The Presence of Aflatoxin B1 and Fungi in Traditional Drugs in Vietnam

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Abstract- To explore the presence of aflatoxin B1 (AFB1) and fungi in traditional drugs collected in Vietnam. Materials and Methods: 505 samples of 88 different traditional drugs were obtained from 10 hospitals in Nghe An, a central province of Vietnam. AFB1 contamination was determined by a high-performance liquid chromatography (HPLC) assay. Fungal contaminants were determined according to WHO regulations, and the obtained *Aspergillus* strains were characterized via morphological and molecular identification. Results: 24 samples (4.75% of the total samples) were contaminated with AFB1, and the average concentration was 0.062±0.030 µg/kg (ranging from 0.009 to 0.097 µg/kg). Fungal isolates were detected from 174 samples (34.45%). The genus *Aspergillus* was predominant (82.76% of the isolates), but *Rhizopus*, *Alternaria*, *Corynespora*, and yeast were also found in a few samples. Among 144 strains of *Aspergillus* recovered, *A. niger* (105 strains) was most frequently found, followed by *A. tubingensis* (31 strains), *A. oryzae* (4 strains), and *A. flavus* (4 strains). Conclusion: This study suggests a low risk of aflatoxin B1 exposure to consumers of traditional drugs in Vietnam.

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Keywords: Traditional drug; Mycotoxin; Aflatoxin; Fungi; Aspergillus; Vietnam

Introduction

Mycotoxins are low-molecular-weight natural compounds produced by filamentous fungi (molds) and are toxic to animals in low concentrations. Aflatoxins are a group of about 20 different mycotoxins. The four major naturally produced compounds are aflatoxin B1, B2, G1, and G2. Among them, aflatoxin B1 (AFB1) is the most toxic, carcinogenic, and mutagenic (1-3). Human exposure to aflatoxins is usually from the consumption of food or drugs that are contaminated by aflatoxin-producing molds. Ingestion of aflatoxins can result in acute or chronic toxicity, with the main target organ being the liver. Acute toxicity in humans (although less likely) can result in mortality, with the acute lethal dose for adults being approximately 10 to 20 mg of aflatoxin (4). Chronic exposure to aflatoxin can result in mutations that cause liver cancer (1). In developed countries, regulations on the levels of aflatoxin total and AFB1 in food work relatively well to protect human populations from significant aflatoxin ingestion. But in many developing countries, aflatoxin ingestion remains high, especially where similar regulations are not enforced or nonexistent (5).

Many species of the *Aspergillus* genus can produce aflatoxins. The most important aflatoxin-producers are *A. flavus* and *A. parasiticus* (2). Other less common species are *A. bombycis*, *A. ochraceoroseus*, *A. nomius*, *A. pseudotamari*, and *A. niger* (2,6). Aflatoxin - producing strains of *Aspergillus* generally produce 2-3 aflatoxins, one of which is always aflatoxin B1, so AFB1 is the most prevalent worldwide (2).

The aflatoxin-producing fungi can colonize a variety of natural products that are usually used as traditional (non-conventional) medicines (7,8). About 70-80% of the world's population rely on non-conventional medicine in their primary healthcare (9), so the risk of aflatoxin exposure after consumption of these drugs cannot be

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ignored.

Vietnam is a developing country and traditional medicine still play an important role in the treatment of different diseases. About 75% of people use traditional drugs as their primary source of treatment (10). The ingredients for traditional medicine are derived from wholly natural sources such as plant, animal, and mineral products. About 1500 licensed and many more unlicensed remedies are used throughout the country (10). One reason for the popularity of traditional drugs is the belief that they produce few or no side effects (10). However, this kind of drugs may be contaminated with pathogens or toxins. The Vietnamese Ministry of Health has issued the National Technical Regulation on mycotoxin contamination in food, limiting the AFB1to a maximum of 12 µg/kg (11). However, this regulation does not cover natural products used as medicine and there is a lack of awareness of the regulation among traditional medicine practitioners and consumers in Viet Nam (10). It is essential to provide the general public with adequate information to facilitate a better understanding of the potential risks associated with the use of natural products and to ensure that all medicines are safe and of adequate quality. This paper aimed at evaluating the occurrence of aflatoxin AFB1 and aflatoxin-producing molds in natural products stored in some hospitals of Nghe An, a central province of Vietnam.

