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Molecular Identification and Sequencing of Fungi Associated With Selected Grain Legumes in Four States of the North Central Nigeria

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The purpose of this work was to carry out molecular identification and sequencing of the fungi associated with grain legumes of bambara nuts, soybeans and cowpeas sold in Kogi, Nasarawa, Niger and Plateau State respectively. 1 kilogram each of Bambara nuts, soybeans and cowpeas were purchased from each States and taken the Plant Science & Biotechnology, Laboratory, Federal University of Lafia. 10 grains each were sterilized in 1% Sodium Hypochlorite, rinsed in deionized water and plated in sterile in three replicates in Petri Dishes moist Blotter paper and left on the bench for 4- 7 days. Pure cultures obtained were inoculated in McCartney bottles containing sterile PDA slants. These were shipped to the Centre for Agriculture and Bioscience, CABI, UK for identification and sequencing. Results showed that Aspergillus oryzae, and Aspergillus flavus were the dominant species associated with Bambara Nuts in Kwande, Aspergillus niger, A. welwitschiae and A. awamori were the dominant species associated with Bambara Nuts in Lamingo, Jos East, Plateau State respectively; A. welwitschiae and A. niger were associated with Bambara Nuts in Lapai City, Niger State; A. welwitschiae, A. niger and A. lacticoffeanus associated with Bambara Nuts in Yelwa, Wushishi, Niger state. A. flavus, A. oryzae were associated with Bambara Nuts, in Wushishi, Niger E0000895004. A. flavus was associated with Cowpeas in Itobe, Ajaokuta, Kogi State; A. aflatoxiformans, A. oryzae, A, agricola and A. toxicus niger, were associated with bambara Nuts in Angwa Tiv, Akwanga, Nasarawa State A. flavus produces only AFB_1, and AFB_2 but A. parasiticus excretes AFB_1, AFB_2, AFG_1, and AFG_2 but not identified here. Curiously soybeans didn't have any contamination; Cowpeas only had a couple and Bambara had 80% of the contamination.

Keywords: *bambara nuts, soybeans, cowpeas, North Central Nigeria. CABI, Aspergillus*

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I. Introduction

The incidence of aflatoxin contamination in crops and livestock is a serious problem in many parts of the world as aflatoxin invasion crops and feedstock compromises public health and development efforts (Jallow *et al*., 2021). The United Nations Food and Agriculture Organization (FAO), says that 25% of world food crops are affected by aflatoxin contamination, and countries that are situated between 40ºN and 40ºS suffer the most risk (Eskola *et al.*, 2020). More than 4.5 billion people in developing countries are at risk of chronic aflatoxin exposure (Williams *et al*., 2004). If the current level of aflatoxin content of crops and livestock feeds are not well managed, food security and improvement of health will not be achieved. Because aflatoxins are confined to crops consumed by animals, they have become a major challenge for animal feeds. also. The most susceptible animals here are rabbits, turkeys, chickens, pigs, cows and goats (Lizárraga-Paulín *et al*., 2011). The molds that excrete aflatoxin on legume grains also invade the aerial parts of the plant by coercion; they are soil-borne, and survive on decaying vegetation, hay, and on grains, cultivated in the tropics (Marin *et al*., 2013), surviving through anaerobic respiration (Willger *et al*., 2009). Aflatoxins are therefore, poisonous metabolites of *Aspergillus flavus* and *Aspergillus parasiticus* (Jef *et al*., 2015) causing different cancers and the degeneration of the immune system in man. Harmful bye-products released when aflatoxin passes through the liver, are expelled through the renal flux (Bbosa, *et al*., 2013).

Bambara Nuts

Poorly dried bambara nut seeds are eaten by insects and they become susceptible to fungal attack (De Vires *et al*., 2002). These molds excrete aflatoxin which cause various illnesses, when the grains are consumed, especially in some African countries, where poor environmental conditions, poor food handling practices and poor storage practices encourage fungal growth (Doku, 1995).

II. Soybeans

Soybean is one of the world's most important grain legumes, containing high-quality protein, vegetable oil, milk, fibre and phytochemicals for human and livestock consumption (Fukushima, 2000; Kerley and Allee, 2003; Palacios *et al*., 2004; Lusas and Riaz, 1995). The protein content rates as a protein digestibility-corrected amino acid score (PDCAAS) of 0.99 (the maximum value for individual protein is 1.0 or 100%) (WHO/FAO/UNU, 2007). Soybean is susceptible to seed-borne diseases and contaminated by sixty-six (66) fungal species, six (6) bacterial and eight (8) viral species (Sinclair, 1977). These seed-borne micro-organisms deteriorate the crop, reduce seed germination or seedling emergence, cause blights, leaf spots and other diseases on mature plants.

Cowpeas

The challenge of cowpea production is storage, (Folefack, *et al*, 2013). Aflatoxin contamination is known to alter the appearance, colour and taste of grains (Kouadio, 2012; Hell *et al*., 2000; Udoh *et al*., 2000). Cowpeas are however, not very susceptible to aflatoxin contamination because the tannins of the seed coat possess antifungal properties (Digrak *et al.,* 1999). The tannin is highly concentrated in the seed coat and prevents fungal invasion and colonization (Vincenzo *et al*., 2005; Plahar *et al*., 2001). Fifty-six (56%) of cowpeas and wheat samples collected from some North Central States of Nigeria however, had unacceptable levels of AFB_1 (Makun *et al*., 2009).

III. Materials And Methods

3.1 Sample Collection

One (1) kilogram each of bambara nuts, soybeans and cowpeas were randomly purchased from markets in Kogi, Nasarawa, Niger and Plateau. In each state, three local governments or three senatorial districts or agricultural zones were sampled. In the three local governments, six towns were sampled, that is, two towns/local government. 24 samples were purchased from each of the 4 states respectively. The purchased samples were dispensed into sterile polythene bags, sorted and taken to the Plant Science & Biotechnology Laboratory at the Federal University of Lafia, Nasarawa State for further processing.

