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Aflatoxigenic fungi and aflatoxin contamination of *Cochlospermum tinctorium* root powder (Kwata) used for soup preparation.

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Abstract: *Cochlospermum tinctorium root powder (Kwata) is commonly used for food and medicinal purposes in West African sub-region. This research evaluated the aflatoxigenic fungi and aflatoxin contamination of C. tinctorium root powder. The fungi were isolated and identified using a standard mycological method. Aflatoxin concentration of the 'Kwata' obtained from different sellers in Sokoto market was evaluated using Enzyme-Linked Immunosorbent Assay technique (ELISA). The total aflatoxin concentration of the different Kwata samples obtained in Sokoto market had concentration from 125 to 580 ppb while the aflatoxin B1 concentrations in the samples were found to be 35.5 ppb, 24.5 ppb, 32 ppb, 23.8 ppb and 45 ppb all of which are above the limits (20 ppb for adult food and 0 ppb for infant food) stipulated by regulatory bodies in Nigeria. The percentage occurrence of fungal species isolated from Kwata samples include; Aspergillus niger-50% in sample A and above 25% in other samples, Aspergillus flavus-50% in sample A and 25% in other samples, Aspergillus fumigatus 33% in samples B and E and 20% in the sample. In conclusion, it was discovered that Kwata sold within Sokoto metropolis is contaminated with aflatoxin and may pose serious public health problems in the long term primarily due to the consumption of soups prepared from Kwata by children population.*

Key words: *Aspergillus flavus, AgraQuant, Aflatoxin, Kwata, ELISA*

Introduction

Kwata (*Cochlospermum tinctorium*) is a bushy plant that is about 50 cm in height with widespread occurrence in Savannah and shrubs land throughout the drier areas of the West African region. It has common names in Nigeria which are: Rawaya or kyamba (Hausa), obazi or obanzi (Igbo) and sewutu (Yoruba). The plant is commonly used for medicinal purposes in West African sub-region for management of various conditions such as pain and inflammation. The roots of the plant are used traditionally to cure fever, hepatitis, and abdominal pain. They are

also used as a remedy for the treatment of gonorrhoea, jaundice and gastrointestinal diseases (Ahmad *et al.,* 2011).

An extract of the root of kwata (*Cochlospermum tinctorium*) is taken to treat malaria in Burkina Faso. In Nigeria, a concoction of the root with tamarind fruits is used to cure snake bites. A decoction is used in a bath to treat urogenital disorders, kidney pain and pain between the ribs. The body is washed with water extract of the root to cure skin diseases. In cote d'voire, powder of the root is applied topically to treat skin diseases; also the root is chewed as a tonic (Burkill, 2000).

Fungi, during their metabolic processes often produce secondary metabolites called mycotoxins; these are poisonous chemical compounds that are capable of causing disease and death in humans and livestock (Marta *et al.,* 2016; Bennett and Klich, 2003). Mycotoxins are not necessary for the growth and development of fungi; they are thought to be used by the fungus to weaken its host as a strategy to make the environment conducive for fungal proliferation (Hussein and Brasel, 2001). The presence of mycotoxins in agricultural products pose severe threats to human health and also cause significant economic losses in several countries (Horn 2003; Wu. F *et al.,* 2014).

Among the several types of mycotoxins, aflatoxins are of major concern with aflatoxin B1 being the most toxic to humans and animals (Olivier *et al.,* 2017). Generally, aflatoxins are genotoxic, carcinogenic, immunosuppressive substances and can cause both acute and chronic toxicity. Worldwide, aflatoxins are estimated to cause 28% of the total cases of the most common liver cancer - hepatocellular carcinoma (HCC) (Wu, 2014). Wu, (2014) suggest that approximately 172,000 cases of HCC per year are caused by consumption of aflatoxin-contaminated diet, and the majority of cases occur in sub-Saharan Africa. In addition to HCC, consumption of aflatoxin-contaminated foods can cause stunted growth in children, acute poisoning and immune-system dysfunction (Groopman *et al.,* 2008). Related health problems are difficult to diagnose, mainly due to cryptic, long-term and chronic exposures. However, as previously shown and recognised by the Kenyan government in 2004 and 2005, hundreds of human death cases can be ascribed to the consumption of aflatoxin-contaminated products (Lewis *et al.,* 2005).

