGGCCCCGCCTTCGGGCGGCCCGGGGCCTGCCCCC GGGACCGGCCCCGCCGGGGACCCCAATGGAAAACTGTTTTGAAAGCGTGCAGTCTGAGT CGATTGATACCAATCAGTCAAAACTTTCAACAATGGATCTCTTGGTTCCGGCATCGATGA AAAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTT TTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTTT CTCCCCTCCAAGCCCCGCTGGTTGTTGGGCCGCGCCCCCCCGGGGGCGGGCCTCGAGAG AAACGGCGGCACCGTCCGGTCCTCGAGCGTATGGGGCACTGTCACCCGCTCTATGGGCC CGGCCGGGGCTTGCCTCGACCCCCAATCTTCTCAGATTGGCCTCGCTCAAAAGACAGAT CGAAGCCGAACTTGGGATT
AWF4	CGAGTGTCTGGGTCCTTCGGGGCCCCAACCTTTCCCACCCCGTGGCTTACCCGTTCCCTG TTGCTTCGGCGGGGCCCCGCCTTCGGGGCGGGCCCGGGGCCTGCCCCCGGGACCCCGCC CCGCCCGGAGACCCCAATGGAACCCTTTCTGAAAGCGTGCAGTCTTGAAGTCGATTGAT ACCAATCAGTCAAAACTTTCAACACATGGATTCTCTTGGTTCCGGCATCGATGAAAGAA CGCAAGCGAAATGCGATTAACTAATTTGAATTGCAGAATTCAGTGAATCATCCGAGTCT TTGAACCGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTTC TCCCCTTCCAGCGCCCGCTGGGGTGGTGGGCGCGCCCCCCGGGGGCGGGCCTCGAGAGA AACGGCGGCACCGTCCGGTCCTCGAGCGTATGGGGCTCTGTCACCCGCTCTATGGGCCC GGCCGGG--GCTTGCCT-CGACCGGTAAGTCCGGGTGGGAGGTGGACCCGAAGACCCAGACCGACCTTCCTTAAG
AWF7	GCAGTCTGAGTCGATTGATACCAATCAGTCAAAACTTTCAACAATGGATCTCTTGGTTCC GGCATCGATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATTCAGTG AATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCC GAGCGTCATTTCTCCCCTCCAGCCCCGCTGGTGGTGGTGGGCCGCGCCCCCGGGGGCGG GCCTCGAGAGAAACGGCGGCACCGTCCGGTCCTCGAGCGTATGGGGCTCTGTCACCCGC TCTATGGGCCCGGCCGGGGCTTGACTCTACGGTAAGTCTTCACGGGTGGGAGGTGGGCC GAAGACACAGACCGATGCCCAACTTTGGC
AWF9	CAGCGGAAGGATCATTACCGAGTGTAGGGTTCCTAGCGAGCCCAACCTCCCACCCCGTG TTTACTGTACCTTAGTTGCTTCGGCGGGCCCGCCATTCATGGCCGCCGGGGGCTCTCAGC CCCGGGCCCGCGCCCGCCGGAGACACCACGAACTCTGTCTGATCTAGTGAAGTCTGAGT TGATTGTATCGCAATCAGTTAAAACTTTCAACAATGGATCTCTTGGTTCCGGCATCGATG AAGAACGCAGCGAAATGCGATAACTAGTGTGAATTGCAGAATTCCGTGAATCATCGAGT CTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTG CTGCCCATCAAGCACGGCTTGTGTGTTGGGTCGTCGTCCCCTCTCCGGGGGGGACGGGC CCCAAAGGCAGCGGCGGCACCGCGTCCGATCCTCGAGCGTATGGGGCTTTGTCACCCGC TCTGTAGGCCCGGCCGGCGCTTGCCGAACGCAAATCAATCTTTCCAGGTGACCTCGATC AGTACGACCCGCCC

