

INTRODUCTION

Mycotoxins are toxic mold metabolites produced during the growth phase of molds in various foods and grains. One of the most important mycotoxins are Aflatoxins.

Aflatoxins are toxic metabolites mostly produced by many fungal species as: *Aspergillus flavus*, *Aspergillus parasiticus* and *Penicillium puberulum* in cereal grains, groundnuts, corn and rice, aflatoxins include aflatoxin (B₁, B₂, G₁, G₂, M₁ and M₂) (**Ki et al., 1987**).

They are considered as potent carcinogens, teratogens, mutagens and immunosuppressives, they play an important role in the development of many human diseases including: haemorrhagic liver necrosis, hepatocellular carcinoma, lung, breast cancers, bile duct proliferation, Reyes syndrome and Kwashiorkor disease (**Hayes et al., 1984; Dvorackova, 1990 and Hendrickse, 1991**), they also cause stunted growth in children who are exposed to aflatoxins at neonatal stages. This due to its capacity to cross placental barrier and cause genetic defects at foetal stages itself (**Maxwell et al., 1998 and Gong et al., 2002**).

In animals, aflatoxins cause suppression of immunity, growth retardation and increase susceptibility to infectious diseases (**Patten, 1981; Miller and Wilson, 1994**).

The economic impact of aflatoxins occurs directly from crop and livestock losses as well as indirectly from cost of regulatory programs designed to reduce risks to animal and human health.

The Food and Agriculture Organization (FAO) estimated that aflatoxin contamination costs US producers more than \$100 million per year on average including \$26 millions to peanuts (*FAO web library, 2000*).

Wu (2004) presented the complex effects of regulatory limits for mycotoxins on price, trade, public health, selling and purchasing.

The developed countries face economic losses as a result of mycotoxin contamination this due to the low quality crops are consumed locally while the highest quality crops are exported, exports of agricultural products particularly ground nut from developing countries have dropped in recent years resulting in major economic losses to producing countries (*Bhat and Visconti, 1999 and Otzuki et al., 2001*).

According to world bank estimate, the policy change by the European Union will reduce by 64% imports of cereals, nuts and dried fruits from: Chad, Egypt, Mali, Senegal, Sudan, South Africa, Gambia, Nigeria and Zimbabwe, this will cost the African countries about \$760 millions per year (*Kellerhals, 2000*).

As a results of the great economic loses due to aflatoxins especially in the human feeds as well as food stuffs of animals, the world public researches directed to examined different techniques to minimize the presence of mold producing aflatoxins.

One of the main techniques is the use of antifungal products of many bacterial species as: lactic acid bacteria and *Bacilli* species which are well documented and have been investigated but more researches are needed.

The lactic acid bacteria have an effect on mold growth and mycotoxin production, so they have the potential to be used as biological control agents in foods to prevent mold growth and mycotoxin production.

Hence, the present study aimed to investigate the following: Search for isolation, purification, and characterization of antiaflatoxigenic substances produced by bacteria that could be safe, cheap and commercially exploitable used in biological control of mycotoxins in stead of using other fungicides which have harmful effects on human and animal health as well as environment.

REVIEW OF LITERATURE

Before 1900, in Italy, researchers there believed that consumption of moldy corn by children led to development of illness.

In Southeastern United State in the early 1950`s there are hundreds of wild pigs foraging in cultivated corn field become ill and many died. Teams of veterinarians and mycologists isolated number of different fungi from the moldy corn and inoculated each fungus on moist corn that had been sterilized and then fed them to pigs. The consumption of corn inoculated with *Aspergillus flavus* cause so called moldy corn disease but no toxins isolated.

In 1960, approximately 100,000 turkeys and lesser number of other domestic birds died in England due to consumption of contaminated ground nut meal imported from Brazil (**Blount, 1961**) and a toxigenic fungus was isolated from this groundnut which identified as *Aspergillus flavus* and a toxin called Aflatoxin, this disease called Turkey x disease, "x" indicated that cause of disease is unknown.

Over 300 Mycotoxins have been reported (**Coker, 1979**), based on extensive analytical studies **International Agency of Research on Cancer (IARC, 1993)**.

Bankole (1994) mentioned that fungi produce toxins in food classified into field fungi and storage fungi based on their ecological requirements for growth.

