EFFECTS OF AFLATOXINS CONTAMINATING FOOD ON HUMAN HEALTH

Magda Carvajal and **Pável Castillo**
Departamento de Botánica, Instituto de Biología, Universidad Nacional Autónoma de México. Ciudad Universitaria, Colonia Copilco, Delegación Coyoacán. 04510 México, D.F.(Institute of Biology, National Autonomous University of Mexico).

Keywords: Mycotoxins, aflatoxins, cancer, mutagenesis, food contamination, DNA adducts, biomarkers, hepatic diseases, cirrhosis, hepatitis, toxicology, chemical mutations.

Contents

Summary

Aflatoxins (AF) are toxic metabolites of the moulds *Aspergillus flavus*, *A. parasiticus* and *A. nomius.* AF link to DNA, RNA and proteins and affect all the living kingdom, from viruses to man, causing acute or chronic symptoms, they are mutagens, hepatocarcinogens, and teratogens.

The impact of AF contamination on crops is estimated in hundreds of millions dollars. FAO declared that 25% of all crops in the world have AF, mainly cereals, oilseeds and spices. The responsible factors for AF production are temperature, grain and environmental humidity, damage by insects, rodents, genetic inheritance, etc. AF are economically important due to the damages to health, also because they act in traces and are accumulated in the nucleic acids of man and animals, and after years of consuming AF, they develop chronic illness such as cancer. When AF link to DNA they form adducts that are biomarkers of risk of disease.

The preventive measures to avoid the fungal production of AF is to dry, all stored food products and their derivates, to take crops or foods to a humidity level lower than the needed by the fungus, to use plant varieties resistant to insects and fungal attacks, extra crop irrigations, chemical products or fungicides. Decontaminate the affected crop with ammonium or other chemical, use of chemosorbents with feed.
In the Sections 1.0 to 4.0 of the present chapter all the main features of AF has been briefly developed, their definition, conditions for their production, occurrence, chemical structure, types, physicochemical and biological properties, their biosynthetic pathway, biotransformation, analytical methods, metabolism.

The Section 5.0 describes the toxic effects of AF in plants, animals and man, giving some details of their behavior as mutagens, AF-DNA adduct formation, proto-oncogene activation and carcinogenesis. Sections 6.0 to 8.0 deal with economic losses, control measures and legislation. The chapter ends with general conclusions and bibliography.

1. Aflatoxins, Production, Occurrence, Chemical Structure

1.1 Definition of Aflatoxins

The word “aflatoxin” comes from a = *Aspergillus*, fla = *flavus* and toxin = venom. Aflatoxins (AF) are fungal secondary metabolites that form a group of toxic compounds that chemically correspond to furan coumarins.

AF were discovered in Great Britain in 1960, after the death of one hundred thousand turkeys that were fed with AF contaminated peanuts from Brazil, the flour was contaminated with the mould *Aspergillus flavus*.

1.2. Aflatoxin Producing Fungi and Production Conditions

1.2.1. Aflatoxin Producing Fungi

Mainly three fungi *Aspergillus flavus* Link, *Aspergillus parasiticus* Speare *and Aspergillus nomius* Kurtzman synthesized AF by their metabolism. *A. bombycis*, *A. ochraceoroseus* and *A. nomius*, are also AF producing mould species, but they are not found frequently.

The morphological characteristics that are more upstanding in *Aspergillus* are globose or column heads with green yellowish or olive green color. Phyalids grow in one or two series frequently from the same fungal strain or even in the same head. The fungal species lack a sexual reproductive stage, they do not reproduce by meiosis, and it is common the presence of brown reddish, purple brown or black sclerotia when they are mature, sclerotia can be globose, sub global or vertically elongated.

The fungi *Aspergillus flavus* produce only AFB_1 and AFB_2 with a higher proteolytic activity, and some strains can produce cyclopiazonic acid. *Aspergillus parasiticus* synthesize mainly AFB_1, that is the most toxic compound of the group, but it can also produce the four common aflatoxins B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1) and G_2 (AFG_2) with more lipolytic activity. Not all the species of *Aspergillus spp*. are capable of producing AF.

These fungi contaminate seeds in the field, where they can grow as saprophytes in crop debris on the soil, and in warehouses. A source of primary contamination can be sclerotia formed in damaged grains and in healthy maize. During dry seasons the plants are more susceptible to insect invasions that carry spores, beginning the development of the fungi. Aflatoxins can remain longer time after the producing fungi die, therefore, grains can have dangerous levels of AF although they have not a moldy appearance.