Plants and herbs are used in most households for culinary purposes; they are often used as additives to enhance flavour and aroma. The consumption of plants or herbs contaminated with mycotoxins may cause ill effects rather than improving the well-being of an individual. This scenario can be averted if there is information on the levels of aflatoxin in commercialised plant root powder. This study aimed to isolate the aflatoxigenic fungi and determine the aflatoxin B1 profile of Kwata (*Cochlospermum tinctorium*), a popular root powder used in Nigeria for preparing soups. In addition, a key aflatoxin biosynthesis gene was amplified from some of the isolated aflatoxigenic organisms.

Materials and Methods

Sample collection

Samples of Kwata (*Cochlospermum tinctorium*) root powder were collected at Sokoto central market and then transported to Microbiology laboratory in Usmanu Danfodiyo University Sokoto, for further analysis.

Isolation and culture condition

The fungi associated with the 'Kwata' was isolated following serial dilution and inoculation on Sabouraud Dextrose Agar (SDA) at 25 ^{o}C for seven days and stored as spore's suspension on 20% glycerol for further analysis (Olivier *et al.,* 2017). Morphological and growth characteristics were carried on Sabouraud Dextrose Agar (SDA). The physiological analysis was carried out on Desiccated Coconut Agar (DCA) (Frisvad and Samson 2004), and yeast extract agar (YES) (Pitt *et al.,* 1983).

Identification of isolates

Isolates were identified using cultural and morphological features such as growth pattern, conidial morphology and pigmentation (Tafinta *et al.,* 2013). Microscopic observation was then carried out by placing a drop of lactophenol cotton blue stain on a glass slide, to this a portion of the fungal mycelia from a pure culture was added and covered with a coverslip, avoiding air bubbles in the process. Viewing was then carried out using the x10 and x40 objective lens and organisms were identified using a comprehensive fungi atlas by Samson and van Reenen-Hoekstra, 1988 (Oyeleke and Manga, 2008).

DNA extraction from Isolates

The DNA isolation was performed using the zymoBIOMICs kit (Zymo research). The cultured spores were added into zymoBIOMICs lysis tube, and 750 µl of zymoBIOMICs lysis solution was then added. It was secured in a bead beater fitted with a 2 ml tube holder assembly and processed at maximum speed for 5 min. The lysis tube was then centrifuged in a microcentrifuge at 10000 x g for 1 min. 400 µl of the supernatant was transferred to a Zymo-spin and centrifuged at 8000 x g for 1 min. 1200 µl of zymoBIOMICs DNA binding buffer was added to the filtrate. 800 µl of the mixture (DNA binding buffer + centrifuged supernatant) was transferred to a Zymo-spin in a collection tube and centrifuged at 10000 x g for 1 minute. The flow from the collection tube was then discarded, and the step was repeated. 400 µl zymoBIOMICs DNA wash buffer 1 was added to the Zymo-spin in a new collection tube and was centrifuged at 10000 x g for 1 min; the flow was discarded. 700 µl of zymoBIOMICs DNA wash buffer 2 was added to the Zymo-spin in a collection tube and centrifuged at 10000 x g for 1 min and the flow through was discarded. 200 µl of DNA wash buffer 2 was added into Zymo-spin and centrifuged at 10000 x g for 1 min. The Zymo-spin was transferred to a clean 1.5 ml microcentrifuge tube and 100 µl (50 µl minimum) zymoBIOMICs DNase/RNase free water was

added directly to the column matrix. It was incubated for 1 min, then centrifuged at 10000 x g for 1 min to elute the DNA.