Field fungi require grain moisture above 20 % in cereals and often causes ear rot disease and toxin production before harvest when the crop

still in the field, the important genera of field fungi include *Fusarium*, *Alternaria* and *Cladosporium*, while the storage fungi usually grow in grain with moisture content in equilibrium with 70-90 % relative humidity, which corresponds to less than 18 % moisture content in cereals, and the most important genera are *Aspergillus* and *Penicillium*.

The most agriculturally important toxins from fungi are Aflatoxin, Ochratoxin A, Fumonisin, Zearalenone, Deoxynivalenol (Vomitoxin) and Tricothecene (T₂- toxin).

1.1: Types of mycotoxins:

Fumonisin: *International Agency of Research on Cancer "IARC" (1993)* reported that grains contaminated with *Fusarium moniliforme* containing fumonisins and fusaric acid which are possible human carcinogens.

The most important field fungi of maize in Africa and worldwide are *Fusarium* spp. It was discovered in South Africa in 1988, *Fusarium verticillioides* and *Fusarium proliferatum* are the most widespread fungi, the fungus is most frequent in preharvest and stored maize, they known to produce over 1400 secondary metabolites that can adversely affect human and animal health (*Ekpo and Banjoko, 1994; Marasas, 1995; Essien, 2000 and Visconti, 2001*).

Some correlation studies suggested a link between consumption of contaminated maize and high incidence of human esophageal carcinoma in certain parts of south Africa and China, also fumonisins induce apoptosis in cultured human cells and in rats kidneys (*Yoshizawa et al., 1994; Tollenson et al., 1996 and International Program on Chemical Safety "IPCS", 2000*).

Fumonisin are hepato and neurotoxic for many animals causing number of animal diseases such as: Leukoencephalomalacia in equines which involves a massive liquefaction of the cerebral hemisphere of brain, abnormal movement, aimless circling, they also cause rat liver cancer, porcine pulmonary oedema and haemorrhage of rabbit brain (**Marasas, 1995 and Howard et al., 2001**).

Doko et al. (1995) reported that average fumonisins levels is 640µg/kg in maize from Benin.

Hell et al. (1996) found that levels from fumonisins detected in maize samples decreased from south to north in Benin.

In a 2- years study conducted by US food and drug administration, it was shown that at high exposure, fumonisin B₁ produced liver cancer, decreased life span in female mice and induced liver cancer in male rat but not decrease life span (**National Toxicology Program "NTP" 1999**).

Insect carry spores of *fusarium* from plant surfaces to the interior of the stalk or kernels or create infection wounds due to the feeding of the larvae on stalk or kernels (**Munkvold and Hellminch, 2000**).

The united state FDA (food and drug administration) had proposed a guide line for tolerance level of 2mg/kg total fumonisins in corn for human consumption (**FDA, 2001**).

Avantaggio et al. (2002) found that insect damage in maize is good predictor of *fusarium* contamination, and can serve as early warning of fumonisins contamination.

Bankole et al. (2003) detected fumonisin B₁ levels in maize in Nigeria with concentrations varied from 65 to 1830µg/kg with mean level in positive samples of 390µg/kg.

Ochratoxin A: Ochratoxin A "OTA" is produced by different species of *Aspergillus* and *Penicillium viridicatum*, it was first isolated from cultures of *Aspergillus ochraceus* (**Van der Merwe et al., 1965**).

OTA is found as natural contaminants in many foodstuff including cereals, dried fruits, cocoa, wine, poultry eggs, milk and common in stored corn, it is immunosuppressive, teratogenic, genotoxic and mutagenic

IARC (1993) had classified it into group 2B as possibly human carcinogens, the joint expert committee on food additives of the WHO and FAO set a provisional maximum -intake of 100 ng/kg body weight, while the scientific committee on food of the European Union proposed the maximum daily intake of OTA should not exceed 5ng/kg body weight (**World Health Organization "WHO", 1996**).

OTA can increase mutagenic ability of aflatoxin B₁ in the case of the two simultaneously occurring in the same crop, it is frequently associated with crops grown in semidried and temperate regions and it is not consider a major problem under tropical climate (**Sedimikova et al., 2001**).

OTA is a problem in cocoa which exported from west Africa, the EU presently contemplating on introducing regulatory limits in cocoa and cocoa products and industries have been mandated to implement preventing measures to reduce OTA.

Scientists at International Association of Chocolate, Biscuits and confectionery Industries (CABI) had collected samples from all stages of cocoa production from the tree through the finished product to determine the points at which OTA enters food (**CABI, 2001**).