Materials and Methods

Sampling

Five hundred and five samples of natural products, composed of 88 different species, were evaluated to assess the presence of aflatoxin B1 and fungal strains. The products were chosen based on their availability and popularity of use and obtained from the stores of ten hospitals in Nghe An province, Vietnam. About 30 g of each kind of medicinal product were put into two sterile polythene bags and sealed properly. These bags were labeled with the code of hospital, type of medicine, and time of sampling. One bag of each medicine was transported to the Department of Laboratory, Nghe An General Friendship Hospital for fungal culture and the other to the National Institute of Medicinal Materials to analyze aflatoxin AFB1. The detailed information of each sample was given as Additional file 1: Table S1. The samples were kept at 4° C until use and processed as soon as possible to avoid second contamination.

Table S1. Samples collection information

Vietnamese name	Scientific name	Number of samples
Bac ha	Mentha arvensis	06
Bach mao can	Rhizoma imperatae cylindricae	03
Ba tu nhan	Semen platycladi orientalis	11
Bach phuc linh	Poria cocos	08
Bach thuoc	Radix paeoniae lactiflorae	08
Bach truat	Rhizoma atractylodis macrocephalae	08
Ban ha	Rhizoma pinelliae	08
Bo cot toai	Rhizoma drynariae	03
Cau dang	Ramulus cum unco uncariae	08
Cau ky tu	Fructus lycii	08
Chi tu	Gardeniae fructus	03
Cho de rang cua	Herba Phyllanthus amari	03
Co xuoc	Radix achyranthis asperae	03
Cot khi cu	Radix polygoni cuspidati	03
Dai tao	Fructus ziziphi jujubae	08
Dan sam	Radix salviae miltiorrhizae	08
Dang sam	Radix codonopsis	08
Day dau xuong	Caulis tinosporae tomentosae	03
Dia long	Lumbricus.	08
Do trong	Cortex eucommiae	08
Duong quy	Radix angelicae sinensis	08

Cont. table S1

Ha diep	Folium nelumbinis	03
Ha kho thao	Spica prunellae	08
Hanh nhan	Semen armeniacae amarum	08
Hau phac	Syzygii cuminii	03
Hoang cam	Radix scutellariae	08
Hoang lien	Rhizoma coptidis	08
Hong hoa	Flos carthami tinctorii	07
Hoai son	Dioscoreae rhizoma	03
Hoang ba nam	Cortex oroxyli indici	03
Hoe hoa	Flos Styphnolobii japonici imaturi	03
Huyen sam	Radix scrophulariae	07
Huong phu	Rhizoma cyperi	03
Huyet giac	Lignum dracaenae cambodianae	03
Hy thiem thao	Herba siegesbeckiae	03
Ich mau	Herba leonuri japonici	03
Ich tri nhan	Fructus alpiniae oxyphyllae	07
Ke noi kim	Endothelium Corneum Gigeriae Galli	03
Ke dau ngua	Fructus xanthium strumarium	07
Khuong hoat	Rhizoma et radix notopterygii	07
Khuong hoang	Rhizoma curcumae longae	03
Kim ngan hoa	Lonicerae Flos	03
Kim tien thao	Herba Desmodii styracifolii	03
Kinh gioi	Herba Elsholtziae ciliatae	03
La khoi tia	Folium Adisae	03
Lac tien	Herba passiflorae	04
Moc thong	Caulis clematidis	03
Ma hoang	Herba ephedrae	07
Mach mon	Radix ophiopogonis japonici	07
Mau don bi	Cortex paeoniae suffruticosae	08
Moc huong	Radix saussureae lappae	08
Ngọc truc	Rhizoma polygonati odorati	03
Nhan tran	Herba adenosmatis caerulei	03
Ngu vi tu	Fructus schisandrae	08
Ngu linh chi	Feaces trogopterum -pteropodae	08
Nguu tat	Radix achyranthis bidentatae	08
O tac cot	Os sepiae	03
Phong phong	Radix ledebouriellae seseloidis	08
Phuc than	Poria	08
Que chi	Ramulus cinnamomi	03
Que tam	Cortex cinnamomi	03
Sai dat	Herba wedeliae	03
Sai ho	Radix bupleuri	08
Sinh dia	Radix rehmanniae glutinosae	08
Son thu	Fructus corni officinalis	08