The Zymo-spin IV-HRC spin filter was prepared by removing the base of the Zymo-spin IV-HRC spin filter and placed into a clean collection tube centrifuged at 8000 x g for 3 min and the flow through was discarded. The cap was removed, and 400 µl zymoBIOMICs DNase/RNase free water was added to the Zymo-spin IV-HRC spin filter. The Zymo-spin IV-HRC spin filter was loosely capped and centrifuged at 8000 x g for 2 minutes. The eluted DNA was transferred to a prepared Zymo-spin IV-HRC spin filter in a clean 1.5 ml microcentrifuge tube. The Zymo-spin IV-HRC spin filter was loosely capped and centrifuged at precisely 8000 x g for 1 min.

PCR confirmation of aflatoxigenic fungi

The confirmation of aflatoxigenic fungi was performed as described previously (Medeiros *et al.,*2017). In order to optimise the PCR amplification assay for direct detection of mycotoxigenic fungal species by targeting the aflatoxin biosynthesis gene in the fungi; the primers used for species-specific detection and amplification of the gene involved in mycotoxin biosynthesis were tested and confirmed. The PCR amplification was standardised by empirically varying critical factors that affect amplification such as primer concentration, amount of template and annealing temperature. The set of primers used for amplification are avf723F (5'-ATGGTCACATACGCCCTCCTCGGG-3') and avf1675R (5'-GCCTCGCATTCTCTCGGCGACCGAA -3'), with annealing temperature 58^{o}C. These primers amplify the *avfA* gene that is involved in the conversion of averufin (AVF) to versiconal hemiacetal acetate (VHA). The expected amplicon size is 950 bp (Yu *et al.,* 2000).

The PCR reaction was performed in 0.2 ml thin-wall PCR tubes with flat frosted caps in 17.5 µl PCR reaction volume containing 3µl of template DNA, 1µl each of primer avf723F and avf1675R, 12.5 µl Taq master mix - containing 20 µM of deoxynucleoside triphosphates (dNTP), Taq DNA polymerase and Tag buffer with $MgCl_2$.

The PCR thermocycling conditions include Initial heat activation of DNA polymerase at 95^{o}C for 15 min; followed by 35 cycles of denaturation at 94 ^{o}C for 30 secs, annealing at 58^{o}C for 1 min, extension at 72^{o}C for 90 seconds, and a final extension at 72^{o}C for 10 min. The PCR products were electrophoresed on a 1% agarose gel with a 100bp DNA size marker at 96V for 1 hour (Medeiros *et al.,*2017). The PCR products were then purified using PCR product purification kit, and the purified PCR products were sent to Inqaba Biotech South Africa for sequencing service. The quality of the sequences from Inqaba was analysed using finch TV, and sequence analysis were performed by comparing the sequences against the known sequences in the NCBI database.

Quantification of Aflatoxin in Kwata

The total aflatoxin and aflatoxin B1 was determined by Animal care services Konsult (Nig) Ltd laboratory. The analysis of total aflatoxin and aflatoxin B1 (AFB1) content of the 'Kwata' samples were performed using ELISA kit AgraQuant® ELISA total and Aflatoxin B1 (Romer Labs, Singapore). Five grams (5g) of Kwata was weighed and added to 25 ml of methanol (70%). It was allowed to stand for 10 mins in order to aid aflatoxin extraction, then filtered using a No. 1 Whatman filter paper. 50 µl of sample filtrate and aflatoxin standard were dispensed in separate dilution wells, and each was with 100 µl of the conjugate. 100 µl from the filtrate/standard-conjugate mixture was taken and dispensed in the antibody-coated wells. It was then incubated at room temperature for 15 mins. The content of the wells was discarded, and the wells were washed 3 – 4 times with distilled water. 100 µl of substrate was added to each well and incubated for 5 mins to allow for colour change (different shades of blue to colourless). 100 µl of stop solution was added which converts the blue end-point to yellow, then the mixture was read with an ELISA plate reader at 450nm. The optical densities of standards (0 ppb, 4 ppb, 10 ppb, 20 ppb and 40 ppb) and those of samples were recorded. A standard curve was generated which was used to extrapolate the concentrations of total aflatoxin and aflatoxin B1 of the samples. Samples with high levels of aflatoxin were diluted further with 70% methanol to either 1/10th or 1/20th (or more) of the original concentration in order to obtain readings within the range of the standard curve.

