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AFLATOXIN: A BRIEF INTRODUCTION OF ORIGIN, THE IMPLICATION IN HUMAN AND ANIMAL HEALTH AND MANAGEMENT STRATEGIES

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Each year a large quantity of stored cereal, pulses, and oilseed became unsuitable for human and animal consumption due to different factors, including mycotoxins. Among several mycotoxins, aflatoxin is responsible for unprecedented economic loss and toll on human and animal health. Aflatoxin is a highly carcinogenic, highly toxic secondary metabolite produced in stored grains, oilseeds, nuts, dried fruits, and vegetables by a plethora of fungi, most prominently by *Aspergillus flavus* and *A.parasiticus* and less commonly by *A. pseudotamarii, A. nomius, A. parvisclerotigenus, and A. nomius.* In 2002 World Health Organization had noted that "aflatoxin contamination possess significant risk human and animal health hazard along with economic loss".

The word "Aflatoxin" itself is derived from the name of its causal organism, where "A" denotes *Aspergillus*, "fla" denotes flavus, followed by "toxin" or poison. The identification of aflatoxin as a potent mycotoxin goes back to the 1960s when more than a lakh of turkey birds died for no apparent reason, which the only shared connection was that all these birds

Fig1: *Aspergillus flavus* colony in agar medium

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were feed mold-infested poor quality groundnut seeds. This incident had been reported in the scientific literature in 1961 and termed as "turkey X" disease. On the compositional basis, aflatoxin is a coumarin ring in which dihydrofuran and tetrahydrofuran fractions are infused. There are numerous variants of aflatoxin that exist in the open environment; however, four major variants, i.e., B_1 (AfB_1), B_2 (AfB_2), G_1 (AfG_1), and G_2 (AfG_2), are most commonly encountered. The "B" and "G" stand for blue and green, respectively, while 1 and 2 denote higher and lower migration distances visible in UV chromatography. Among all these variants, a more significant concern is associated with the AfB_1 derived AfM_1 and AfB_2 derived AfM_2 commonly detected in cow milk if the cattle are fed with contaminated feeds, especially groundnut cakes, as even a minimal amount of aflatoxin can cause life-threatening issues for infants and children.

Figure2:*Aspergillus* infested groundnut and maize kernel

Aspergillus and the Origin of Aflatoxin

The most common form of *Aspergillus*, the *Aspergillus* flavus, can grow on complex protein and carbohydrate substrates, enabling more extensive host specificity, including plants, animals, and even humans. *Aspergillus* is a fungus from the Ascomycota phylum and Trichomaceae family and is extensively found in tropical and sub-tropical climates where temperature lingers around 27-30^{0}C and relative humidity above 80% although unlike A. *parasiticus*; A. flavus can survive the much drier arid climate. *Aspergillus* can be found in different soils worldwide, present as spores, hyphae, and sclerotia. Colour and morphological characteristics distinguish different species of *Aspergillus*, while the colonies are typically flat, granular, and powdery in texture. As the temperature decreases during the winter, *A. flavus* produces its microscopic structure for survival, a compact and hard mass of mycelium known as **sclerotia**.

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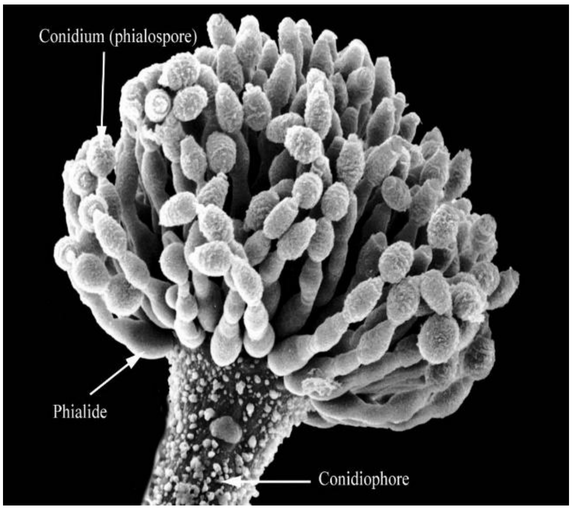

Figure 3: Conidiophores of *Aspergillus*

Implication on Human and Animal Health

Ingestion of aflatoxin results in many complexions, including severe toxicity, liver necrosis, cancer, or death. Repeated ingestion in even tiny quantities can cause undernourishment, immune suppression, a complication in reproductive organs, hepatic cell carcinoma, and impaired growth and development in humans and animals.

The severity of the symptom and disease is often regulated by the frequency and quantity of aflatoxin present in food or feed. Very high consumption is termed acute aflatoxicosis, which is rare but upon occurring causes fatty and decolorized liver, hemorrhages due to altered clotting mechanism of blood, reduction of liver serum protein, glomerular nephritis, and lung congestion. In ingestion in a lower quantity for a prolonged period, immunosuppression is the most common symptom. One of the primary variants, $AfB_{1,}$ is the most lethal naturally occurring hepatocarcinogen to humans. Repeated exposure to aflatoxin significantly increases the risk of hepatocellular carcinoma. The severity increases even up to 30 times if the person is already suffering from Hepatitis B. Besides the complications above, aflatoxin produces ROS, toxic compounds such as b-nitropropionic acid, cyclopiazonic acid, kojic acid, sterigmatocystin, aflatrem, aspertoxin, *Aspergillus* acid, and gliotoxin.