Cont. table S1

Tam sen	Embryo nelumbinis	04
Tan di	Flos magnoliae liliflorae	08
Tan giao	Radix gentianae macrophyllae	08
Tang ky sinh	Herba loranthi gracifilolii	08
Thach vi	Herba pyrrosiae cheareri	03
Thach xuong bo	Rhizoma acori graminei	03
Thao quyet minh	Semen cassiae torae	03
Thien nien kien	Rhizoma homalomenae occulatae	03
Thuyen thoai	Periostracum cicadidae	03
To moc	Lignum sappan	03
Trach ta	Rhizoma alismatis	03
Te tan	Herba asari	07
Thang ma	Rhizoma cimicifugae	07
Thien ma	Rhizoma gastrodiae elatae	07
Thien mon	Radix asparagi cochinchinensis	07
Tho phuc linh	Rhizoma smilacis glabrae	07
Thong thao	Medulla tetrapanacis	08
Thuc dia	Radix rehmanniae glutinosae	08
Thuong truat	Rhizoma atractylodis lanceae	08
Ty giai	Rhizoma dioscoreae	08
Vien chi	Radix polygalae	08
Xich thuoc	Radix paeoniae	08
Xuyen khung	Rhizoma ligustici wallichii	08

Quantitative determination of aflatoxin B1

The HPLC assessment was performed on a Shimadzu (Kyoto, Japan) HPLC system equipped with an LC-20AD pump, SIL-20A HT autosampler, detector SPD-M20A, CTO-10AS VP column oven. All used reagents were of analytical reagent grade. AFB1 standard solution (250 ng/mL) was obtained from Rhone-diagnostics technologies (UK). Chromatographic separation was carried out on a Shim-pack GIST C18 column (250 x 4.6 mm, 5 µm). The reverse-phase HPLC assay was carried out using a C18 column with a flow rate of 1 mL/min, injection volume of 20 µL, and a column temperature of 25° C. The mobile phase was acetonitrile-phosphate, and the detection wavelength was set as 350 nm.

Evaluation of fungal contamination

The isolation and identification of fungi from herbal medicines were tested according to WHO regulations (9). Ten grams of each sample were mechanically homogenized in 90 mL 0.1% Tween-20 for 2 minutes. The mixture was filtrated through a disposable syringe with sterile cotton and then centrifuged at 2600×g for 10 min. The collected pellet was plated onto Petri dishes 9-10 cm in diameter containing Sabouraud dextrose agar (SDA) with chloramphenicol (0.1 g/L). Plates were incubated at room temperature and observed every day for a maximum of 7 days before negative results were noted. Colonies with different morphological characteristics were picked and subcultured onto fresh SDA slants to obtain pure cultures. The genus identification was based on the morphological characteristics of their colonies and spores. Those strains belonging to the *Aspergillus* genus were further identified into species-level followed the taxonomic schemes of Raper and Fennel (12).

To confirm the species identification, some representative strains of different *Aspergillus* species were subjected to molecular analysis in the laboratory of the Department of Parasitology, Vietnam Military Medical University. The fungal DNA was extracted using the Fungi/Yeast Genomic DNA Isolation Kit (Norgen Biotek Corp, Canada) under the manufacturer's instructions. The primers ITS5 (13) and NL4 (5′- (14) were used to amplify a partial sequence of small subunit ribosomal RNA gene, the complete sequence of internal transcribed spacer (ITS) 1, 5.8S ribosomal RNA gene, ITS2, and partial sequence of large subunit ribosomal RNA. The yielded PCR products were sequenced, and the

obtained nucleotides were compared to database sequences in GenBank for the identification. Some of the sequences in the current study were deposited in GenBank under the code MF599709.1, MF599710.1, MF599715.1.