Results

Aflatoxin B1 concentrations in Kwata (*Cochlospermum tinctorium* root powder) obtained from old market Sokoto and its limits for food in Nigeria showed that sample E had the highest concentration of aflatoxin B1(45 ppb), sample A (35.5 ppb), sample B (24.5 ppb), sample C (32 ppb), and sample D has the lowest concentration (23.8 ppb) (**Figure 1**).

The fungi associated with the contamination of Kwata (*Cochlospermum tinctorium* root powder) were determined in this work. Morphological and microscopic identification of the isolated fungi showed that the *C. tinctorium* roots powder were contaminated with four organisms of which three were identified as *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus*. The fourth organisms could not be identified based on the chart used for mycological identification (**Table 1**).

The frequency of occurrence of fungi associated with *Cochlospermum tinctorium* root powder obtained from Sokoto market was determined, and the result shows that *A. niger* and *A. flavus* had frequency of 50% in sample A and had more than 25% frequency in all the other Kwata samples (**Figure 2**). *A. fumigatus* had frequency of occurrence of 33.3% in both samples B and E, 20% in sample C, and 20% in sample D. The uncharacterised organism appeared in sample B and D with occurrence frequencies of 16.6% and 20% respectively.

PCR analysis detected the presence of the aflatoxin biosynthesis gene (*avfA*) in *A. flavus* and the uncharacterised organism from the Kwata. The molecular analysis confirmed the presence of the *avfA* gene in the *A. flavus* with 99% identity to *A. flavus* NRRL 3357 and the uncharacterised organism had 100% identity to an *avfA* gene from *A. flavus* isolate AF70 (**Table 2**).

Phylogenetic analysis based on neighbour-joining tree showed that our the aflatoxin biosynthesis genes from our isolates had some degree of difference based on their nucleotide sequences with the divergent from the root at 0.2 confidence limit based on the bootstrap analysis (**Figure 3**).

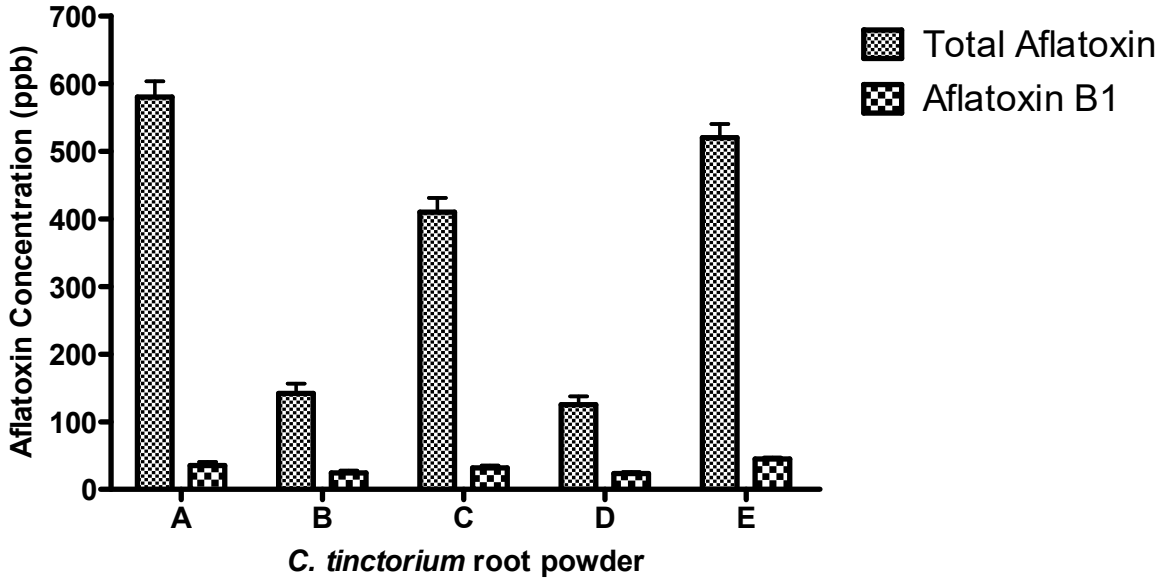

Figure 1: Total Aflatoxin and aflatoxin B1 concentrations in different *C. tinctorium* root powder samples obtained within Sokoto Market, Nigeria. Columns represent means of triplicate measurements and error bars are standard deviations (SD) from the means. The Nigerian limits for AFB_1 concentrations in adults and infants foods are 20 and 0 ppb (Tiffany, 2013) respectively.