3.2 Standard Blotter Method:

The blotter method. which was developed by Doyer in 1938 was later included in the International Seed Testing Association (ISTA) rules of 1966. The method was later used by Singh *et al.,* (1974)

Ten (10) grain legumes were randomly selected, washed in running water to remove chemicals and debris before immersing in 1% and 2% (v/v) respectively in sodium hypochlorite for 1 minute and rinsed four times in dionized water. The seeds were incubated on a damp blotter (Whatman's Filter paper) placed in Petri Dishes, labelled and left on the bench at 26℃ for 7 days under cycles of 12 h NUV light/12h in darkness (McGee, 1994; Singh *et al*., 1974). Daily observation of growth of fungi were recorded. At the end of 7 days, the final readings were recorded. The Petri Dishes containing infected seeds were selected for further processing and empty seeds were discarded.

3.3 Isolation and identification of associated fungi

The Petri Dishes containing contaminated grains were collected for further processing. Potato Dextrose Agar (PDA) media were prepared for the isolation of the observed fungal growth. The PDA was autoclaved at a temperature of 121°C and 15psi for 30 minutes. Then the fungi observed in the Petri Dish were aseptically inoculated into these freshly prepared PDA. The Petri Dishes were labelled and placed on the bench for 7 days. This is to enable the development of pure cultures of the organisms. Having obtained the pure cultures, PDA slants were prepared and inoculated with the pure cultures for identification and characterization. These were sent to the Microbial Identification Service, CABI (Center for Agriculture and Bioscience International), Bakeham Lane, Egham, Surrey, England. Pictures of the pure culture were documented.

Identification of samples

DNA Lysis

At CABI, all original samples were subjected to purity checks. Molecular assays were performed on each sample using nucleic acid as a template. A proprietary formulation (microLYSIS (MLP), Microzone, UK) was

subjected to the rapid heating and cooling of a thermal cycler, to lyse fungal cells and release the deoxyribonucleic acid (DNA).

Polymerase Chain Reaction

After DNA extraction, Polymerase Chain Reaction (PCR) was used to amplify copies of the (Recombinant DNA) rDNA in vitro. The quality of the PCR product was assessed by undertaking gel electrophoresis. The PCR purification step was carried out to remove unused (Deoxyribonucleotide triphosphates) dNTPs containing deoxyribose, primers, polymerase and other PCR mixture compounds to obtain a highly purified DNA template for sequencing. This procedure also allows the concentration of low yield amplicons.

Sequencing

Sequencing reactions were undertaken using BigDye® Terminator v3.1 kit from Applied Biosystems (Life Technologies, UK) which uses fluorescent labelling of the chain terminator Dideoxynucleotides (ddNTPs), to permit sequencing. The excess unincorporated dye terminator was removed to ensure a problem-free electrophoresis of fluorescently labelled sequencing reaction products on the capillary array AB 3130 Genetic Analyzer (DS1) DyeEx™ 2.0 (Qiagen, UK). Samples were loaded onto the AB 3130 Genetic Analyzer and sequencing performed to determine the order of the nucleotide bases, adenine, guanine, cytosine, and thymine in the DNA oligonucleotide. At the end of the sequencing processes, fungal identifications were undertaken by comparing the sequence obtained with those available from the European Molecular Biology Laboratory (EMBL) database via the European Bioinformatics Institute (EBI).

IV. Results And Discussion

CABI NO. E0000895001 (Bambara nuts from Kwande, Shendam, Plateau State)

The ITS sequence (see appendix) obtained from this sample produced top matches at >99% identity to members of *Aspergillus* section Flavi. The top matches included sequences from type material published in peer-reviewed literature, e.g. 100% identity to sequence EF661560 from the type strain of *Aspergillus oryzae* (NRRL 447) and 99.6% identity to sequence NR111041 from the type strain of *A. flavus* (ATCC 16883) (Schoch *et al*., 2014). Members of *Aspergillus* section Flavi are common worldwide and are frequently isolated from soil, air and as contaminants of a wide variety of foods, e.g. cereals, nuts and fruits. Many species in this group are both halotolerant and thermotolerant and also grow well in conditions of low water activity. Many strains produce mycotoxins, including both A and G aflatoxins and cyclopiazonic acid. All members of the genus *Aspergillus* are assigned by Advisory Committee on Dangerous Pathogens (ACDP) (UK) to hazard group 2.

CABI NO. E0000895002 (bambara nuts from Lamingo, Jos East, Plateau State)

The ITS sequence (see appendix) obtained from this sample produced top matches at 100% identity to members of *Aspergillus* section Nigri. The best matches included type strains of a number of species in this group e.g. sequence AY373852 from the type strain of *A. niger* (ATCC 16888), sequence FJ629340 from the type strain of *A. welwitschiae* (CBS 139.54) and sequence MH862766 from the type strain of *A. lacticoffeanus* (CBS 101883). These results were confirmed by partial calmodulin gene sequencing which also produced top matches at >98% identity members of *A.* section Nigri including sequence KF288119 from the type strain of *A. awamori* (NRRL 4948), sequence KC480196 from the type strain of *A. welwitschiae* (CBS 139.54) and sequence EF661154 from the type strain of *A. niger* (NRRL 326) Samson *et al.,* (2011).

CABI NO. E0000895003 (Bambara nuts, Lapai City, Niger State)

Partial calmodulin gene sequencing (see appendix) produced top matches at >98% identity members of A. section Nigri including sequence KC480196 from the type strain of *A. welwitschiae* (CBS 139.54) and sequence EF661154 from the type strain of *A. niger* (NRRL 326), Samson *et al*. (2011). Members of this group are common and widespread, occurring on a wide range of substrata such as soils and plants and as contaminants in indoor environments and on foodstuffs including fruit, vegetables and nuts. They have been reported to produce such mycotoxins as malformin and naphthopyrones and some strains are known to produce ochratoxin. Members of this group infect man and animals causing superficial and local infections (cutaneous infections, otomycosis, tracheobronchitis), infections associated with damaged tissue (aspergilloma, osteomyelitis), pulmonary infections and clinical allergies (allergic bronchopulmonary aspergillosis, rhinitis, farmers' lung). However, the majority of infections relate to immunocompromised individuals. Members of the genus *Aspergillus* are assigned to hazard group 2 by ACDP (UK). Partial calmodulin gene sequencing produced top matches of members of Aspergillus section Nigri, sequence KC480196 from type strain *A. welwitschiae* (139.54), sequence EF661154 from type strain *A. niger* (NRRL326) Samson *et al*., 2011. These two organisms are same as in CABI 2, that is, the organisms in Lamingo, Jos East, Plateau are the same as those identified in Lapai, Niger State.

