ISSN : 0974 - 7532 Volume 7 Issue 6

Trade Science Inc.

Research & Reviews in

BioSciences

Regular Paper

RRBS, 7(6), 2013 [243-248]

Aflatoxin contamination of medicine plant powders sold in Sokoto metropolis

S.A.Kabir[1], R.M.Aliyu[2]*, S.A.Shinkafi[1], U.-K.Muh'd[1], A.D.Ibrahim[1]

[1]Department of Microbiology, (NIGERIA)

[2]Department of Veterinary Microbiology, Usmanu Danfodiyo University, Sokoto, (NIGERIA)

E-mail : rabiuabdul2005@yahoo.com

ABSTRACT

This Study was conducted to determine the mycotoxigenic and aflatoxin contamination of traditional medicinal plants powder in Sokoto metropolis. Total aflatoxin was determined using AgraQuant Total Aflatoxin kit and the mycotoxigenic flora was determined using standard methods. The fungal species identified were *Aspergillus niger, Aspergillus flavus* and *Rhizopus oryzae*. Analysis of total aflatoxin concentration in the medicinal plants powder in Sokoto revealed that all the samples were contaminated with aflatoxin with the highest concentration being 187 ppb and the least was 14.5 ppb. Results from this study revealed that traditional powdered medicine are the study area are contaminated with fugal species which exposes consumers to the probable toxic metabolites produced by these fungi.

KEYWORDS

Aflatoxin;
Aspergillus spp;
Medicinal plants.

INTRODUCTION

From ancient time up to today, Plants are being used worldwide as a traditional medicine in prevention, treatment and cure of disorders, diseases and other home remedies in a variety of healthcare. World Health Organization (WHO) survey report indicates that about 70-80% of the world population particularly in the developing countries rely on non-conventional medicines mainly of herbal sources in their primary health care[4].

The increasing popularity of natural drugs made their use a Public Health problem due to the lack of effective surveillance of the use, efficacy, toxicity and quality of these natural products. The premise that traditional use of these medicinal products for generations establishes their safety does not necessarily attest to their safety and efficacy. Indeed, the adverse effects of long-term herbal use, adulteration with toxic compounds and contamination by pathogenic microbials or natural toxins like mycotoxins have been reported for herbal products and medicinal plants[1].

The term "mycotoxin" refers to all toxins produced by various types of fungi when they grow on agricultural products before or after harvest or during transportation or storage[65]. Mycotoxins have been reported to be carcinogenic, teratogenic, tremorogenic, haemorrhagic, and dermatitic to a wide range of organisms and to cause hepatic carcinoma in man[41]. Aflatoxins are the most common and toxic mycotoxins produced by filamentous fungi *Aspergillus*, particularly *A. flavus* and *A. parasiticus* and there are four naturally occurring AFs, designated B1, B2, G1 and G2 with

Regular Paper

AFB1[41].

The concern over quality of these products is mainly due to their potential contamination, considering their natural origin. Practices used in harvesting, handling, storage, production and distribution make medicinal plants subject to contamination by various fungi, which may be responsible for spoilage and production of mycotoxins. In spite of the extensive research on the occurrence of mycotoxins in foods, there are some reports available on the incidence of toxigenic mycoflora and mycotoxins in medicinal plants and phytotherapic compounds worldwide[1].

Due to the little information on the toxigenic moulds and their ability to produce mycotoxins in medicinal plants in the study area, the objectives of the present study were to evaluate the predominant mycoflora isolated from indigeneous medicinal formulations and their ability to produce aflatoxins.

MATERIALS AND METHODS

Sample collection

Thirty samples of powdered medicinal plants were collected from different sellers within Sokoto metropolis. Each sample was put in a sterile polyethylene bag, sealed and transferred to the mycology research laboratory for analysis.