Table 2: The Phenotypic identity of fungal species isolated from Kwata (*Cochlospermum tinctorium* root powder) obtained within Sokoto market.

Identified organism	Colony description	Microscopy
Aspergillus niger	It is black having a round shape, and it is powdery. The reverse is yellow.	The conidiophore terminates in vessels, and the conidia are in chains.
Aspergillus flavus	It is green in colour and powdery.	They have hyphae bearing conidiophores
Aspergillus fumigatus	It is blue and smooth	They have hyphae with conidiophores
Uncharacterized	Black in colour with crystals	They have hyphae conidiophores

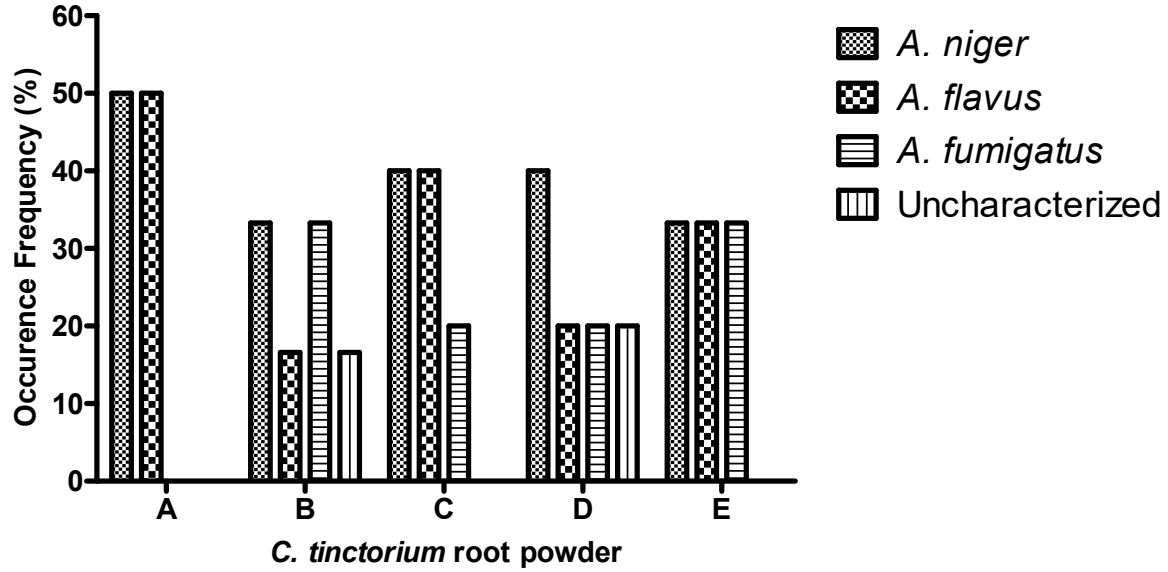

Figure 3: Frequency of occurrence of fungi associated with *C. tinctorium* root powder obtained from Sokoto market, Nigeria.