CABI NO. E0000895004 (Bambara nuts, Yelwa Wushishi, Niger State)

The ITS sequence (see appendix) obtained from this sample produced top matches at 100% identity to members of *Aspergillus* section Nigri. The best matches included type strains of a number of species in this group e.g. sequence AY373852 from the type strain of *A. niger* (ATCC 16888), sequence FJ629340 from the type strain of *A. welwitschiae* (CBS 139.54) and sequence MH862766 from the type strain of *A. lacticoffeanus* (CBS 101883). These results were confirmed by partial calmodulin gene sequencing which also produced top matches at >98% identity members of *A.* section Nigri including sequence KC480196 from the type strain of *A. welwitschiae* (CBS 139.54) and sequence EF661154 from the type strain of *A. niger* (NRRL 326), Samson *et al*., (2011). Members of *Aspergillus* section Flavi are common globally and are frequently isolated from soil, air and as contaminants of a wide variety of foods, e.g. cereals, nuts and fruits. Many species in this group are both halotolerant and thermotolerant and also grow well in conditions of low water activity. Many strains produce mycotoxins, such as AFB_1, AFB_2, AFG_1 and AFG_2 and cyclopiazonic acid. All members of the genus *Aspergillus* are assigned by ACDP (UK) to hazard group 2. *Aspergillus flavus* in literature (Donner *et al*., 2015; Yu *et al*., 2011; Dhanasekaran *et al*., 2011; Abdel-Hadi *et al*., 2011). produces only AFB_1, AFB_2 while *Aspergillus parasiticus* produces all four aflatoxins but was not identified as an associated fungus. The ITS sequence (see appendix) obtained from the sample produced top matches at 100% to members of Aspergillus section Nigri and the best matches included type strains of sequence AY373852 from the type strain of *A. niger* (ATCC 16888), sequence FJ629344 from the type strain of *A. welwitschiae* (CBS 139.54) and sequence MH862766 from the type strain of *A. lacticoffeanus* (CBS 101883). Partial calmodulin gene sequence further confirmed KC480196 from the type strain *A. welwitschiae* (CBS 139.54) and sequence EF661154 from the type strain *A. niger* (NRRL 326) Samson *et al*., 2011. These organisms are same as those identified in Lamingo, Jos East, Plateau State.

CABI NO. E0000895005 (Bambara nuts, Wushishi City, Niger State)

The ITS sequence (see appendix) obtained from this sample produced top matches at >99% identity to members of *Aspergillus* section Flavi. The top matches included sequences from type material published in peer-reviewed literature, e.g. 99.4% identity to sequence EF661560 from the type strain of *Aspergillus oryzae* (NRRL 447) (Peterson, 2008) and 99.0% identity to sequence NR111041 from the type strain of *A. flavus* (ATCC 16883) Schoch, *et al*. (2014). Members of *Aspergillus* section *Flavi* are common globally and are frequently isolated from soil, air and as contaminants of a wide variety of foods, e.g. cereals, nuts and fruits. Many species in this group are both halotolerant and thermotolerant and also grow well in conditions of low water activity. Many strains produce mycotoxins, including AFB_1, AFB_2, AFG_1 and AFG_2 and cyclopiazonic acid. All members of the genus *Aspergillus* are assigned by ACDP (UK) to hazard group 2. *Aspergillus flavus* in literature (Donner *et al*., 2015; Yu *et al*., 2011; Dhanasekaran *et al*., 2011; Abdel-Hadi *et al*., 2011). produces only AFB_1, AFB_2 while *Aspergillus parasiticus* produces all four aflatoxins but was not identified as an associated fungus. The ITS sequence (see appendix) obtained from the sample produced top matches at >99% identity to members of the Aspergillus section Flavus. 99.4% sequence EF661560 from type strain *Aspergillus oryzae* (NRRL 447) (Peterson, 2008) and 99% sequence NR111041 from type strain *A. flavus* (ATCC 16883) Schoch *et al*., 2014. Here two *Aspergillus* species were identified in association with Bambara Nuts in this location; 99.4% *Aspergillus oryzae* and 99% *A. flavus.*

CABI NO. E0000895006 Cowpea (w) Itobe, Ajaokuta, Kogi State

From morphological examination, this sample was identified as a member of *Aspergillus* section Flavi. Features observed were consistent with the description for this group as given in published taxonomic keys, e.g. (Klich, 2002). Here, the DNA failed to be amplified and this can be due to a number of factors such as impurity of the sample, previous growth on antibiotic media or the presence of inhibitory substances. Members of *Aspergillus* section *Flavi* are common worldwide and are frequently isolated from soil, air and as contaminants of a wide variety of foods, e.g. cereals, nuts and fruits. Many species in this group are both halotolerant and thermotolerant and also grow well in conditions of low water activity. Many strains produce mycotoxins, such as both A and G aflatoxins and cyclopiazonic acid. All members of the genus *Aspergillus* are assigned by ACDP (UK) to hazard group 2. Morphological examination identified the sample as a member of the *A. flavus* using taxonomic keys.