Maize seeds can be invaded by fungi during plant formation, as well as in post harvest, transportation and storage periods. The fungi reduce viability, nutritional and sanitary qualities to seeds and grains.

1.2.2. Conditions for Aflatoxin Production

Aflatoxins appear when a **producing strain** of *A. flavus* or *A. parasiticus* grows in a substrate, where environmental conditions favor their development. The genotype of each species, some biological, environmental and chemical factors determine the amount of AF produced by aflatoxicogenic strains in the substrate.

The fungi *Aspergillus spp*. is stimulated by **physical factors** such as **temperature**, **relative humidity** of the atmosphere and **substrate**, or by biological, biochemical and environmental factors.

The principal factors for AF production are: the aflatoxicogenic fungus, the substrate, environmental and substrate humidity (an environmental relative humidity of 85% corresponds to a wetness content of 16.5-18% of the grain), temperature, associated mycoflora, oxygen of the storage atmosphere and time of storage.

The AF production in the substrate can happen in the field and in storage conditions between 20 and 40°C with a 10-20% of humidity, and 70-90% of relative humidity in the air. Aflatoxins can be formed in storage conditions or in the cereal fields before harvest. These fungi grow at temperatures from 8 to 55 °C being 36 to 38 °C the optimal ones, and 25 a 35 °C for AF production; beginning at 11 to 14 °C and stopping AF production below 10 °C or more than 45 °C. *A. parasiticus* grows mainly in tropical or subtropical areas; *A. flavus* develops in a wide range of temperatures in substrates with high hydrocarbon content, nevertheless many non aflatoxicogenic strains do the same, therefore the fungi presence do not indicate the existence of aflatoxins.

Mycotoxins are produced at the end of the exponential phase or at the beginning of the stationary phase of the mould growth. The development of the fungus is favored if the grains are damaged by insects or rodents. Some spores of the substrate bud and grow as mycelia generators of AF because, when breathing, they produce water increasing the humidity of the seeds or grains.

Aflatoxin production by *Aspergillus* reaches its maximum production rate at the fifth day, that is, when the mould comes up to the stationary growth phase. Its AF production diminishes from the sixth to the eight days. After 24 hours, *Aspergillus* reaches the optimal conditions of temperature (27-30 °C) and relative humidity (85%) to produce aflatoxins, its rate of production increases as far as 12 days that is when it has its highest production, later this rate lowers progressively.

Aspergillus producer species require to be in pure culture to generate AF, because there are other species, such as *A. chevalieri*, *A. candidus* and *A. niger*, that compete with them at the same conditions of temperature and humidity and interfere the AF production These facts have been applied in the biological control, where some other fungi and bacteria are used as contenders of the aflatoxicogenic strains.

-
-
-

Bibliography

Asao, T., Büchi, G., Abdel-Kader, M.M., Chang, S.B., Wick, E.L. and Wogan, G.N. 1965. The structures of aflatoxins B1 and G1. *Journal of American Chemistry Society*, 87:882-886. [The importance of this reference is that it given the structure of the most important aflatoxins for the first time since their discovery in 1960].

Bray, G.A. and Ryan D.H. 1991. *Mycotoxins, Cancer and Health*, Vol. I. Pennington Center Nutrition Series Louisiana State University Press, Baton Rouge and London. 331 pp. [This book has four chapters. Part I: Epidemiology and economics, with several articles of natural occurrence of mycotoxins, aflatoxins as risk factors for primary hepatocellular carcinoma in humans, their detection and metabolites in human fluids, economic significance of mycotoxicoses, interaction with hepatitis B virus. Part II: Biochemistry of carcinogenesis and aflatoxin synthesis, with cell and molecular biology of carcinogenesis, production and repair of AFB_1-DNA damage, bioactivation of aflatoxins. Part III: Comparative aspects of toxicity. Prenatal toxicity carcinogenicity in rainbow trout, immunomodulators. Part IV: Prevention and remediation: prevention of aflatoxicosis, chemoprotection, biosynthesis and reduction of preharvest aflatoxin in corn].

CAST, 1989. *Control and Management of Mycotoxins in: Mycotoxins Economic and Health Risks*, Reports No. 116. Iowa, USA. 66. [This book presents the mycotoxin control, management and it recommends good practices of manufacturing. It gives advances in sampling and chemical analysis, with a review on prevention methods associated to the field (pre-harvest), harvest and storage. The manufacturing processes, establishment of quality controls, strategies of detoxification by physical, biological and chemical methods].