Table 4: Molecular confirmation of aflatoxigenic fungi based on PCR amplification of aflatoxin biosynthetic gene (*avfA*)

Seq ID	Best Hit	% Query coverage	% Identity	Accession
AF1	*Aspergillus flavus* NRRL 3357 SAGA Complex component (sgfl3)	69	99	XM002382146.1
SB	*Aspergillus flavus* isolate AF70 aflatoxin biosynthesis gene cluster complete sequence	100	100	XM002382146.1

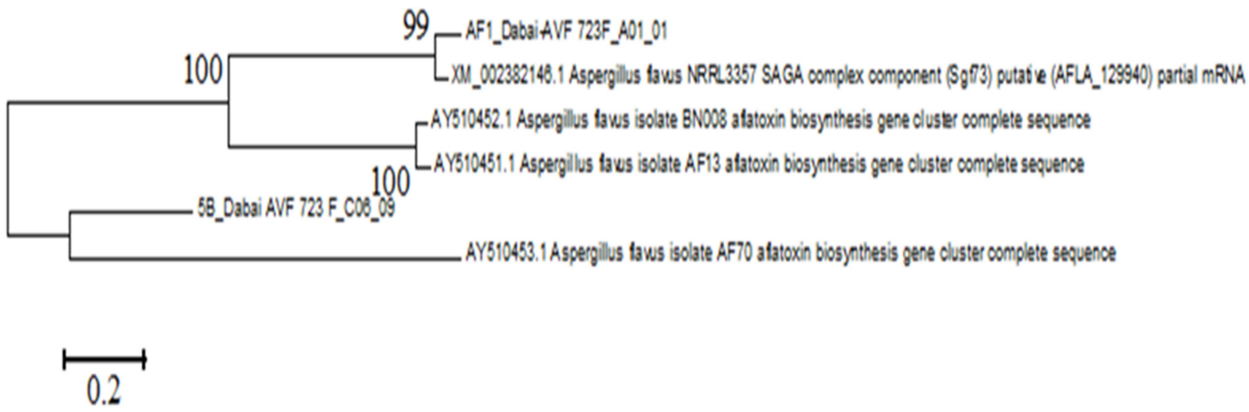

Figure 3: Neighbour-joining tree showing the relationship of the aflatoxin biosynthesis genes of identified isolates to their closest relatives available on the NCBI database.

Discussion

In this study, we have detected the presence of aflatoxins and isolated aflatoxigenic fungi in Kwata obtained from Sokoto market, northwestern Nigeria, a root powder widely known for its culinary importance. High concentrations of aflatoxin B1 (AFB_1) were found in all the *Cochlospermum tinctorium* root powder samples; the concentrations were in the range of 23.8 ppb to 45 ppb which are all higher than the maximum acceptable limits of aflatoxins in foods. Therefore, the Kwata obtained from Sokoto market is not safe for human or livestock consumption. Aflatoxins are correlated to adverse health effects such as cancer; the most toxic amongst them is aflatoxin B1 (AFB_1), it is a potent carcinogen and has been directly related to liver cancer in several animals. AFB_1 is carcinogenic because it is metabolized by the liver to the highly reactive and electrophilic epoxide intermediate which causes hepatotoxicity (Dohnal and Kuča, 2014).

The mycotoxin producing organisms associated with Kwata (*Cochlospermum tinctorium* root

powder) were isolated and characterized. These organisms include *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus* and another uncharacterized organism. The presence of these organisms is not surprising as they could come from soil or field (preharvest) or during storage (postharvest) (Marta *et al.,* 2016). *Aspergillus spp*. are widely distributed and are the major sources of mycotoxins. *Aspergillus* is found in food storage places and produces mycotoxins at suitable moisture and temperature conditions (Surekha *et al*., 2011). Amongst all the *Aspergillus spp*., *A. flavus* is the major producer of AFB_1 and AFB_2. Other species such as *A. parasiticus* also synthesizes AFB_1 and AFB_2 alongside other aflatoxins such as AFG_1 and AFG_2 (Bennett and Klich, 2003). *A. niger* and *A. fumigatus* have not been reported to produce aflatoxin; however, some strains were found to produce ochratoxin A. and gliotoxin respectively (Schuster *et al.,* 2002; Nieminen *et al.,* 2002). The biological, chemical and physical conditions of *Aspergillus* influence the production of aflatoxins (Kumar *et al.,* 2017).