CABI NO. E0000895007 Cowpea (w) Angwa Tiv, Akwanga, Nasarawa State

The ITS sequence (see appendix) obtained from this sample produced top matches at >99% identity to members of Aspergillus section Flavi. The top matches included sequences from type material published in peer-reviewed literature, e.g. 100% to sequence MG662388 from the type strain of Aspergillus aflatoxiformans strain DTO 228-G2 Frisvad, *et al.,* (2019) and sequence EF661560 from the type strain of *Aspergillus oryzae* (NRRL 447) Peterson, (2008). Partial calmodulin gene sequencing (see appendix) results supported this identification further, with top matches at >98% identity members of *Aspergillus* section Flavi, including 99.2%

to sequence MN987053 from the type strain of *Aspergillus agricola* (NRRL 66869) and 98.6% to sequence MN987092 from the type strain of *Aspergillus toxicus* (NRRL 66898) Singh, *et al.,* (2020**)**. There was insufficient distinction between species, a section-level identification is therefore given. Members of Aspergillus section Flavi are common worldwide and are frequently isolated from soil, air and as contaminants of a wide variety of foods, e.g. cereals, nuts and fruits. Many species in this group are both halotolerant and thermotolerant and also grow well in conditions of low water activity. Many strains produce mycotoxins, including AFB_1, AFB_2, AFG_1 and AFG_2 and cyclopiazonic acid. All members of the genus Aspergillus are assigned by ACDP (UK) to hazard group 2. The ITS sequence (see appendix) of sample obtained produced top matches at >99% identity to members of the *Aspergillus flavus*. The top matches are sequences from type strained published in peer-reviewed literature, e. g. 100% to sequence MG662388 from type strain *A. aflatoxiformans* strain DTO 228-G2 (Frisvad *et al*., 2019) and sequence EF661560 from type strain of *A. oryzae* (NRRL 447) (Peterson, 2008). Partial calmodulin gene sequencing supports this identification with top matches at >98% to members of A. section Flavi which includes 99.2% to sequence MN987053 from type strain of *A. agricola* (NRRL 66869) and 98.6% to sequence MN987092 from type strain of *A. toxicus* (NRRL 66898) (Singh *et al*., 2020).

CABI NO. E0000895008 (Bambara Nuts, Wakwa, Lafia, Nasarawa State)

From morphological examination, this sample has been identified as a member of *Aspergillus* section Flavi. Features observed were consistent with the description for this group. Klich (2002). Here again, the DNA failed to be amplified and this may be due to a number of factors such as impurity of the sample, previous growth on antibiotic media or the presence of inhibitory substances. Members of *Aspergillus* section *Flavi* are common worldwide and are frequently isolated from soil, air and as contaminants of a wide variety of foods, e.g. cereals, nuts and fruits. Many species in this group are both halotolerant and thermotolerant and also grow well in conditions of low water activity. Many strains produce mycotoxins, including both A and G aflatoxins and cyclopiazonic acid. All members of the genus *Aspergillus* are assigned by ACDP (UK) to hazard group 2. Morphological examination was used to identify the fungus as *Aspergillus flavus* (Klich, 2002).

CABI NO. E0000895009 (Bambara Nuts, Mada, Keffi, Nasarawa State)

The ITS sequence (see appendix) obtained from this sample produced top matches at 100% identity to members of *Aspergillus* section Nigri. The best matches included type strains of a number of species in this group e.g. sequence AY373852 from the type strain of A. niger (ATCC 16888), sequence FJ629340 from the type strain of *A. welwitschiae* (CBS 139.54) and sequence MH862766 from the type strain of *A. lacticoffeanus* (CBS 101883). These results were confirmed by partial calmodulin gene sequencing which also produced top matches at >98% identity members of *A.* section Nigri including 99.2% to sequence KC480196 from the type strain of *A. welwitschiae* (CBS 139.54) and 98.8% to sequence EF661154 from the type strain of A. niger (NRRL 326) Samson *et al*., (2011). Members of this group are common and widespread, occurring on a wide range of substrata including soil and plants and as contaminants in indoor environments and on foodstuffs including fruit, vegetables and nuts. They have been reported to produce mycotoxins such as malformin and naphthopyrones and some strains are known to produce ochratoxin. Members of this species group have been implicated in human and animal infections including superficial and local infections (cutaneous infections, otomycosis, tracheobronchitis), infections associated with damaged tissue (aspergilloma, osteomyelitis), pulmonary infections and clinical allergies (allergic bronchopulmonary aspergillosis, rhinitis, framers' lung).

CABI NO. E0000895010 (Bambara Nuts, Odo-Ere, Kogi State)

The ITS sequence (see appendix) obtained from this sample produced top matches at 100% identity to members of *Aspergillus* section Nigri. The best matches included type strains of a number of species in this group e.g. sequence AY373852 from the type strain of *A. niger* (ATCC 16888) and sequence FJ629340 from the type strain of *A. welwitschiae* (CBS 139.54). These results were confirmed by partial calmodulin gene sequencing which also produced top matches at >98% identity members of A. section Nigri including 99.2% to sequence KC480196 from the type strain of *A. welwitschiae* (CBS 139.54) and 98.8% to sequence EF661154 from the type strain of *A. niger* (NRRL 326), Samson *et al.,* (2011). Members of this group are common and widespread, occurring on a wide range of substrata including soil and plants and as contaminants in indoor environments and on foodstuffs such as fruits, vegetables and nuts. They have been reported to produce mycotoxins which includes malformin and naphthopyrones and some strains are known to produce ochratoxin. Members of this species group have been implicated in human and animal infections such as superficial and local infections (cutaneous infections, otomycosis, tracheobronchitis), infections associated with damaged tissue (aspergilloma, osteomyelitis), pulmonary infections and clinical allergies (allergic bronchopulmonary aspergillosis, rhinitis, Framers' lung). However, the majority of infections relate to immunocompromised individuals. Members of the genus *Aspergillus* are assigned to hazard group 2 by ACDP (UK).

