



Biological control strategies for mycotoxin mitigation in groundnuts (*Arachis hypogaea* L.): A review

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ABSTRACT

Introduction. Mycotoxins are ubiquitous food contaminants that pose significant public health, food safety, and economic challenges worldwide, particularly in developing countries. Groundnut is especially vulnerable to mycotoxin contamination due to its biochemical composition and geocarpic growth habit, with the risk further exacerbated by the warm and humid climatic conditions prevalent in sub-Saharan Africa. In recent years, biological control has emerged as a promising, cost-effective, and environmentally sustainable approach to mitigating mycotoxin contamination by using antagonistic microorganisms, enzymes, and plant-derived compounds that suppress mycotoxigenic fungi, enhance host resistance, or detoxify mycotoxins.

Literature. This review synthesises current knowledge on the occurrence and health impacts of mycotoxins in groundnut and critically evaluates the potential of biological control agents, including fungi, bacteria, enzymes, and plant-derived extracts, as sustainable strategies to prevent fungal growth and reduce mycotoxin contamination. The underlying mechanisms of action, recent advances, and the advantages and limitations of these biological approaches are also discussed.

Conclusion. Mycotoxin contamination remains a major constraint to groundnut safety, public health, and economic sustainability, particularly in sub-Saharan Africa. Biological control agents represent environmentally friendly, sustainable, and cost-effective alternatives for mitigating mycotoxin contamination and improving groundnut quality and safety. Nevertheless, further research is required to optimise their efficacy under field conditions and facilitate their integration into comprehensive mycotoxin management strategies that support safe foodstuff production and enhance food security.

Keywords: Biological agents' control, biological control, Mycotoxin, groundnut, microorganisms.

RESUME

Stratégies de lutte biologique pour l'atténuation des mycotoxines chez l'arachide (*Arachis hypogaea* L.) : synthèse bibliographique

Introduction. Les mycotoxines sont des contaminants alimentaires ubiquitaires qui représentent un défi majeur pour la santé publique, la sécurité sanitaire des aliments et l'économie, en particulier dans les pays en développement. L'arachide et d'autres denrées alimentaires sont particulièrement sensibles à cette contamination en raison de leur composition biochimique, de leur mode de fructification géocarpique et des conditions climatiques chaudes et humides de l'Afrique subsaharienne. La lutte biologique est aujourd'hui considérée comme une approche durable, économique et respectueuse de l'environnement, reposant sur l'utilisation de microorganismes antagonistes, d'enzymes et de composés d'origine végétale pour inhiber les champignons mycotoxinogènes ou détoxifier les mycotoxines.

Littérature. Cette revue synthétise les connaissances actuelles sur l'occurrence et les risques sanitaires associés aux mycotoxines dans les denrées alimentaires, et évalue le potentiel des agents de lutte biologique, notamment les champignons, les bactéries, les enzymes et les extraits végétaux, pour prévenir le développement des champignons mycotoxinogènes et réduire la contamination. Les mécanismes d'action, les avancées récentes ainsi que les avantages et les limites de ces approches sont également examinés.

Conclusion. La contamination des denrées alimentaires, comme l'arachide, par les mycotoxines demeure un enjeu majeur de santé publique et de sécurité alimentaire, notamment en Afrique subsaharienne. Les agents de lutte biologique offrent des solutions durables et sûres pour l'environnement pour réduire cette contamination et améliorer la qualité sanitaire de l'arachide. Toutefois, des recherches complémentaires sont nécessaires afin d'optimiser leur efficacité au champ tout comme en stockage et de favoriser leur intégration dans des stratégies globales de gestion des mycotoxines visant à renforcer la sécurité alimentaire.

Mots-clés: Lutte biologique, agents de lutte biologique, mycotoxines, arachide, microorganismes

1. INTRODUCTION

Groundnuts (*Arachis hypogea*), often known as peanuts, are significant legume crops grown in tropical and semiarid regions. They serve as a significant source of vegetable protein and edible oil. It ranks fourth globally in terms of importance as an oilseed crop and thirteenth globally as a food crop (Manizán *et al.*, 2018). It was first grown in South America, in what is now southern Bolivia and northwest Argentina, around 1000 BC. These days, it's grown in areas 40°C north and south of the equator, with 500–1,200 mm of above-average rainfall and 20°C average daily temperatures (Ncube and Maphosa, 2020; Ilunga *et al.*, 2023).

World annual groundnut production is estimated at 54, 238,360 tons. The main groundnut-producing countries in the world are China, India, Nigeria, the United States and Myanmar. The major producers and exporters of groundnuts in Africa are Nigeria, Senegal, Sudan, Chad, Ghana, Niger and Mali, which account for more than 26% of world production (World Bank, 2022). In Sub-Saharan African countries, groundnuts are a valuable source of protein, fat, vitamins, and minerals that contribute to a nutrient-rich diet in the region, where 43 % of the population, particularly children and pregnant and breastfeeding women, suffer from chronic malnutrition, characterised by vitamin A and iron deficiencies. The Food and Agriculture Organisation (FAO, 1990) suggested a handful (~30 g) of peanuts a day could solve most cases of malnutrition in developing countries, such as Sub-Saharan.