Amongst the four organisms isolated, *A. niger* has the highest frequency of occurrence in all the samples. Interestingly, *A. niger* has been shown to inhibit the biosynthesis of AFB_1 in *A. flavus* through down-regulation of the expression of major biosynthetic genes. Remarkably, 19 out of 20 aflatoxin biosynthetic genes were reported to be down-regulated by *A. niger* (Xing *et al.,* 2017). Despite this crucial biological role played by *A. niger* in inhibiting AFB_1 biosynthesis, the concentration of AFB_1 in our samples is alarming as it is at least 4-fold higher than the acceptable limit.

PCR analysis detected the presence of a critical aflatoxin biosynthesis gene (*avfA*) in our isolated *A. flavus* and the uncharacterized organism from the Kwata. The molecular analysis confirmed the presence of the gene in our *A. flavus* isolate with 99% identity to that from *A. flavus* NRRL 3357 and the uncharacterised organism showed 100% identity to aflatoxin biosynthesis gene from *A. flavus* isolate AF70. This finding further confirms that our isolates are indeed aflatoxigenic organisms.

Although it is difficult to prevent aflatoxin formation in food before harvesting due to heavy rainfall, temperature and moisture content; however, it is possible to reduce their level by good hygienic conditions during transport and storage (Marta *et al.,* 2016; Zinedine and Maes, 2009). Decreasing or controlling fungal growth and eliminating aflatoxins formation in foods for human consumption and animal feed is essential for food security and health.

Conclusion

In conclusion, our findings show that kwata (*C. tinctorium* roots powder) obtained within Sokoto metropolis harbours fungal species that are capable of producing aflatoxins. Four different fungal species were isolated; three Aspergillus spp (*A. niger, A. flavus* and *A. fumigatus*) and one uncharacterized organism. Furthermore, PCR and molecular analysis of the key aflatoxin biosynthetic gene (*avfA*) confirmed that two of our isolates (A. flavus and uncharacterised) are indeed aflatoxigenic organisms. The 100% identity of the *avfA* gene from

the uncharacterised isolate to *A. flavus* isolate AF70 suggests that the uncharacterised organism is highly likely to be an *A. flavus* strain. The concentrations of total aflatoxins and aflatoxin B1 determined in all the samples exceeded the acceptable limits. Therefore, these results show that *C. tinctorium* root powder is not safe for consumption and it can be used in establishing public health awareness on the consumption of contaminated foods.

References

Ahmad T. S., Magaji, M. G., Yaro, A. H Musa, A. M. and Adamu A.K (2011). Aqueous methanol extracts of *Cochlospermum tinctorium* anti-inflammatory activities. *Journal of young pharmagologist* **3**(3) 237-242.

Bennett, J.W. And Klich, M.,(2003). Mycotoxins. *Clinical microbiology reviews,* **16**(3), 497-516.

Burkill HM (2000). "*The useful plants of west Tropical Africa*". 2nd Edition. Royal Botanical Garden. Kew. **1**: 387

Dohnal, V., Wu, Q. and Kuča, K.,(2014). Metabolism of aflatoxins: Key enzymes and interindividual as well as interspecies differences. *Archives of Toxicology,* **88**(9), pp. 1635-1644.

Frisvad J.C., Samson R.A(2004). "*Polyphasic taxonomy of Penicillium subgenus Penicillium. Aguide to identification of food and air-borne terverticilliate Penicillia and their mycotoxins*". Studies in Mycology; **49** :1–174.

Groopman, J.D., Kensler, T.W. and Wild, C.P., (2008). *Protective interventions to prevent aflatoxin-induced carcinogenesis in developing countries.*

Horn BW (2003). "*Ecology and population biology of aflatoxigenic fungi in soil*". Journal of Toxicology Toxin Review, **22**:355-383.

Hussein, H.S. and Brasel, J.M., (2001). Toxicity, metabolism, and impact of mycotoxins on humans and animals. *Toxicology,* **167**(2), pp. 101-134.

Kumar, P., Mahato, D.K., Kamle, M., Mohanta, T.K. and Kang, S.G., (2017). Aflatoxins: A global concern for food safety, human health and their management. *Frontiers in Microbiology,* **7**(January), 2170.