Results

During 2017, a total of 505 samples, belonging to 88 species, of traditional medicines were examined for the presence of AFB1. Among them, 24 samples (4.75%) were AFB1-contaminated with the average concentration of 0.062±0.030 µg/kg (range of 0.009-0.097 µg/kg). The contaminated samples belonged to Rhizoma imperative cylindricae, Semen platycladi Orientalis, Radix achyranthis asperae, Rhizoma dioscoreae persimilis, Endothelium corneum gigeriae galli, Herba desmodii styracifolii, Embryo nelumbinis, Rhizoma acori graminei, Radix salviae miltiorrhizae, Spica prunellae, Fructus schisandrae, Radix ledebouriellae seseloidis, and Rhizoma ligustici wallichii species. Among them, Endothelium corneum gigeriae galli (3/3 samples), Rhizoma imperatae cylindricae, Semen platycladi orientalis, Rhizoma dioscoreae persimilis, Rhizoma acori graminei (2/3 samples) had the highest frequency of AFB1-contaminated samples (Table 1).

Among 505 investigated samples in this study, 174 specimens (34.45%) contained fungal contamination. The genus *Aspergillus* was predominant among the identified fungi (82.76% of the isolates) (Table 2).

Among 144 strains of *Aspergillus* recovered, *A. niger* (105 strains) was the most frequently encountered, followed by *A. tubingensis* (31 strains), *A. oryzae* (4 strains), and *A. flavus* (4 strains) (Table 3).

Table 1. Distribution of AFB1 among traditional drugs analyzed in this study

Drug name	n	Positive
Rhizoma imperatae cylindricae	03	2
Semen platycladi orientalis	03	2
Radix achyranthis asperae	03	1
Rhizoma dioscoreae persimilis	03	2
Endothelium corneum gigeriae galli	03	3
Herba desmodii styracifolii	03	1
Embryo nelumbinis	04	2
Rhizoma acori graminei	03	2
Radix salviae miltiorrhizae	08	2
Spica prunellae	08	1
Fructus schisandrae	08	2
Radix ledebouriellae seseloidis	08	2
Rhizoma ligustici wallichii	08	2
Total	65	24
Mean (µg/kg)		0,062
Standard deviation (µg/kg)		0,030

(* the species without any positive samples were not expressed here)

Table 2. Frequency of different fungi isolated from traditional drugs

Fungi	n	Percentage (%)	
		Among infected samples (n=174)	Among total samples (n=505)
Aspergillus	144	82.76	28.51
Rhizopus	33	18.97	6.53
Alternaria	10	5.75	1.98
Corynespora	9	5.17	1.78
Yeast	24	13.79	4.75
Total	220	100.00	34.46

Table 3. Frequency of Aspergillus species isolated from traditional drugs

Aspergillus species	n	Percentage (%)	
		Among *Aspergillus* strains (n=144)	Among the total of samples (n=505)
Aspergillus niger	105	72.92	20.79
Aspergillus tubingensis	31	21.53	6.14
Aspergillus oryzae	4	2.78	0.79
Aspergillus flavus	4	2.78	0.79

Discussion

The presence of aflatoxin in traditional drugs

Contamination with mycotoxins, especially AFB1, in natural products used for food or medicines has been extensively investigated worldwide, but data in Vietnam is scarce, despite the climatic conditions in Vietnam being very favorable for aflatoxin-producing fungi (15). The current study was carried out to investigate the occurrence of mycotoxins and fungi, focusing on AFB1 and AFB1-producing molds to assess the risk to consumers in Vietnam.

Our findings showed that 24 out of 505 tested samples (4.75%) were contaminated with AFB1. This prevalence was lower than that (19.1%) in some agricultural products (maize, rice, peanut, and sesame) in Vietnam (15) but still showed the potential for mycotoxin contamination in traditional drugs. Other surveys carried out in some neighboring countries showed a wide range of aflatoxin incidence in traditional drugs (from 8.7% to 35% of the tested samples) (16-19). The traditional drug samples were highly contaminated (64%-70.8% of herbal drugs) in some studies (20,21) and very low or even free of contamination in others (22). The formation of mycotoxins depends on many factors such as the fungus, substrate, environmental factors, and time, which may account for such variation (23). Our result did not reveal an association between specific types of traditional drugs and AFB1 contamination, given the low frequency of positive samples (Table 1).