Trichothecenes toxins (T-2) toxins: It is produced by *Trichothecium roseum* and *Fusarium tricinctum*, it cause alimentary toxic aleukia (ATA) where number of leucocytes were reduced in affected person, other symptoms include nose and throat bleeding and multiple subcutaneous hemorrhages, it is responsible for feed refusal, emesis and poor growth in swine and associated with hemorrhagic syndrome in poultry and with variety of symptoms and lesions in other animals.

Some of trichothecenes are extremely toxic when consumed or when contact with skin, they were used in biological weapon as yellow rain in the mid of 1970s, they cause all animals and people dead, their bodies were bleeding from the nose and ears, their skin were blistered and yellow, it cause also shortness of breath and sick of stomach before death (**Coulombe, 1993**).

Deoxynivalenol (vomitoxin, DON): It is produced by *Fusarium graminearum*, which grow in the ears of corn and on the head of cereal grain before harvest, it makes grain unpalatable to swine.

Wet, rainy, warm and humid weather from flowering time promotes infection of corn and cereals also low temperatures following infection may increase the production of DON by *Fusarium* species resulting in ear rot in corn and scab or head blight in wheat, barley, oats and rye.

Feeds that contain 1part per million (ppm) of DON may result in significant reduction in swine feed consumption and weight gain (*Christensen et al., 1975*).

Zearalenone: Zearalenone is produced by *Fusarium graminearum* that contribute to the ear and stalk rot complex growing in the ears of corn and on the heads of cereal grains standing in the field or in stored ear corn in the corn belt, when consumed by swine at more than 0.1 to 5 (ppm), these compounds cause estrogenic syndrome, which is characteristic externally by vulvar and mammary swelling in females, uterine hypertrophy and enlarged mammary glands and shrinking of the testes in young males leading to infertility (*Christensen et al., 1975*).

Ergot and Ergotism: Ergot toxicity caused by the fungus *Claviceps purpurea*, differ from other mycotoxicosis since it results from consumption of considerable amount of fungal tissue, the ergot fungus infects the flowers of cereals and many grasses forming characteristic black, spur – like sclerotia that replace the ovaries, the sclerotia or ergot bodies contain toxic alkaloids when consumed regularly in small amount results in ergotism (*Christensen et al., 1975*).

Sterigmatocystin: It is produced by *Aspergillus versicolor* this species is a storage fungus, it is not found as the only fungus or as the predominating fungus in deteriorating cereals, this species, under right conditions, produce sterigmatocystin, it is a toxic compound cause lung and liver tumors in lab animals (*Christensen et al., 1975*).

Fumagillin: It is produced by *Aspergillus fumigatus*, this species known to be an animal pathogen, infection occurs through inhalation of

spores and affects lungs, infection may also occur in eggs and the fetuses of cows, producing metabolic product may be considered a toxin or an antibiotic, it is a thermophilic species, found in substrate of high temperature up to 50 °C.

It is found in materials in advanced stages of decomposition where substrate temperature is raised due to microbial decomposition, under proper conditions, *Aspergillus fumigatus* produces fumagillin, which used as amoebicids in humans, correct dosage of the compound is critical, a little bit more than you need to get rid of amoebae and patient will get rid of life (*Christensen et al., 1975*).

1.2: Aflatoxins:

Aflatoxins are toxic secondary metabolites produced by the fungi *Aspergillus parasiticus*, *Aspergillus flavus*, *Aspergillus nomius* and *Aspergillus tamari*. These fungi are naturally occurring in soil and decaying vegetation.

Aflatoxins have been recognized as significant agricultural contaminant, it is first isolated in 1960 after death of 100,000 turkeys and many hundreds of other birds due to eating ground nut meal imported from Brazil to England, it known as turkey X- disease before the discovery of aflatoxin (*Blount, 1961*).

The toxigenic fungus identified as *Aspergillus flavus* isolated from ground nut and toxic principle was named aflatoxin meaning a flavus toxin.

Food products that contaminated with aflatoxin include cereals (maize, sorghum, rice and wheat), oilseeds (ground nut, soybean, sunflower,

cotton), spices (chilies, black pepper, coriander, zinger, turmeric) tree nuts (almonds, pistachio, walnuts, coconuts) and milk.

Aflatoxins are fluorescence strongly in ultraviolet light at 365 nm, B₁ and B₂ produce a blue fluorescence while G₁ and G₂ produce green fluorescence (**Hartely et al., 1963**).