Hazard of Aflatoxin Contamination of Food and Feed

In recent times agricultural production and distribution systems are not close circuits; instead, the whole world is connected by the different agricultural circulatory systems. As a result, although aflatoxin contamination is predominant in the hot and humid climate, any region of the world can be susceptible to potential aflatoxin contamination. Although numerous agricultural commodities are susceptible to aflatoxin, groundnuts, maize seeds, cotton seeds, and tree nuts are the most common commodities. Research investigations indicate that *A. flavus* and *A.parasiticus*invades maize, cottonseeds, and groundnuts, respectively, before the crop reaches its maturity.

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Aspergillus predominantly targets plants/ grains with previous insect attacks and mainly targets areas with wounds common in maize seeds. Infection through silk is also reported in maize. Maize cultivated in dry and dryland areas is subjected to seed cracking which accelerates the attack of insects followed by *Aspergillus* invasion. At the initial stage, *Aspergillus* infests embryo tissue and aleurone layer and subsequently infests endosperm.

On the other hand, groundnut is susceptible to both *A. flavus* and *A.parasiticus,* but infestation occurs only when the plant is damaged or facing environmental stress such as drought or increased soil temperature to 30 degrees Celcius during maturity and harvesting period. When there is rain in between digging up the pegs and harvesting, the chances of contamination increase. The mechanical damage during transportation also plays a critical role in *Aspergillus* contamination. Generally, uncultivated soils contain fewer *Aspergillus*; however, cultivated groundnut has a higher number of conidia which further increases drought. If the kernel moisture is above 20%,the chances of *Aspergillus* invasion increase manifold.

A.flavus also infested cotton, and the aflatoxin level in cottonseed cakeexceeds the toxic limit. The infestation is much more common in the case of bolls which were previously attacked by pink bollworms. If the mean daily temperature rises above 24^{0}C accompanied by high relative humidity or rainfall, the chances of aflatoxin formation areincredibly high.

Detection of Aflatoxin

Awareness regarding aflatoxin contamination has been increased over time across the world. The awareness is mainly increased in Asian and African countries. However, as in Africa and Asia,the small farmers do most of the farming; proper monitoring and awareness regarding aflatoxin arecomplex. The upper limit of the aflatoxin is different in different countries; in the USA its 20 µg/kg, in the European union 0.10 µg/kg for AB_1 and 0.025 µg/kg for AM_1 for infant meals and India its 30 µg/kg for all foods.

Figure4: Rapid aflatoxin detection kit

In the majority of the countries, the limit lingers around fourµg/kg. AM_1 is more important from in dairy products perspective, and it directly depends on the AfB_1 content in the feed; the safe limit of which is set 20-300 µg/kg depending on feeds.

The detection procedure of aflatoxin is widely varied from the older thin layer chromatography (TLC) to newer generation high-performance liquid chromatography (HPLC), enzyme-linked immunosorbent assay (ELISA), and liquid chromatography-mass spectroscopy (LC/MS). Most of these procedures are based on the aflatoxin compound's fluroscene characteristics, which requires extraction of aflatoxin from food or feed followed by separation from other contamination and concentration upto a measurable limit. Different strip-based measurement systems are also available in the market for more straightforward and rapid detection.

Aflatoxin Prevention Measure

Over the years, several pre-harvest and postharvest methods have been developed to control *Aspergillus* and aflatoxin contamination. Avoidance of contamination is the most straightforward yet efficient measure to control aflatoxin. Preharvest agronomic management strategies, including cultivating resistant crop varieties, good cultural practices, fungicides, pesticides, proper water management to avoid drought stress, and harvesting in the proper maturity stage are essential tools to prevent aflatoxin contamination. Modern biotechnological methods, including the transfer of fungal infection-resistant genes,are helping to curb this toxicity.

Postharvest strategies include rapid drying, maintain proper storage conditions, and checking chemical change. However, this procedure requires proper structure and investment seldom found with the small farmers. As an alternative, some makeshift procedures can be used to prevent contamination. For example, maize kernels can be cooked for 45-60 minutes in a slightly alkaline solution to prevent aflatoxin contamination.

Conclusion

Aflatoxin contamination by *Aspergillus* is one of the most potent mycotoxins in the agriculture and allied sector, which can cause severe damage to the liver, acute toxicity, and hepatic cell carcinoma, or even death of both animals and humans. The toxicity issue is even more serious when infants and small children are fed with contaminated milk resulting from

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feeding milch cows *Aspergillus* contaminated feeds. Despite such severity of Aflatoxin contamination, the awareness and prevention measures are pretty limited, especially in Asian and African countries, as agricultural activities are primarily driven by marginal and small farmers who lack mechanized and sophisticated crop management, harvesting, and postharvest technological expertise. However, technological advancement paved a way to integrate aflatoxin management with proper crop management; integration of biotechnological and omics tools, development of rapid kit or strip-based detection procedure, and development of country-specific and strain-specific toxicity limits finally increased awareness.

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