Fungal isolation

On each of the dried Sabouraud Dextrose Agar plates to which penicillin and streptomycin had been incorporated were inoculated in the processed samples using sterile forceps. The inoculated plates were incubated at 25 ^{0}C for 5 days. All observed colonies were subcultured to obtain pure cultures[18].

Fungal identification

The growth rate, colour, texture, colonial morphology and diffusible pigmentation of each sample were examined macroscopically. Microscopic features such as spore and hyphae morphology were observed and compared with the standard colour atlas as described by Ochei and Kolhatkar (2000).

Sample preparation/extraction

One hundred grams each of traditional medicinal plant was sieved using 20 microlitre-meshes. Twenty grams of the sample was weighed into clean bottles and 100 ml of Acetonitrile/water extraction solution was added and the bottles were sealed tightly. It was shaken vigorously for 3 minutes and allowed to settle. The top layer of extract was filtered using Whatman No 1 filter paper and the filtrate collected, cleaned with a mycoses 112 column and 1 ml used for total aflatoxin analysis.

Total aflatoxin analysis

The AgraQuant Total Aflatoxin kits were used for total aflatoxin assay. The AgraQuant Total Aflatoxin assay is direct competitive enzyme-linked immunosorbent assay (ELISA). ELISA is a serological assay in which bound antigen or antibody is detected by an antibody that is conjugated to an enzyme. The enzyme converts a colourless substrate to a coloured product reporting the antibody capture of the antigen.

RESULTS

Mycotoxigenic fungi associated with traditional powdered medicine consumed in Sokoto metropolis was isolated, identified and the results presented in TABLE 1. From the result, the mycotoxigenic flora of the traditional powdered medicine consumed in Sokoto metropolis was identified as *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus oryzae*.

Aspergillus niger and *Aspergillus flavus* were the most frequently isolated (TABLE 2), from which *Aspergillus niger* has the highest frequency of 53.3% and *Rhizopus oryzae* with the least frequency of 20%.

TABLE 1: Result of colonial (macroscopic) and microscopic identification of isolates

Aflatoxins	Morphological characteristics	Occurance in the samples
Aspergillus niger	Black large and powdery colonies	Maganin sanyi 1, Maganin sanyi 2, Maganin shawara 1, Maganin shawara 2, Maganin kunne, Maganin mara, Maganin gaba, Maganin iska.
Aspergillus flavus	Light green and powdery colonies	Maganin zafi, Maganin daji, Maganin dankanoma, Maganin kashi, Taura, Maganin Ciwon kai, 'Ya 'yan kalgo, Maganin daji.
Rhizopus oryzae	Pigmented rhizoids, sporangiophores and numerous stolons	Maganin atuni, Maganin ciwon kai (madacci), Maganin ciwon hannu, Maganin atuni, Gawo, Maganin dankanoma.

Analysis of the total aflatoxin content of traditional powdered medicine consumed in Sokoto was conducted (TABLE 3). From the result, all the samples consumed in Sokoto are contaminated with aflatoxin and "Maganin ciwon mara" had the highest total aflatoxin concentration range of 187 ppb while "Maganin ciwon gaba (madacci)" had the least total aflatoxin concentration with a range of 14.5 ppb.

TABLE 2 : Result showing the overall frequency of the Aflatoxins found in the various samples of the traditional powdered medicine

Aflatoxins	Frequency (%)
Aspergillus niger	53.3
Aspergillus flavus	26.7
Rhizopus oryzae	20

TABLE 3 : Total Aflatoxin concentration of traditional powdered medicine collected from different sellers within Sokoto Metropolis

Sample	Total aflatoxin (ppb)
"Maganin Sanyi 1"	35.7
"Maganin Sanyi 2"	86.8
"Maganin Shawara 1"	68.7
"Maganin Shawara 2"	80.4
"Maganin Zafi"	18.45
"Maganin Atuni"	19.22
"Maganin Ciwon ciki (Madacci)"	185.6
"Maganin Ciwon kunne (rinin samri)"	16.8
"Maganin Ciwon daji"	35.8
"Maganin Ciwon mara"	187
"Maganin Ciwon dankanoma"	89
"Maganin Ciwon hannu (tsamiya)"	67.6
"Maganin Ciwon gaba (madacci)"	14.5
"Maganin Ciwon kashi"	46.6