Isolate Code	Sequences
AWF12	CCCTGTTGCTTCGGCGGGCCCGCCTTCGGGCGGCCCGGGGCCTGCCCCCGGGACCGCGC CCGCCGGAGACCCCAATGGAACACTGTTTGAAAGCGTGCAGTCTGAGTCGATTGATACC AATCAGTCAAAACTTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGC GAAATGCGATAACTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCA CATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTTCTCCCCCTCCA ACCCCGCTTGGTTGTTGGGCCGCGCCCCCCCGGGGGCGGGGCCTCGAGAGAAAACGGCG GCACCGTCCGGTCCCTCGAGCGTATGGGGCTCTGTCACCCGCTCTATGGGCCCGGCCGA
AWF13	CACCCCGTGGCTTACCCGTTCCCTGTTGCTTCGGCGGGGCCCCGCCTTCGGGGCGGGCCC GGGGCCTGCCCCCGGGACCCCGCCCCGCCCGGAGACCCCAATGGAACCCTTTCTGAAAG CGTGCAGTCTTGAAGTCGATTGATACCAATCAGTCAAAACTTTCAACACATGGATTCTCT TGGTTCCGGCATCGATGAAAGAACGCAAGCGAAATGCGATTAACTAATTTGAATTGCAG AATTCAGTGAATCATCCGAGTCTTTGAACCGCACATTGCGCCCCCTGGTATTCCGGGGGG CATGCCTGTCCGAGCGTCATTTCTCCCCTTCCAGCGCCCGCTGGGGTGGTGGGCGCGCCC CCCGGGGGCGGGCCTCGAGAGAAACGGCGGCACCGTCCGGTCCTCGAGCGTATGGGGC TCTGTCACCCGCTCTATGGGCCCGGCCGGGTTGCTTGCCTACGA
AWF24	GGGTTAGATTATGTTAGTGTTTTTTTAGTGCCGGAAGTTTTTAGTTTTGGTGGGGCTTACG GGGATCTTGGGCCACTCCACCCGTGCTTCCGTACCCTGTTGCTTCGGCGGGCCCGCCTTC GGGCGGACCGGGGCCTGCCCCCGGGACCACGCCCGCCGGAGACCCCAATGGAACACTG TCTGAAAGCGTGCAGTCTGAGTCAATTGATACCAATCAGTCAAAACTTTCAACAATGGA TCTCTTGGTTCCGGCCTCGATGAAGAACGCACCGAAATGCGATAACTAATGTGAATTGC AGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGCATTCCGGGGG GCATGCCTGTCCGAGCGTCATTTCTCCCCTCCAGACCCGCTGGTTGTTGGGCCGAGCCCC CCCGGGGGCAGGCCTCGAGAGAAACGGCGGCACCGTCCGGTCCTCCAGCGTATGGGGC TCTGTCACCCGCTCTATGGGCCCGGCCGGGGCTTGCCTCCACCCCCAATCTTCTCAGATT GACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAACCGAAGGAA
AWF27	GGGGTCGAGGCAAGCCCCGGCCGGGCCCATAGAGCGGGTGACAGTGCCCCATACGCTC GAGGACCGGACGGTGCCGCCGTTTCTCTCGAGGCCCGCCCCCGGGGGGCGCGGCCCAAC AACCAGCGGGGCTTGGAGGGGAGAAAATGACGCTCGGACAGGCATGCCCCCCGGAATA CCAGGGGGCGCAATGTGCGTTCAAAAACTCGATGATTCACTGAATTCTGCAATTCACAT TAGTTATCGCATTTCGCTGCGTTTTTCATCGATGCCGGAACCAAGAGATCCATTGTTGAA AGTTTTGACTGATTGGTATCAATCGACTCAGACTGCACGCTTTCAAAACAGTTTTCCATT GGGGTCCCCGGCGGGGCCGGTCCCGGGGGCAGGCCCCGGGCCGCCCGAAGGCGGGGCC CGCCGAAGCAACAGGGTACGGTAAGCACGGGTGGGGAAGGTTGGGCCCCGAAGGACC
AWF31	CATTACCGAGTGCTTGTGTCCCTTGGGGGGCCCACACTCCCCACCCGTGGCTTAACGTAA CCCTGTGGTGGTTTGGCGGGGCCCGGCCCTCTTGGCCCGCCCCGGGGGCCCTGCCCCCC GGGGAACCGGGGCCCGCCGGAGGACCCCCCAATATGAACCCACGTTCTGGAAAACGTG GCAGTCTGAATTCTGATTGGATACCCAATTCAGGTCAAAAACTTTTCAACAATGGAGAT CTCCTTGTGTTCCCCGCCATTCGATGAAAGAAACGCAGCGAAAATGCGCGTAAACTAAT GGTGAATTGCCAGAATTTCAATGAATCAATCGAGTCTTTTGAACCGCACATTGCGCCCCC TGGTATTTCTGGGGGGGCATGCCTGTCCGAGCGTCCATTTCTTCCCTCCACCCCCCGCTG GTTGGTGGGCCGCGCCCCCCGGGGCGCGGCTCGAGAGAAGAAACGCCGGCACCGTCCG GTCCTCGAGCGTATGGGGCTCTGT

Table 2: Similarity between the Sequences of Fungal Isolates.