The range of AFB1 in the current study was 0.009-0.097 µg/kg, lower than the regulatory limit of mycotoxin in Vietnam (11). The low concentration of aflatoxin in our finding is consistent with other surveys evaluating the concentration of AFs in traditional drugs (16-17,19-20). Nevertheless, the level of AFB1 (290.80 µg/kg) was high - enough to cause serious health problems, has been reported in Chinese products (18). These findings reveal the high variation of aflatoxin in traditional drugs, so local and updated data on that issue is needed.

The presence of fungi in traditional drugs

Molds are a large group of around 100,000 species that are widely distributed in nature. Given their ability to multiply on various raw materials or under unfavorable conditions, molds are one of the most widespread environmental contaminants. The growth of molds in traditional drugs may result in drug spoilage and the production of mycotoxins. In the current study, discoloration and musty smell due to fungal growth was noted in some samples at collection time. Results of the present study show that the traditional drugs were frequently contaminated by different fungal species, however, with lower prevalence (34.45%) compared with that in some other reports (7,21,24).

The observation of *Aspergillus* as the most frequent contaminant is consistent with previous reports (8,24-25). Among *Aspergillus* strains, *A. niger* was the most common, followed by *A. tubingensis* and others. The low prevalence of *A. flavus*, one of the most important producers of aflatoxin (26), is in line with other studies (7,22) and may explain the low concentration of AFB1 in traditional drugs in the present study. The dominance of *A. niger* aligns with some previous findings (8,22,24-25,27). *A. niger* can grow in a wide range of temperatures (6-47^0 C) and pH (1.4-9.8); thus, it is ubiquitous in the natural environment (28). Although *A. niger* is not a major aflatoxin-producer, some isolates of *A. niger* can produce aflatoxin B1, B2, and G2 (6). *A. tubingensis* has been isolated from traditional drugs, but their production of aflatoxin has not been reported (21). *A. oryzae* is a species of the section Flavi (containing important aflatoxin-producing *A. flavus* and *A. parasiticus*) and economically important in food fermentation and industry (26). *A. oryza* species is usually considered atoxigenic, but the production of aflatoxin B1, B2, or G2 by some strains of *A. oryzae* has been reported (29,30). From the present investigation and other studies, it is conceivable that *A. niger* is a mold that should be considered concerning mycotoxin production and fungal contaminant. The high contamination with fungi that are capable of producing aflatoxin in traditional drugs should raise attention because mycotoxins are stable chemical compounds and cannot be destroyed during most processing operations (7).

This study suggests that natural products used for medicinal purposes in Vietnam are usually contaminated with fungi that are capable of producing mycotoxins. There appears to be a low risk of aflatoxin to consumers who occasionally use traditional drugs because no samples used in this study had levels of AF contamination above current country regulation limits. However, people who use traditional drugs more frequently or with other sources of aflatoxin may be at a higher risk of exposure. The occurrence of aflatoxin B1 in tested samples should raise public awareness of the potential health hazards associated with traditional medicines. More studies are needed to investigate the contamination of traditional drugs by mycotoxin and mycotoxin-producing fungi in more areas of Vietnam.