DISCUSSION

This study revealed that traditional powdered medicine consumed in Sokoto harbours the toxigenic fungi *A. flavus*. *A. flavus* produces the carcinogenic mycotoxin, aflatoxin which often contaminates foods such as nuts. The exposure of these powdered medicinal plants to the ubiquitous fungi during processing and their spores in the air and the aerobic condition of the atmosphere in the market would ordinarily encourage more fungal growth. The presence of these fungi is an indication that the medicinal plant that are intended for human consumption in Sokoto are of low quality and could constitute public health hazard to the Sokoto populace. The degree of pathogenicity of fungi is based on immune state of individual. When immunosuppressed individual consume food that is contaminated with these pathogenic fungi such person may come down with mycotic diseases. Aspergillus species have been the cause of a vital disease in man called Aspergillosis. Aspergillosis may be in form of allergy due to inhalation of the conidia as in asthmatic patient that is developing IgE antibody to the surface antigen, in others it may colonize the bronchial tree. Invasive Aspergillosis do occur following ingestion of the conidia. Invasive disease develops as an acute pneumonia process. Individual with leukaemia, lymphoma is at high risk. Aspergillus species are also capable of causing disease in poultry especially in chicks, if they are being fed with Aspergillus contaminated feeds. *Rhizopus stolonifer* is one of the most predominant fungi that can be found on contaminated food substances and it is one of the major causes of food spoilage. It has not been reported to cause disease in man but it cause moldiness and ropiness of food substances. *Aspergillus niger* was found to have the highest frequency. The rare infections caused by aspergillus include pneumonia and fungus ball. The total aflatoxin analysis revealed that all the samples are contaminated. 'Maganin ciwon mara' has the highest concentration of total aflatoxin of 187 ppb while 'maganin ciwon gaba' has the lowest concentration of total aflatoxin of 14.5 ppb. Those who consume these powdered medicine are prone the fatal disease conditions due to the presence of aflatoxin. Since traditional powdered medicine is highly consumed in Sokoto, the result is important in enacting laws, creating awareness, and improving the standard of these powdered medicines.

CONCLUSION

The study found that powdered medicinal plants sold in Sokoto metropolis are contaminated with *Aspergillus niger* (53.3%), *Aspergillus flavus* (26.7%) and *Rhizopus oryzae* (20%). All the samples were contaminated with aflatoxin with "Maganin ciwon mara" has the highest concentration of total aflatoxin of 187 ppb while "Maganin ciwon gaba" has the lowest concentration of total aflatoxin of 14.5 ppb in the sample.

Regular Paper

The result of the study could be useful in developing and establishing public health standards and in creating awareness on the consumption of traditional powdered medicine.

REFERENCES

[1] A.Bunyo, A.B.Almodovar, T.A.Pinto, M.Sabino; Braz.J.of Microb., **37**, 47-51 **(2008)**.

[2] A.Goflieb; Cause of Mycotoxins Silagos Field to Food Bank. **(1997)**.

[3] A.O.Aduku; Tropical Foodstuff Analysis Table, ABU Zaria, 27 **(1993)**.

[4] A.Sareen, R.Ahirwar, A.Gautam, R.Bhadauria; Sci.&Cult., **76(3-4)**, 118-120 **(2010)**.

[5] A.Z.Joffe; Fusarium Species, Their Biology and Toxicology, John Wiley and New York, **(1986)**.

[6] A.Z.Joffe, T.D.Willie, More L.G.J.Hoiuse, Fusarium; Poae and Fusarium Sporotricides as principal causal Agents of Alimentary toxic mycotoxin case, an Encyclopaedia handbook, 3 New York, Marcel Dieker, 21-86 **(1978)**.