Lewis L, M. Osongo, Henry N, H. Schurzrogers, G. Luber, S. Kreszak, J. Nyamongo, L. Backer, A. M Dahiye, A Misore, K. Decock, C. Rubin (2005). "*Aflatoxin contamination of commercial maize products during an outbreak of acute Aflatoxicosis in Eastern and Central Kenya*". Journal of Environmental Health perspective **113**(12):1763-1767

Marta Tola, Bedaso Kebede & Fatih Yildiz (2016) Occurrence, importance and control of mycotoxins: A review, Cogent Food & Agriculture, 2:1, DOI: 10.1080/23311932.2016.1191103

Medeiros, F. A. C., Gomes, L. I., Oliveira, E., de Souza, C. S. A., Mourão, M. V., Cota, G. F.,Rabello, A. (2017). Development and validation of a PCR-ELISA for the diagnosis of symptomatic and asymptomatic infection by Leishmania (Leishmania) infantum. *Journal of tropical medicine.*

Nieminen, S.M., Kärki, R., Auriola, S., Toivola, M., Laatsch, H., Laatikainen, R., Hyvärinen, A. and Von Wright, A., (2002). Isolation and identification of Aspergillus fumigatus mycotoxins on growth medium and some building materials. *Applied and Environmental Microbiology,* **68**(10), pp. 4871-4875.

Olivier p., Amaranta C. C., Ama L. M., S. Tadrist, D. K. Akaki, R. K.Nevry, G. G. Moore, S. O. Fapohunda, S. Bailly, D. Montet, I. P. Oswald, S. Lorber, C. Barbet (2017). "*Aspergillus korhogoensis, A Novel Aflatoxin producing species from the cote d'voire*". Toxins 1-18.

Oyeleke, A. and Manga, S.B. (2008). Essential of Laboratory Practice, 3rd edition, Tobes Publishers, Minna, Niger State, Nigeria.12-29.

Pitt, J.I., Hocking, A.D. and Glenn, D.R. (1983). "*An improved medium for the detection of Aspergillus flavus and A. parasiticus*". Journal of General Microbiology **101**, 35-40.

Schuster, E., Dunn-Coleman, N., Frisvad, J. and Van Dijck, P.,(2002). On the safety of *Aspergillus niger.* A review. *Applied Microbiology and Biotechnology,* **59**(4-5), 426-435.

Surekha, M., Saini, K., Reddy, V. K., Reddy, A. R., & Reddy, S. (2011). Fungal succession in stored rice (Oryza sativa Linn.) fodder and mycotoxin production. *African Journal of Biotechnology, **10***(4), 550.

Tafinta I. Y., K. Shehu, H. Abdulganiyyu, A. M. Rabe and A. Usman (2013). Isolation and identification of fungi associated with spoilage of sweet oranges (citrus sinensis) fruits in Sokoto state. Nigerian journal of basic and applied science (3)**21**:193-196.

Tiffany, I., (2013). The implication of aflatoxin contamination for local food safety in Senegal. *World Mycotoxin Journal **5***, 225-256.

Wu F., Groopman J.D., Pestka J.J (2014). "*Public health impacts of foodborne mycotoxins*". Annuual Review of Food Science Technology; **5**:351–372. doi: 10.1146/annurev- food-030713-092431.

Xing, F., Wang, L., Liu, X., Selvaraj, J.N., Wang, Y., Zhao, Y. And Liu, Y.,(2017). Aflatoxin B1inhibition in Aspergillus flavus by Aspergillus niger through down-regulating expression of major biosynthetic genes and AFB1degradation by atoxigenic A. flavus. *International journal of food microbiology,* **256**, 1-10.

Yu, J., Woloshuk, C.P., Bhatnagar, D. and Cleveland, T.E.,(2000). Cloning and characterization of avfA and omtB genes involved in aflatoxin biosynthesis in three Aspergillus species. *Gene,* **248**(1-2), 157-167.

Zinedine, A., & Mañes, J. (2009). Occurrence and legislation of mycotoxins in food and feed from Morocco. *Food Control,* ***20***(4), 334-344.