Isolate Code	Blast Search Request ID	Sequence length blasted (bp)	Highest coverage (%)	E Value	% identity (accession no.)	Identified name of samples
ASF6	G0DMCXFU015	526	99	0.0	99 (KJ175418.1)	Aspergillus flavus
ASF9	G0EHD4PR015	524	87	0.0	98 (FJ037755.1)	Aspergillus niger
ASF11	G05BM8GM015	456	100	0.0	100 (FJ037755.1)	Aspergillus niger
ASF18	BFMDV5AU015	260	78	2e-88	96 (HE818077.1)	Aspergillus fijiensis
ASF20	G0BJJVME015	554	89	0.0	98 (FJ037755.1)	Aspergillus niger
ASF21	G06YC1MM014	535	93	2e-142	85 (JX501404.1)	Aspergillus flavus
AVD4	G0A44383014	490	99	0.0	100 (KP055655.1)	Aspergillus flavus
AVD12	BA6H5J32014	553	93	0.0	97 (EU833207.1)	Aspergillus japonicus
AWF4	G0F6GC2N014	546	90	0.0	93 (FJ037755.1)	Aspergillus niger
AWF7	BAB6PEKA014	534	88	0.0	97 (KF938958.1)	Aspergillus aculeatus
AWF9	G0AVWG5S01R	548	99	0.0	99 (EF409776.1)	Aspergillus flavus
AWF12	BEX7Y1V7015	414	99	0.0	98 (FJ037755.1)	Aspergillus niger
AWF13	BF3PN8C1015	460	99	0.0	93 (FJ037755.1)	Aspergillus niger
AWF24	G0CCSKVJ01R	591	85	0.0	98 (KJ588207.1)	Aspergillus aculeatus
AWF27	G0GH4UUP01R	469	100	0.0	97 (KF938958.1)	Aspergillus aculeatus
AWF31	BF9FKT6J015	497	100	7e-101	82 (FJ037755.1)	Aspergillus niger

Table 3: Production of Blue Fluorescence on CAM and Growth Measurement in PDB.

Isolate Code	Fungal Identity	Blue Florescence on CAM visible at 365nm	Growth on PDB Measured at 365nm
ASF_6	*Aspergillus flavus*	+	0.263
ASF_9	*Aspergillus niger*	+	0.323
ASF_{11}	*Aspergillus niger*	+	0.270
ASF_{18}	*Aspergillus fijiensis*	+	0.343
ASF_{20}	*Aspergillus niger*	+	0.597
ASF_{21}	*Aspergillus flavus*	+	0.720
AVD_4	*Aspergillus flavus*	+	0.307
AVD_{12}	*Aspergillus japonicus*	+	0.509
AWF_4	*Aspergillus niger*	+	0.270
AWF_7	*Aspergillus aculeatus*	+	0.420
AWF_9	*Aspergillus flavus*	+	0.358
AWF_{12}	*Aspergillus niger*	+	0.320
AWF_{13}	*Aspergillus niger*	+	0.220
AWF_{24}	*Aspergillus aculeatus*	+	0.420
AWF_{27}	*Aspergillus aculeatus*	+	0.358
AWF_{31}	*Aspergillus niger*	+	0.151

Key: PDB – Potato Dextrose Broth, CAM – Coconut Agar Medium.

الجودة الفطرية والتوصيف الجزيئي لأنواع الفطر Aspergillus في مياه الري والخضروات الطازجة في جنوب غرب نيجيريا

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ملخص

في هذه الدراسة، تم عزل الأنواع الفطرية من مياه الري والتربة الزراعية ، وقد تم الحصول على عينات الخضار الطازجة من حقل الخضروات في جنوب غرب نيجيريا. كما تم تحديد مجموعة Aspergillus عن طريق استخدام التضخيم والتسلسل من المنطقة الخاصة (ITS) بإنتاج الأفلاتوكسين باستخدام القياس الطيفي. وقد كان (nm365) أعلى متوسط f فطر 3.2 SD x 103 ± 0.03 CFU / g 3.23و x 104 ± 0.01 CFU / g 3.07 و x 102 ± 0.13 CFU / ml 8.67 لعينات من الخضار والتربة والماء على التوالي. وقد تم تحديد 32 سلالة فطرية معزولة افتراضيا باسم Aspergillus س. (50٪ (، Fusarium sp. (6.25 ٪ (، Chrysonilia sitophila (3.13 ٪ (، Acremonium sp. (6.25٪ (، Mucor sp. (12.5 ٪ (، و Rhizopus sp. (21.87 ٪ .(وقد كشف التوصيف الجزيئي لذرات الرشاشيات عن (Aspergillus aculeatus، Aspergillus fijiensis، Aspergillus flavus، Aspergillus japonicusjaponicas، Aspergillus niger. وقد أظهرت جميع الأنواع Aspergillus 16 عن إنتاج الأفلاتوكسين B1 مع Aspergillus japonicus (AWF27) المعزول من مياه الري ذات أعلى تركيز (106 (µg / kg و Aspergillus fijiensis (ASF6) المعزولة من التربة ذات التركيز الأقل (93 .(µg / kg) وكشفت هذه الدراسة عن احتمال تعرض الإنسان للسموم الفطرية من خلال تناول الخضراوات الطازجة الملوثة. ويلزم بذل المزيد من الجهود لا سيما في البلدان النامية لزراعة الخضروات الطازجة في بيئة صحية تسقى بمياه ري نظيفة.

الكلمات الدالة: fungiFungi، Aspergillus ، الأفلاتوكسين، الخضروات، التربة، مياه الري.

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