[7] Anon.Botany 135 syllabus, www.botany.hawail.edu/faculty, **(2003)**.

[8] AOAC, Official Methods of analysis and association of official Analytical Chemists, Washington DC, U.S.A. **(1995)**.

[9] B.A.Anhwange, V.O.Ajibola, F.G.Okibe; Fisheries, **1(2-4)**, 73-76 **(2006)**.

[10] B.Coulibaly; The Problem of Aflatoxin Contamination of Groundnut and Groundnut Products as seen by the African Groundnut Council, In D.MC Donald and V.K Mehan, (Eds); Aflatoxin Contamination of Groundnuts, Proceedings of the International Workshop, Patancheru, India, International Crops Research Institute for the Semi-Arid Tropics, 47-55 **(1989)**.

[11] B.Seglar; Case Studies that implicate Silage Mycotoxins as the Cause of Dairy herd problems in, Silage, Field to feed bank NRAES-99, Northeast Regional Agricultural Engineering service Ithaca, N.Y., 242 **(1997)**.

[12] L.Berry; The Pathology of Mycotoxins, **(1988)**.

[13] C.N.Haenan, K.J.Shaw, J.I.Pitt; J.Food Mycol., 67-72 **(1998)**.

[14] Commission of European Communities, Commission Regulation (EC) No. 1525/98 of 16 July 1998, Official Journal of European Communities L20/143, **(1998)**.

[15] D.M.Johnson, J.A.N.Johnson, D.M.Bedoll, J.P.Pringer, K.K.Chexal, J.C.Chordy, R.H.Cox; J.Agric.Food Chem., **25**, 856 **(1997)**.

[16] D.N.S.Boyaeiogiu, S.Hettiararcheny, R.W.Stack; Science **72**, 93 **(1992)**.

[17] F.S.Chu, I.G.Y; Applied Environmental Microbiology, **60**, 847-845 **(1994)**.

[18] ICFM, Methods of the International commission on Food mycology, **(2007)**.

[19] International agency for research on cancer (IAROC) some naturally occurring substances, Food Items and Constituents Heterocyclic Aromatic Amines and mycotoxins monograph 56, Lyon, WHO International Agency for Research on cancer, **(1993)**.

[20] J.Stoloff, J.V.Rodricks, C.W.Hesseltine, M.A.Mehlman; Mycotoxin in Human and Animal Health, Park Forest south IL, 7-28 **(1997)**.

[21] J.D.Miller, H.L.Trenholm, (Eds); Mycotoxin in grain St Paul, M.N.Eagan, **(1996)**.

[22] J.D.Miller, H.L.Trenholm, J.M.Beardall; Mycotoxins in Grain, Compounds Other Than Aflatoxins, Eagan Press, St.Paul, MN **(1994)**.

[23] J.E.Smith, M.O.Mosses; Mycotoxin formation Analysis and significance, John Wiley and sons, Chichester Britain, 1-143 **(1985)**.

[24] J.I.PiH; Penicilium An application of environmental microbiology, **53**, 266-269 **(1987)**.

[25] J.I.Pitt, A.O.Hocking, B.F.Miscable; The Mycoflora of Food Commodities from Indonesia, Journal of Food Mycology, **1**, 41-60 **(1998)**.

[26] J.I.Pitt, A.D.Hocking, K.Bhudhasamaij, B.F.Miscamble, K.A.Whether, E.K.Tanboon; Intl.Food Microbio., **20**, 211-226 **(1993)**.

[27] J.Lacey; Natural occurrence of aflatoxin contamination of groundnut and groundnut products as seen by the African groudnut council, In D.M.C.Donald, V.K.Mohan, (Eds); Aflatoxin contamination of groundnut proceeding of the international workshop, 47-55 **(1991)**.