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References

1. Coulombe RJ. Biological action of mycotoxins. J Dairy Sci 1993;76:880-91.
2. Bennett JW., Klich M. Mycotoxins. Clin Microbiol Rev 2003;16:497-516.
3. Squire R. Ranking animal carcinogens: a proposed regulatory approach. Science 1981;214:877-80.
4. Pitt JI. Toxigenic fungi: Which are important? Med Mycol 2000;38:17-22.
5. Krishnamachari K, Bhat R, Nagarajan V, Tilak T. Investigations into an outbreak of hepatitis in parts of Western India. Indian J Med Res 1975;63:1036-48.
6. Al-Abdalall AHA. Production of aflatoxins by Aspergillus flavus and Aspergillus niger strains isolated from seeds of pulses. J Food Agric Environ 2009;7:33-9.
7. Zheng R, Wang W, Tan J, Xu H, Zhan R, Chen W. An investigation of fungal contamination on the surface of medicinal herbs in China. Chin Med 2017;12:2.
8. Efuntoye MO. Fungi associated with herbal drug plants during storage. Mycopathologia 1996;136:115-8.
9. WHO. WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues 2007. Geneva:Switzerland;2007.
10. Van NDN, Tap N. An overview of the use of plants and animals in traditional medicine systems in Viet Nam. Ha Noi, Vietnam: TRAFFIC Southeast Asia, Greater Mekong Programme;2008.
11. MOH. National technical regulation on the limits of mycotoxins contamination in food (QCVN 8-1:2011/BYT). Ha Noi, Vietnam: Vietnam Ministry of Health; 2011.
12. Raper K, Fennell DI. The Genus Aspergillus. Philadelphia: Williams and Wilkins; 1965.
13. White T, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. Protocols and Applications - A Laboratory Manual. 1st ed. United States: Academic Press; 1990:315-22.
14. Guadet J, Julien J, Lafay J, Brygoo Y. Phylogeny of some Fusarium species, as determined by large- subunit rRNA sequence comparison. Mol Biol Evol 1989;6:227-42.
15. Do TH, Cao Tran S, Le CD, Thi Nguyen HB, Thi Le PT, Thi Le HH, et al. Dietary exposure and health risk characterization of aflatoxin B1, ochratoxin A, fumonisin B1, and zearalenone in food from different provinces in Northern Vietnam. Food Control 2020;112:107108.
16. Tassaneeyakul W, Razzazi-Fazeli E, Porasuphatana S, Bohm J. Contamination of aflatoxins in herbal medicinal products in Thailand. Mycopathologia 2004;158:239-44.
17. Chien MY, Yang CM, Huang CM, Chen CH. Investigation of aflatoxins contamination in herbal materia medica in a Taiwan pharmaceutical factory. J Food Drug Anal 2018;26:1154-9.
18. Liu L, Jin H, Sun L, Ma S, Lin R. Determination of aflatoxins in medicinal herbs by high-performance liquid chromatography-tandem mass spectrometry. Phytochem Anal 2012;23:469-76.
19. Roy AK, Chourasia HK. Mycoflora, mycotoxin producibility and mycotoxins in traditional herbal drugs from India. J Gen Appl Microbiol 1990;36:295-302.
20. Chourasia HK. Mycobiota and mycotoxins in herbal drugs of Indian pharmaceutical industries. Mycol Res 1995;99:697-703.
21. Chen L, Guo W, Zheng Y, Zhou J, Liu T, Chen W, et al. Occurrence and Characterization of Fungi and Mycotoxins in Contaminated Medicinal Herbs. Toxins (Basel) 2020;12:30.
22. Hitokoto H, Morozumi S, Wauke T, Sakai S, Kurata H. Fungal contamination and mycotoxin detection of powdered herbal drugs. Appl Environ Microbiol 1978;36:252-6.
23. Diener UL, Davis ND. Aflatoxin formation by Aspergillus flavus. In: Goldblatt LA, ed. Aflatoxin Scientific Background Control and Implications. New York: Academic Press; 1969:13-54.
24. Siakrwar P, Mahajan S, Gupta A, Asthana M. Isolation and Identification of Fungal Contamination in Stored Medicinal Plants. Am J Pharmacol Pharmacother 2014;1:52-8.
25. Singh P, Srivastava B, Kumar A, Dubey NK. Fungal Contamination of Raw Materials of Some Herbal Drugs and Recommendation of Cinnamomum camphora Oil as Herbal Fungitoxicant. Microb Ecol 2008;56:555-60.
26. Klich MA. Aspergillus flavus : the major producer of aflatoxin. Mol Plant Pathol 2007;8:713-22.
27. Kong W, Wei R, Logrieco AF, Wei J, Wen J, Xiao X, et al. Occurrence of toxigenic fungi and determination of mycotoxins by HPLC-FLD in functional foods and spices in China markets. Food Chem 2014;146:320-6.
28. Gautam AK, Sharma S, Awasthi S, Bhadauria R. Diversity, pathogenicity and toxicology of A. niger: An important spoilage fungi. Res J Microbiol 2011;6:270-80.
29. Basappa S, Jayaraman A, Sreenivasamurthy V, Parpia H. Effect of B-group vitamins & ethyl alcohol on aflatoxin production by Aspergillus oryzae. Indian J Exp Biol

1967;5:262-3.

30. Akinola SA, Ateba CN, Mwanza M. Polyphasic Assessment of Aflatoxin Production Potential in Selected Aspergilli. Toxins (Basel) 2019;11:692.