V. Conclusion And Recommendation

Bambara Nuts sold in Kwande, in Shendam LGA, Plateau is that 100% identity is associated with sequence EF661560 from type strain *A. oryzae* (NRRL477) and 99.6% identity to sequence NR111041 from type strain *A. flavus* (ATCC 16888) Schoch *et al*., (2014**).** Bambara Nuts purchased from Lamingo in Jos East LGA was associated with sequence KF288119 from type strain *A. awamori* (NRRL 4948), sequence KC480196 from type strain *A. welwitschiae* (CBS 139.54) and sequence EF661154 from type strain *A. niger* (NRRL 326) Samson *et al*., (2011). The Bambara Nuts purchased from Lapai City, were associated with *Aspergillus* sequence KC 480196 from type strain *A. welwitschiae* (CBS 139.54) and sequence EF661154 from type strain *A. niger* **(**NRRL 326) Samson *et al*., (2011). Bambara Nuts purchased from Yelwa, Wushishi was associated with sequence KC480196 from the type strain *A. welwitschiae* (CBS 139.54) and sequence EF661154 from type strain *A. niger* **(**NRRL 326**).** Bambara Nuts from Wushishi City, Niger State was associated with sequence EF661560 from type strain *A. oryzae* (NRRL 447) Peterson, 2008 and sequence NR111041 from type strain *A. flavus* (ATCC 16883) Schoch *et al*., (2014). Morphological examination identified the fungus from Cowpeas purchased from Itobe, Ajaokuta, Kogi State as *A. flavus*, using taxonomic keys because the sequencing procedure failed. The Cowpeas purchased from Angwa Tiv, Akwanga, Nasarawa State was associated sequence MG662388 from the type strain A. aflatoxiformans strain DTO 228- G2 (Frisvad et al., 2019) and sequence EF661560 from type strain A. oryzae (NRRL 447) Peterson, 2008. However, partial calmodulin confirmed sequence MN 987053 from type strain *A. agricola* (NRRL 66869) and sequence MN987092 from type strain of *Aspergillus toxicus* (NRRL 66898) (Singh *et al*., 2020). The Bambara Nuts from Wakwa, Lafia, Nasarawa State was associated *Aspergillus flavus.* Morphological examination identified the associated fungus as *A. flavus* (Klich, 2000) was undertaken, because the sequencing process was unsuccessful. Bambara Nuts from Mada, Keffi, Nasarawa was associated with sequences AY373852 from type strain A. niger (ATCC 16888), sequences FJ629340 from the type strain of *A. welwitschiae* (CBS 139.54) and sequence MH862766 from the type strain of *A. lacticoffeanus* (CBS 101883). Bambara Nuts from Odo-Ere, Kogi State was associated with sequence AY 373852 from the type strain of A. niger (ATCC 16888) and sequence FJ629340 from the type strain of *A. welwitschiae*. Given that *Aspergillus* fungi, the cause of aflatoxicosis is so pervasive in all four states, farmers and store owners should begin to use preventive methods to mitigate the spread of the fungi. Store owners who warehouse the harvest should ensure proper ventilation of their shops to reduce humidity and temperatures that encourage the growth and development of the *Aspergillus* species, especially *Aspergillus flavus.*

APPENDICES

>E895001 ITS sequence:

ACCTCCCACCCGTGTTTACTGTACCTTAGTTGCTTCGGCGGGCCCGCCATTCATGGCCGCCGGGGGCTCTCAGCCCCGGGC
CCGCGCCCGCCGGAGACACCACGAACTCTGTCTGATCTAGTGAAGTCTGAGTTGATTGTATCGCAATCAGTTAAAACTTTC
AACAATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAGTGTGAATTGCAGAATTCCGTGA
ATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCATCAA
GCACGGCTTGTGTGTTGGGTCGTCGTCCCCTCTCCGGGGGGGGACGGGCCCCAAAGGCAGCGGCGGCACCGCGTCCGATC
CTCGAGCGTATGGGGCTTTGTCACCCGCTCTGTAGGCCCGGCCGGCGCTTGCCGAACGCAAATCAATCTTTTTCCAGG
TTGACCTCGGATCAGGTAGGGATACCCGCTGAACT

>E895002 ITS sequence:

AACCTCCCATCCGTGTCTATTGTACCCTGTTGCTTCGGCGGGCCCGCCGCTTGTCGGCCGCCGGGGGGGCGCCTCTGCCCC
CCGGGCCCGTGCCCGCCGGAGACCCCAACACGAACACTGTCTGAAAGCGTGCAGTCTGAGTTGATTGAATGCAATCAGTT
AAAACTTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGA
ATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCT
GCCCTCAAGCCCGGCTTGTGTGTTGGGTCGCCGTCCCCCTCTCCGGGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGC
GTCCGATCCTCGAGCGTATGGGGCTTTGTCACATGCTCTGTAGGATTGGCCGGCGCCTGCCGACGTTTTCCAACCATTCTT
TCCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACT

>E895002 Partial calmodulin sequence:

AGACCTTGAAAGCCTCGCGGATTTCCTCCTCGGAGTCGGTGTCCTTCATCTTACGAGCCATCATGGTAAGGAATTCTGGCG
GCAAAAGTCAATCACAACCCAGCCCGCATTTCCGCCTTACAGGCGTAAATCTAACACATACCGGGGAAGTCGATCGTTCC
GTTGTTGTCAGCGTCAACCTCGTTGATCATGTCCTGAAGCTCAGACTCGGAGGGGTTCTGGCCAAGGGAGCGCATCACAG
TGCCGAGCTCCTTGGTGGTGATCTGGCCTGATTCACCGATTAAATTAGTATTATGATCCAAATCATTCTGGGAAATGCTGT
CGAAATCCCGCGGTCGGATCGAGCGCTACAGGTAAAATGTGAAGGGGATAGAATTCCACCCACCATCGCCATCCTTGTCC
TATTAAGTCCGAAAACACATTAGCATTATGCTCCGGTAAAGATAGCTCATCGATCAGGATGATCGGAGGGTCATTCAGGG
AGCACTCACAAAGAGGGAGAAGGCCTCCT