[28] J.Locey; National Occurrence of Mycotoxins in Growing and Conserved Forage Crops in, Mycotoxins and Animals Food, J.E.Smith, R.E.Henderson, (Eds); CRC Press, Boca Raton, Florida, **(1991)**.

[29] J.M.Adamas; Trop.Sci., **19(1)**, 1-27 **(1977)**.

[30] J.Ochei, A.A.Kolhatkar; Medial Mycology In, Medical Laboratory Science, Theory and Practice, Tata-McGraw Hill, 7 West Pael Ngar New Delhi, 1047-1050 **(2000)**.

[31] J.Pestka, G.S.Bondy; Immunotoxic effect of mycotoxins in J.D.Miller, H.L.Trenholem, (Eds); Mycotoxins in Grain St Paul, MN, Eagan, 339-359

(1994).

[32] J.Vara, E.Keror, E.Ringu, J.Teron; Applied Env.Microbio., **62**, 4461-4464.

[33] K.C.Goodman, T.Scott, P.M.Matanable; Regulatory toxic Pharmacology, **7**, 253-306 **(1987)**.

[34] L.W.Whittow, W.M.Hagler; Mycotoxins and Spoilage, Effect of mycotoxins on the animal, The Produces Perspective, In Silage, Field to Feed Bunk, Northeast Regional Agricultural Engineering Service, NRAES-99, Ithaca, N.Y., 222 **(1997)**.

[35] M.A.Iklich, J.I.PiH; Thornas Brown Mycological Science, **91**, 99-108 **(1988)**.

[36] M.K.Wolford; The Silage Fermentation Market Dekker Incorporation, New York, **(1984)**.

[37] M.L.Abarca, M.R.Bragulate, G.Custella, F.J.Cabares; App.Env.Microbiol., **60**, 200-26 **(1994)**.

[38] M.O.Moss; The environmental factor controlling mycotoxin formation in, Mycotoxins and animal foods, J.E.Smith, R.S.Henderson, (Eds); CRC Press Boca Raton Florida, **(1991)**.

[39] M.S.Palmgren, A.W.Hayes; Aflatoxin in Foods, In P.Krogh, (Ed); Mycotoxin in food London, Academic, Press, 65-95 **(1987)**.

[40] M.S.Saeed Alghalibi, M.S.Abdul-Rahman; Mycoflora and mycotoxin contamination of some dried fruits in Yemen republic. Ass.Univ.Environ.Res., **7(2)**, **(2004)**.

[41] M.Tajkarimi, M.H.Shojaee, H.Yazdanpanah, S.A.Ibrahim; Aflatoxin in Agricultural Commodities and Herbal Medicine, Aflatoxins - Biochemistry and Molecular Biology, Guevara-Gonzalez (Ed.); ISBN, 978-953-307-395-8, InTech, **(2011)**.

[42] NRAES-99, Northeast Regional Agricultural Engineering Service, Ithaca, N.Y., 213.

[43] O.O.Atanda, M.C.Ogunrinu, F.M.Olorunfemi; A neutral red desiccated coconut agar far rapid detection of aflatoxigenic fungi and visual determination of aflatoxins, World Mycotoxin J., **4(2)**, 147-155 **(2011)**.

[44] P.Dimanchie; Groundnut exporters in Southern countries penalized by news standards on aflatoxins imposed by the European Union, OCL-Oleagineux, Corps Gras, Lides, **8**, 237-238 **(2001)**.

[45] P.Krogh, B.Hald, P.Englund, L.Rutqvist, O.Acta; Pathol Microbiol Scand B.Microbiol.Immunol., **82(2)**, 301-302 **(1974)**.

[46] P.M.Scott; Penicillium mycotoxins in wglhe T.D.More honest, L.G.(Ed); Mycotoxic fungi, mycotoxins, mycotoxicoses an encyclopedic Handbook, Mycotoxigenic Fungi New York, Marcel Dether J., **1**, 283-356 **(1977)**.