The major members are aflatoxin B₁, B₂, G₁, G₂., aflatoxin B₁ (AFB₁) and AFB₂ mainly produced by *A.flavus* while *A. parasiticus* produce AFG₁, AFG₂, AFB₁, and AFB₂, other aflatoxins M₁, M₂, B₂A and G₂A may produced in minor amounts.

There are number of closely related compounds namely aflatoxin GM₁, parasiticol and aflatoxicol produced by *A. flavus* (**Holzappel et al., 1966; Dutton and Heathcote, 1969; Detory and Hesselstine, 1970 and Stubblefield et al., 1970**).

Aflatoxin M₁ and M₂ are major hydroxylated derivatives of aflatoxin B₁ and B₂ respectively, aflatoxin M₁ can appear in milk of lactating cows consuming significant quantities of aflatoxin B₁, it is not carcinogenic as aflatoxin B₁ but can be toxic (**Verma, 2004**).

Aflatoxin M₁ can be detected in milk products using a rapid and sensitive indirect competitive enzyme-linked immunosorbent assay (ELISA) method using monoclonal antibody (**Lee et al., 2009**) or by using immunoaffinity column chromatography and high performance liquid chromatography with fluorescence detector (**Pei et al., 2009**).

Aflatoxins are soluble in chloroform, acetone, methanol and acetonitrile, pure AFB₁ is odourless pale-white to yellow solid crystalline.

Both AFB₁ and AFB₂ are essentially biologically inactive unless these toxins are first metabolically oxidized to AFG₁ and AFG₂ *in vivo*.

1.2.1: Physical and chemical properties of aflatoxins:

Aflatoxins are normally refers to the group of difuranocoumarins and classified into two broad groups according to chemical structure:

- 1- The difurocoumarocyclopentenone series (AFB₁, AFB₂, AFB₂A, AFM₁, AFM₂ and aflatoxicol).
- 2- The difurocoumarolactone series (AFG₁, AFG₂, AFG₂A, AFGM₁, AFGM₂, and AFB₃).

The aflatoxins display potency of toxicity, carcinogenicity and mutagenicity in the order of AFB₁ > AFG₁ > AFB₂ > AFG₂ as illustrated by their LD₅₀ values(*Williams et al., 2004*).