BLAST results: E895002 (E895019)
>E895003 partial calmodulin sequence:
TGTCGCGGTCGAAGACCTTGAAAGCCTCGCGGATTTCCTCCTCGGAGTCGGTGTCCTTCATCTTACGAGCCATCATGGTAA
GGAATTCTGGCGGCAAAAGTCAATCACAACCCAGCCCGCATTTCCGCCTTACAGGCGTAAATCTAACACATACCGGGGAA
GTCGATCGTTCCGTTGTTGTCAGCGTCAACCTCGTTGATCATGTCCTGAAGCTCAGACTCGGAGGGGTTCTGGCCAAGGG
AGCGCATCACAGTGCCGAGCTCCTTGGTGGTGATCTGGCCTGATTCACCGATTAAATTAGTATTATGATCCAAATCATTCT
GGGAAATGCTGTCGAAATCCCGCGGTCGGATCGAGCGCTACAGGTAAAATGTGAAGGGATAGAATTCCACCCACCATCG
CCATCCTTGTCCTATTAAGTCCGAAAACACATTAGCATTATGCTCCGGTAAAGATAGCTCATCGATCAGGATGATCGGAGG
GTCATTCAGGGAGCACTCACAAAGAGGGAGAAGGCCTCCT
BLAST results: E895003 (E895020)
>E895004 ITS sequence:
CCATCCGTGTCTATTGTACCCTGTTGCTTCGGCGGGCCCGCyGCTTGTCGGCCGCCGGGGGGGCGCCTCTGCCCCCCGGG
CCCGTGCCCGCCGGAGACCCCAACACGAACACTGTCTGAAAGCGTGCAGTCTGAGTTGATTGAATGCAATCAGTTAAAAC
TTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATTCA
GTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCT
CAAGCCCGGCTTGTGTGTTGGGTCGCCGTCCCCCTCTCCGGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCG
ATCCTCGAGCGTATGGGGCTTTGTCACATGCTCTGTAGGATTGGCCGGCGCCTGCCGACGTTTTCCAACCATTCTTTCCAG
GTTGACCTCGGATCAGGTAGGGATACCCGCTGAACT
>E895004 partial calmodulin sequence:
ACCTTGAAAGCCTCGCGGATTTCCTCCTCGGAGTCGGTGTCCTTCATCTTACGAGCCATCATGGTAAGGAATTCTGGCGGC
AAAAGTCAATCACAACCCAGCCCGCATTTCCGCCTTACAGGCGTAAATCTAACACATACCGGGGAAGTCGATCGTTCCGTT
GTTGTCAGCGTCAACCTCGTTGATCATGTCCTGAAGCTCAGACTCGGAGGGGTTCTGGCCAAGGGAGCGCATCACAGTGC
CGAGCTCCTTGGTGGTGATCTGGCCTGATTCACCGATTAAATTAGTATTATGATCCAAATCATTCTGGGAAATGCTGTCGA
AATCCCGCGGTCGGATCGAGCGCTACAGGTAAAATGTGAAGGGGATAGAATTCCACCCACCATCGCCATCCTTGTCCTAT
TAAGTCCGAAAACACATTAGCATTATGCTCCGGTAAAGATAGCTCATCGATCAGGATGATCGGAGGGTCATTCAGGGAGC
ACTCACAAAGAGGGAGAAGGCCTCCTT
BLAST results: E895004 (E895021
>E895005 ITS sequence:
TCCCACCCGTGTTTACTGTACCTTAGTTGCTTCGGCGGGyyhGCCATTCATGGCCGCCGGGGGCTCTCAGCCCCGGGCCCG
CGCCCGCCGGAGACACCACGAACTCTGTCTGATCTAGTGAAGTCTGAGTTGATTGTATCGCAATCAGTTAAAACTTTCAAC
AATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAGTGTGAATTGCAGAATTCCGTGAATC
ATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCATCAAGCA
CGGCTTGTGTGTTGGGTCGTCGTCCCCTCTCCGGGGGGGACGGGCCCCAAAGGCAGCGGCGGCACCGCGTCCGATCCTC
GAGCGTATGGGGCTTTGTCACCCGCTCTGTAGGCCCGGCCGGCGCTTGCCGAACGCAAATCAATCTTTTTCCAGGTTGACC
TCGGATCAGGTAGGGATACCCGCTGAACT
BLAST results: E895005 (E895022)
>E895007 ITS sequence:
ACCTCCCACCCGTGTTTACTGTACCTTAGTTGCTTCGGCGGGCCCGCCATTCATGGCCGCCGGGGGCTCTCAGCCCCGGGC
CCGCGCCCGCCGGAGACACCACGAACTCTGTCTGATCTAGTGAAGTCTGAGTTGATTGTATCGCAATCAGTTAAAACTTTC
AACAATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAGTGTGAATTGCAGAATTCCGTGA
ATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCATCAA
GCACGGCTTGTGTGTTGGGTCGTCGTCCCCTCTCCGGGGGGGACGGGCCCCAAAGGCAGCGGCGGCACCGCGTCCGATC
CTCGAGCGTATGGGGCTTTGTCACCCGCTCTGTAGGCCCGGCCGGCGCTTGCCGAACGCAAATCAATCTTTTTCCAGGTTG
ACCTCGGATCAGGTAGGGATACCCGCTGAACT
>E895007 partial calmodulin sequence:
AACCTTGAAAGCCTCCCGGATCTCTTCCTCAGAGTCGGTATCCTTCATCTTTCTCGCCATCATCGTAAGGAACTCTATTTGA
ACATTAACAGCTATTTCATTTATGAACCGTACGGAAGCCGTCTCGTACCAGGGAAGTCAATGGTGCCATTGTTGTCGGCGT
CAACCTCGTTAATCATGTCCTGGAGTTCCGACTCGGAGGGGTTTTGGCCCAGAGAGCGCATGACAGTGCCCAACTCCTTG
GTGGTGATCTGGCCTGCATATCAAGTCAATTCATCAATGCCAAGACTTCAGCCGATCGAACAGAAACTGGATAAAATTAA
GGCGGCTAAAACATATTGGTCGCATTTGAAGGGAGAATGAATAAAATAAACTGTACTAACCATCACCGTCCTTGTCCTATA
GGAAACCCAAGAAAAGCCAAGTCAGCTTGTGTTCAAATCATGACGACTAACAAAACCAATTTTCACGAACGACGATAACT
ACTTACGAATAGGGAGAAGGCCTCCT
BLAST results: E895007 (E895024)
>E895009 ITS sequence:
CCTCCCATCCGTGTCTATTGTACCCTGTTGCTTCGGCGGGCCCGCCGCTTGTCGGCCGCCGGGGGGGCGCCTCTGCCCCCC
GGGCCCGTGCCCGCCGGAGACCCCAACACGAACACTGTCTGAAAGCGTGCAGTCTGAGTTGATTGAATGCAATCAGTTAA

AACTTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAAT
TCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGC
CCTCAAGCCCGGCTTGTGTGTTGGGTCGCCGTCCCCCTCTCCGGGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGT
CCGATCCTCGAGCGTATGGGGCTTTGTCACATGCTCTGTAGGATTGGCCGGCGCCTGCCGACGTTTTCCAACCATTCTTTC
CAGGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACT

>E895009 partial calmodulin sequence:

AGACCTTGAAAGCCTCGCGGATTTCCTCCTCGGAGTCGGTGTCCTTCATCTTACGAGCCATCATGGTAAGGAATTCTGGCG
GCAAAAGTCAATCACAACCCAGCCCGCATTTCCGCCTTACAGGCGTAAATCTAACACATACCGGGGAAGTCGATCGTTCC
GTTGTTGTCAGCGTCAACCTCGTTGATCATGTCCTGAAGCTCAGACTCGGAGGGGTTCTGGCCAAGGGAGCGCATCACAG
TGCCGAGCTCCTTGGTGGTGATCTGGCCTGATTCACCGATTAAATTAGTATTATGATCCAAATCATTCTGGGAAATGCTGT
CGAAATCCCGCGGTCGGATCGAGCGCTACAGGTAAAATGTGAAGGGGATAGAATTCCACCCACCATCGCCATCCTTGTCC
TATTAAGTCCGAAAACACATTAGCATTATGCTCCGGTAAAGATAGCTCATCGATCAGGATGATCGGAGGGTCATTCAGGG
AGCACTCACAAAGAGGGAGAAGGCCTCCT

BLAST results: E895009 (E895026)

>E895010 ITS sequence:

ACCTCCCATCCGTGTCTATTGTACCCTGTTGCTTCGGCGGGCCCGCCGCTTGTCGGCCGCCGGGGGGGCGCCTCTGCCCCC
CGGGCCCGTGCCCGCCGGAGACCCCAACACGAACACTGTCTGAAAGCGTGCAGTCTGAGTTGATTGAATGCAATCAGTTA
AAACTTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAA
TTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTG
CCCTCAAGCCCGGCTTGTGTGTTGGGTCGCCGTCCCCCTCTCCGGGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCG
TCCGATCCTCGAGCGTATGGGGCTTTGTCACATGCTCTGTAGGATTGGCCGGCGCCTGCCGACGTTTTCCAACCATTCTTTC
CAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACT

>E895010 partial calmodulin sequence:

AGACCTTGAAAGCCTCGCGGATTTCCTCCTCGGAGTCGGTGTCCTTCATCTTACGAGCCATCATGGTAAGGAATTCTGGCG
GCAAAAGTCAATCACAACCCAGCCCGCATTTCCGCCTTACAGGCGTAAATCTAACACATACCGGGGAAGTCGATCGTTCC
GTTGTTGTCAGCGTCAACCTCGTTGATCATGTCCTGAAGCTCAGACTCGGAGGGGTTCTGGCCAAGGGAGCGCATCACAG
TGCCGAGCTCCTTGGTGGTGATCTGGCCTGATTCACCGATTAAATTAGTATTATGATCCAAATCATTCTGGGAAATGCTGT
CGAAATCCCGCGGTCGGATCGAGCGCTACAGGTAAAATGTGAAGGGGATAGAATTCCACCCACCATCGCCATCCTTGTCC
TATTAAGTCCGAAAACACATTAGCATTATGCTCCGGTAAAGATAGCTCATCGATCAGGATGATCGGAGGGTCATTCAGGG
AGCACTCACAAAGAGGGAGAAGGCCTCCT

BLAST results: E895010 (E895027)

>E895011 ITS sequence:

AAACCTTGAAAGCCTCCCGGATCTCTTCCTCAGAGTCGGTATCCTTCATCTTTCTCGCCATCATCGTAAGGAACTCTATTTG
AACATTAACAGCTATTTCATTTATGAACCGTACGGAAGCCGTCTCGTACCAGGGAAGTCAATGGTGCCATTGTTGTCGGCG
TCAACCTCGTTAATCATGTCCTGGAGTTCCGACTCGGAGGGGTTTTGGCCCAGAGAGCGCATGACAGTGCCCAACTCCTT
GGTGGTGATCTGGCCTGCATATCAAGTCAATTCATCAATGCCAAGACTTCAGCCGATCGAACAGAAACTGGATAAAATTA
AGGCGGCTAAAACATATTGGTCGCATTTGAAGGGAGAATGAATAAAATAAACTGTACTAACCATCACCGTCCTTGTCCTAT
AGGAAACCCAAGAAAAGCCAAGTCAGCTTGTGTTCAAATCATGACGACTAACAAAACCAATTTTCACGAACGACGATAAC
TACTTACGAATAGGGAGAAGGCCTCCT

>E895011 Partial calmodulin sequence:

CCAACCTCCCACCCGTGTTTACTGTACCTTAGTTGCTTCGGCGGGCCCGCCATTCATGGCCGCCGGGGGCTCTCAGCCCCG
GGCCCGCGCCCGCCGGAGACACCACGAACTCTGTCTGATCTAGTGAAGTCTGAGTTGATTGTATCGCAATCAGTTAAAAC
TTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAGTGTGAATTGCAGAATTCC
GTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCA
TCAAGCACGGCTTGTGTGTTGGGTCGTCGTCCCCTCTCCGGGGGGGGACGGGCCCCAAAGGCAGCGGCGGCACCGCGTCC
GATCCTCGAGCGTATGGGGCTTTGTCACCCGCTCTGTAGGCCCGGCCGGCGCTTGCCGAACGCAAATCAATCTTTTTCCAG
GTTGACCTCGGATCAGGTAGGGATACCCGCTGAACT

References

[1]. Bbosa, G., S. Kitya, D., Odda, J., and Ogwal-Okeng, J. (2013). "Aflatoxins metabolism, effects on epigenetic mechanisms and their role in carcinogenesis," Health, vol. 5, no. 10, pp. 14–34,

[2]. De Vires, J., W. Trucksess, M., W. and Jackson, L., S. (2002). Mycotoxins and food safety. 7 Kluwer Academic/Plenum Publications, New York, N.Y.