[47] J.I.Pitt, A.D.Hocking; fungi and food spoilage 2nd Edition, London Blackie, **(1997)**.

[48] R.J.Cole, R.A.Hill, P.D.Blanthenship, T.H.Sanders, H.Garron; Development industrial biology, **23**, 299-326 **(1982)**.

[49] R.T.Riley, E.Wang, S.S.Schroeder; Natural Toxins, **4**, 3-15 **(1996)**.

[50] R.V.Bhat, S.Vashanti; Agricultural Development, **23**, 50-56 **(1999)**.

[51] R.V.Bhat, R.B.Sashidar, J.Ramerterishman, K.L.Munshi; Lancet, **1**, 35-37 **(1989)**.

[52] S.C.Benzuidenhout, W.C.A.Gelderblom, C.P.Gort-Allman, R.M.Horak, W.F.O.Morasas, F.Spiteller, R.Vleggar; J.Chem.Soc.Chem.Commun., 743-745 **(1988)**.

[53] S.Drusch, W.Ragab; J.of Food Protein, **66**, 1514-1527 **(2003)**.

[54] S.J.Van Rensbury, J.V.Rodricks, C.W.Itesseltine, M.A.Mehlman; Role of Epidemiology in the Elucidation of mycotoxin Health risk (Eds) mycotoxin in Human and Animal health Park, Forest South, IL, Pathotox, **(1997)**.

[55] J.E.Smith, M.O.Mosses; Mycotoxin formation Analysis and significance John Wiley and sons, Chichester Sutton UK, Wiley, **(1985)**.

[56] T.C.Campbell, L.Stoloff; Agricultural Food Chemistry, **22**, 1006-1015 **(1974)**.

[57] Truckess, Scott; Mycotoxins in botanicals and dried fruits, A review of Food Additives and Contaminants, Part A, **25(2)**, 181-192 **(2008)**.

[58] V.J.D.Miller, H.L.Trencholm, (Eds); Mycotoxins in Grain St Paul, M.M.Eagon, **(1996)**.

[59] V.Paul, P.Eary; What are mycotoxins in, mycotoxin in grain, University of Kentucky Cooperative Extension Service, 7 **(2002)**.

[60] W.C.A.Gelderblom, S.D.Synman, S.Abel; Hepatotoxicity and Carcinogenicity of the fumonisins in roots a review regarding mechanistic implication for establishing risk in humans in, Jackson Ls; J.W.Perries, I.B.Buller man, (Eds); Fumonisins in Food, New York, Plonium, 25-256 **(1996)**.

[61] W.F.O.Marasas, P.G.ThielHorack, R.M.Uleggar, M.P.J.Kriek; Appl.Envir.Microbio., **54**, 1806 **(1988)**.

[62] W.F.O.Marasas, P.E.Nelson, T.A.Tousson; Toxigenic Fusarium Species Identity and Mycotoxicology University Park, P.A, Pennsylvania State, **(1984)**.

[63] W.F.O.Marasas, T.S.Kellerman, W.C.A.Gelderblom, J.A.W.Coetger, P.G.Thiel,

Regular Paper

J.S.Vander Lugt; Onderspiot, **55**, 197-203 **(1988)**.

[64] W.S.O.Marasas, F.C.Wehner, Van S.S.Rensborg, O.J.Van Schalkwyk; Phytopathology, **71**, 792-796 **(1981)**.

[65] M.Weidenborner; Mycotoxins in food safety, New York, Springer, 7-18 **(2008)**.

[66] WHO Energy and Protein Requirements, WHO Technical Report, No.522 **(1975)**.

[67] K.C.Williams, B.J.Blaney, M.H.Magee; Aust.J.Of Agric Resear., **39**, 1095-1105 **(1989)**.

[68] Worldwide Occurrence of Mycotoxin in Foods and Feeds, An updated Journal Association of Analytical Chemistry, **72**, 223-230.