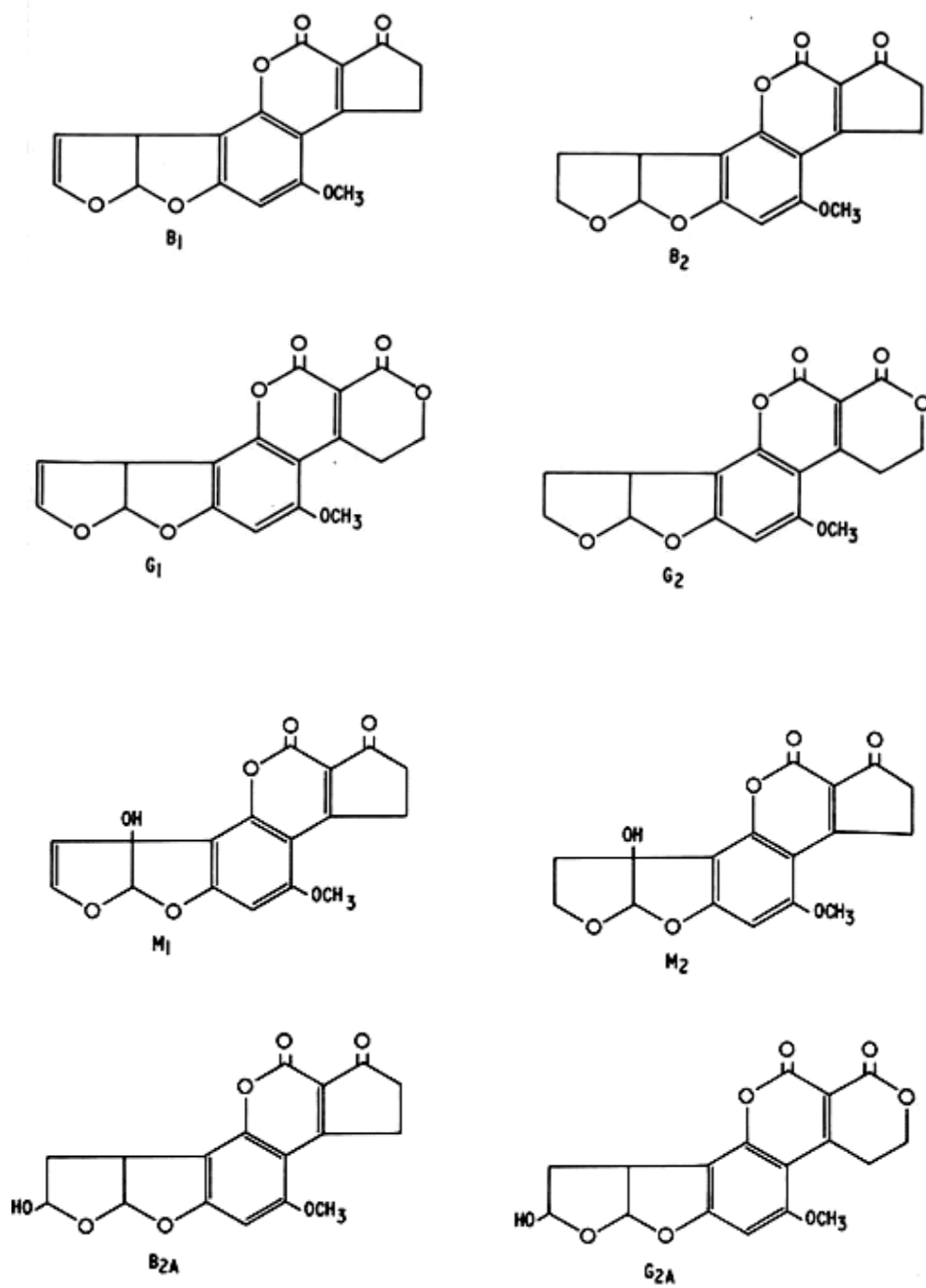


Figure (1): Structure of different aflatoxins (Williams et al., 2004). .

Table (1): Chemical and physical properties of aflatoxins (*Williams et al., 2004*). :

Aflatoxin	Molecular formula	Molecular Weight	Melting Point
B ₁	C ₁₇ H ₁₂ O ₆	312	268-269
B ₂	C ₁₇ H ₁₄ O ₆	314	286-289
G ₁	C ₁₇ H ₁₂ O ₇	328	244-246
G ₂	C ₁₇ H ₁₄ O ₇	330	237-240
M ₁	C ₁₇ H ₁₂ O ₇	328	299
M ₂	C ₁₇ H ₁₄ O ₇	330	293
B ₂ A	C ₁₇ H ₁₄ O ₇	330	240
G ₂ A	C ₁₇ H ₁₄ O ₈	346	190

1.2.2: Chemical reactions of aflatoxins:

The reactions of aflatoxins to various physical conditions and reagents have been studied extensively because of the possible application of such reactions in the detoxification of aflatoxins contaminated material.

i: Heat:

Aflatoxins in dry state are very stable to heat up to melting point, while in the presence of moisture and at elevated temperatures there is destruction of aflatoxin over a period of time, destruction occurs either with aflatoxin in oilseed meal and in roasted peanuts or aflatoxin in aqueous solution at pH7.

Although the reaction products have not been examined in details, it seems likely that the treatment leading to opening of the lactone ring with possibility of decarboxylation at elevated temperatures (*IARC, 1987*).

ii: Alkalis:

In alkaline solution, hydrolysis of lactone moiety occurs, it is reversible. Recyclization occurs following acidification of a basic solution contain aflatoxin, while at higher temperatures (100°C) ring opening followed by decarboxylation and reaction may proceed further leading to the loss of methoxy group from aromatic ring (*IARC, 1987*).

iii: Acids:

In the prescence of mineral acids, aflatoxin B₁ and G₁ are converted to B₂ A and G₂A due to acid –catalyzed addition of water across the double bond in the furan ring.

In the presence of HCl and acetic anhydride, the reaction proceeds further to give the acetoxy derivative, Similar adducts of aflatoxin B₁ and G₁ are formed with formic acid – thionyl chloride, acetic acid –thionyl chloride and trifluroacetic acid (*IARC, 1987*).

iv: Oxidizing agents:

Many oxidizing agents such as H₂O₂, O₃, KMNO₄, chlorine sodium hypochlorite and sodium perborate react with aflatoxin and change the aflatoxin molecule in some ways as indicated by loss of fluorescence,the reaction mechanism is uncertain and reaction product is unidentified (*IARC, 1987*).