[3]. Digrak, M., Hakki, A., Ahmet, I., Selim, S. (1999). Antibacterial and antifungal effects of various commercial plant extracts. Intern J Pharmacognosy 37: 216–2206

[4]. Doku, E., V. (1995). Proceedings of the workshop on conservation and improvement of Bambara groundnut (Vigna subterranea). Harare, Zimbabwe: University of Ghana.

[5]. Eskola, M., Kos, G., Elliott, C., T. Hajsolva, J., Sultan, M., and Krska, R. (2020). Critical Reviews in Food Science and Nutrition. 60 (16):2773-2789

[6]. Frisvad, J., C, Hubka, V., Ezekiel, C., N, Hong, S., B, (2019). Taxonomy of Aspergillus section Flavi and their production of aflatoxins, ochratoxins and other mycotoxins. Stud. Mycol. 93:1–63

[7]. Fukushima, D. (2000). Soybean Processing. In: Nakai, S., Wayne-Modler, H. (Eds) Food Proteins: Processing application. New York; Wiley-VCH Inc. pp 309-342.

[8]. Hell, K., Cardwell, K., F. Setamou, M., Poehling, H., M. (2000). The influence of storage practices on aflatoxin contamination in maize in four agro-ecological zones of Benin, West Africa. J. Stored Prod. Res., 36: 365-382

[9]. Jallow, Abdou, Lee, Xie, Hualix, Tang, Xiaoquin, Qu, Xhang and Li, Peiwu (2021).

[10]. "Worldwide Aflatoxin contamination of Agricultural products and foods; from occurrence to control". Comprehensive Reviews in Food Science and Food safety 20: (3) 2332-2381

[11]. Jef, L., Jia-Sheng, W., Kelly, J. (2015). Serum aflatoxin B_1-lysine adduct level in adult women from Eastern Province in Kenya depends on household socio-economic status: A cross sectional study. Social Science and Medicine Journal 146:104-110

[12]. Kerley, M., S. and Allee, G., L. (2003). Modifications in soybean seed composition to enhance animal feed uses and value: Moving from a dietary ingredient to a functional dietary component. Journal of Agrobiotech Management Economics 6:14-17.

[13]. Klich, M. (2002). Identification of Common Aspergillus Species. Centraal Bureau voor Schimmelcultures, Utrecht, Netherlands

[14]. Kouadio, J., H. (2012). chratoxins A en Côte d"Ivoire: moisissures ochratoxinogènes, exposition humaine et détoxification des aliments. Rev. Ivoir. Sci. Technol., XX, pp. 87-103,

[15]. Lizárraga-Paulín, E., G. Moreno-Martínez, E., and Miranda-Castro, S. P. (2011). Aflatoxins and Their Impact on Human and Animal Health: An Emerging Problem. Aflatoxins- Biochemistry and Molecular Biology, Rijeka, InTech, 255-282.

[16]. Lusas, E., W. and Riaz, M., N. (1995). Soy protein products: Processing and use. Journal of Nutrition 125:573-580.

[17]. Makun, H., A. Anjorin, S., T. Moronfoye, B., Adejo, F., O. Afolabi O., A. Fagbayibo, G.,Surajudeen, A., A. (2010). Fungal and aflatoxin contamination of some human food commodities in Nigeria. African Journal of Food Science

[18]. Marin, S., Ramos, A., J. Cano-Sancho, G., Sanchis, V. (2013). Mycotoxins; Occurrence, toxicology, and exposure assessment. Food and Chemical Toxicology 60:218-37.

[19]. Palacios, M., F. Easter, R., A. Soltwedel, K., T. Parsons, C., M. Douglas, M., W. Hymowitz, T., Pettigrew, J. E. (2004). Effect of soybean variety and processing on growth performance of young chicks and pigs. Journal of Animal Science 82:1108-1114.

[20]. Peterson, S., W, (2008). Phylogenetic analysis of Aspergillus species using DNA sequences from four loci. Mycologia 100 (2): 205-226

[21]. Plahar, W., A. Annan, N., T. Nti, C., A. (2001). Cultivar and processing effect on the pasting characteristics, tannin content and protein quality and digestibility of cowpea (Vigna Unguiculata). Food Research Institute (CSIR) Accra, Ghana. J Food Technol Afr. 6: 50–55

[22]. Samson, R., A, Yilmaz, N, Houbraken, J, Spierenburg, H, (2011). Taxonomic Studies on genus Aspergillus. Studies in Mycology. 69:1-97

[23]. Schoch, C., L, Robbertse, B, Robert, V, Vu, D, (2014). Finding needles in haystacks: linking scientific names, reference specimens and molecular data for Fungi. Database (Oxford).

[24]. Sinclair, l., B. (1977): Soybean seed pathology. Testing for seed-borne micro-organisms. Preprin

[25]. Singh, P., Callicott, K., A, Orbach, M., J, Cotty, P., J (2020). Molecular analysis of S-morphology aflatoxin producers from the United States Front. Microbiol. 11:1236.

[26]. Udoh, J., M. Cardwell, K., F, Ikotun, T. (2000). Storage structures and aflatoxin content of maize in five agroecological zones of Nigeria. J. Stored Prod. Res., 36: 187-201.

[27]. Vincenzo, L., Roberto, T., Nunzia, C., Angela, C., Di, D. V., Vito, L. (2005). Seed coat tannins and bruchid resistance in stored cowpea seeds. J Sci Food Agri 85: 839–846

[28]. WHO/FAO/UNU (2007). Joint WHO/FAO/UNU expert consultation on protein and amino-acid requirements in human nutrition. Geneva, Switzerland. WHO technical report series 935:93-102.

[29]. Williams, J., H. Phillips, T., D. Jolly, P., E. Stiles, J., K. Jolly, C., M. and Aggarwal, D.

[30]. (2004). Human aflatoxicosis in developing countries: a review of toxicology, expo- sure, potential health consequences, and interventions. American Journal of Clinical Nutrition, 80(5), 1106-1122.

[31]. Willger, S., D. Grahl, N., and Cramer, R., A. Jr. (2009). Aspergillus fumigatus metabolism: clues to mechanisms of in vivo fungal growth and virulence. Medical Mycology. 47 suppl1s72–79

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