Diener, U.L., and Davis, N.D. 1986. Biology of *Aspergillus flavus* and *A. parasiticus.* In: Zuber, M.S., Lillehoj, E.B. and Renfro, B.L. Eds. Aflatoxin in Maize: A proceedings of the Workshop. CYMMYT/UNDP/USDA. April 7-11, 1986. El Batán, México, D.F. 298-307. [This book deals with aflatoxin production by *Aspergillus flavus* and *Aspergillus parasiticus* using natural and synthetic media; with the influence of temperature and *Aspergillus* strain on aflatoxin production. Temperatures in the range between 28 and 35 °C promote second metabolite production. Media with 2% of yeast and sucrose favors the secondary metabolite production].

Eaton, D.L. and Groopman J.J. Editors. 1993. *The toxicology of aflatoxins. Human Health, Veterinary and Agricultural Significance.* Academic Press, Inc. San Diego, USA. 544 pp. [This a very complete compendium about aflatoxins each chapter written by one the best specialists. This book encompasses five parts. Part I: Experimental toxicology of aflatoxins, biochemical mechanisms and biological implications of the toxicity of aflatoxins as related to aflatoxin carcinogenesis, biotransformation, pharmacokinetics and excretion, nonhepatic disposition and effects of AFB_1, carcinogenicity in nonmammalian organisms, role of aflatoxin-DNA adducts in the cancer process, mechanisms of aflatoxins to induce mutations, carcinogenesis, nutrition modulation of Af carcinogenesis, Part. II: Human carcinogenicity, molecular dosimetry. Part III: Agricultural and veterinary problems: crop contamination, AF formation, veterinary diseases, AF in milk decontamination. Part IV: methodology of analysis, Sampling, role of antibodies. Part V: human risk assessment, and economic issues].

OPS-OMS. 1983. Micotoxinas. Criterios de la salud ambiental 11: Micotoxinas. Publicación Cientifica N° 453. Washington, USA: Organización Mundial de la Salud [WHO]. 131 pp. [It is a general book that explains many mycotoxins, such as aflatoxins, ocratoxins, zearalenone, trichothecenes. About aflatoxins many subjects are explained: sources, distribution, effects, recommendations over new researches, chemical propertiesanalytical methods, sampling, fungal formation humidity and temperature , their presence in food products, metabolism, effects in animals, occupational exposition, human epidemiological and clinic studies, risk evaluation of aflatoxin exposition, and references].

Sweeney, M.J., and Dobson, A.D.W. 1999. Molecular biology of mycotoxin biosynthesis. FEMS *Microbiology Letters*, 175(2): 149-163. [This reference gives a detailed description, chemical structure and biosynthesis of four important mycotoxins (Aflatoxins, Fumonisins, Sterigmatocistin and Trichothecenes) for agriculture. It refers to the methods employed for clonation and molecular characterization of the genes responsible of the biosynthetic pathways. It shows a discussion about the importance of the knowledge of the molecular organization of biosynthetic pathways and their regulation].

Wyllie T.D., and Morehouse L.G. (1978). *Mycotoxin Fungi, Mycotoxins, Mycotoxicoses. An Encyclopedic Handboook.* Vol 3. 1-202.New York, USA.: Mercel Dekker, Inc. [This Encyclopedia is an excellent guide in the complex subject of mycotoxicology. Several mycotoxicogenic fungi, mycotoxins and mycotoxicosis are described. A detailed analysis of the fungi, their toxic metabolites and toxicogenic cases of diseases related to them. This book has a thorough description of Aleukia Toxic Alimentary in Russia and a description about the effects of aflatoxins in plants and in man].

Biographical Sketches

Magda Carvajal is a Researcher Professor of Mycotoxins at the Institute of Biology of the Universidad Nacional Autónoma de México (National Autonomous University of Mexico) in Mexico City. She carried out his Postdoctoral work at the University of York, UK in the Cancer Research Unit, and also in the University of Bristol, UK in Long Ashton Research Station. Her present research interests are in aflatoxin biomarkers of human cancer, aflatoxins in maize products, in poultry tissues and in eggs, fumonisins in maize products. She has been tutor of 28 thesis, has presented around 250 papers in congresses and has more than 70 publications.

Pavel Castillo-Urueta Studied Food Chemistry (2002) with the thesis "Contamination of marine organisms by polycyclic aromatic hydrocarbons", and his Mastership in Chemical Sciences (2006) with a thesis "Aflatoxins in fructose corn syrups" in the "Facultad de Química de la Universidad Nacional Autónoma de México" (Chemistry School, National Autonomous University of Mexico, UNAM). He is also Microbiology and Chemistry teacher in UNAM, and has set up technical analytical methodologies to determine organic compounds. He has participated in several national and international congresses and nowadays works in aflatoxins of corn products.