Groundnut cultivation is faced with numerous diseases caused by various phytopathogenic moulds. These include damping-off (*Aspergillus niger*, *Rhizopus stolonifer*), cercosporiosis (*Cercospora arachidicola*) and dry rot of roots and pods (*Rhizoctonia solani*), which reduce seed yield and quality, and increase production costs. In addition to diseases, groundnut seeds are susceptible to contamination by moulds, mainly of the genus *Aspergillus*, some species of which produce mycotoxins, such as aflatoxins produced

by *Aspergillus flavus* (Guchi, 2015). These mycotoxins are dangerous to human and animal health, causing many pathological effects, including cancers, and disorders in the functioning of various organs negatively impacting the country's economy (Ncube and Maphosa, 2020).

The World Health Organisation (WHO) (2023) reports that mycotoxins are responsible for around 600 million cases of morbidity, including 420,000 deaths worldwide, and 5 billion people, particularly in poor countries, are at risk of chronic exposure to mycotoxins. This is a serious public health issue, especially in low-income nations like the sub-Saharan region where eco-climatic factors (high humidity and temperature) and people's poverty provide a favourable environment for mycotoxin contamination of foodstuffs (Kamika *et al.*, 2013). The Food and Agriculture Organisation (FAO) (2018) estimated that around a third of all food produced by the world's population, is lost from the field to the consumer, representing around 1.3 billion metric tons per year. Mycotoxin contamination is one of the primary causes of food and feed losses in underdeveloped nations, especially in sub-Saharan Africa (Manizán *et al.*, 2018). Numerous studies have shown that most groundnuts produced in sub-Saharan Africa are subject to toxigenic fungal colonisation before and after harvest and to mycotoxin contamination (Guchi, 2015).

There is, therefore, a need to ensure the quality of the groundnut crop to maintain its significant impact on the national economy and food security. Thus, the prevention and control of mycotoxin contaminants in groundnuts is becoming a priority for producers and consumers. Although chemical pesticides have been crucial in improving production by controlling pests, this has led to several environmental problems, such as the emergence of pesticide-resistant pests, the destruction of beneficial pests and predators, and the release of pesticides into the environment.

Biological control agents (BACs) represent a safe and effective alternative for reducing the impact of mycotoxins in groundnut crops. This can lead to the production of fewer or no toxic intermediates and end products. The field of biological control holds great promise for the future and is a crucial component of the integrated management of plant diseases. It is also inexpensive, easy to formulate and uses antagonists present in the rhizosphere (Hassan *et al.*, 2021). We present this literature review to highlight the use of BACs in the mycotoxin management strategy for groundnut crops to reduce their harmful pre-harvest and post-harvest impact, especially in poor regions such as sub-Saharan Africa.

2. METHODOLOGY

A documentary review was employed as the methodological framework for this study. Relevant literature published between 1960, when mycotoxins were first identified, and 2026 was systematically searched, screened, and critically reviewed. Scientific sources, including peer-reviewed journal articles, books, review papers, theses, dissertations, and technical reports, were retrieved from major academic databases and search engines, such as Google Scholar, ResearchGate, PubMed, and other scholarly repositories. The review focused on publications addressing mycotoxin contamination in groundnuts and other foodstuffs, with particular emphasis on biological control strategies.

Additional references were obtained from the libraries of the University of Kinshasa, the National Pedagogical University, and the Catholic University of Congo. Eligible documents were required to provide substantial information on foodborne mycotoxins affecting groundnuts or other foodstuffs produced or consumed in the Democratic Republic of the Congo, while relevant studies from other countries were also included to broaden the scientific perspective and facilitate comparison of current knowledge and advances in biological control. Following the screening and eligibility

assessment, 48 documents met the inclusion criteria and were retained for qualitative analysis.

3. MYCOTOXINS IN GROUNDNUTS

Greek terms "Mykos" (meaning "Fungus") and "Toxicum" (meaning "Poison") are the source of the word "mycotoxin." Mycotoxins are therefore toxic substances secreted mainly by micromycetes. More specifically, they are the secondary metabolites produced by various moulds (*Fusarium*, *Aspergillus*, *Penicillium*, *Claviceps*, and *Alternaria*) which cause adverse effects in humans and animals. These fungal toxins are chemically diverse, and they belong to different chemical families. Their molecular weight ranges from approximately 200 to 500.

They are among the important food contaminants. Mycotoxicosis is the syndrome of toxicity resulting from the absorption of mycotoxin by humans or animals, generally by ingestion (Ilunga *et al.*, 2022). However, groundnuts, like other agricultural products, are very sensitive to contamination by mycotoxins secreted by certain fungi at any time, before or after harvest, during drying, storage or transport, causing changes in organoleptic and chemical characteristics, resulting in huge production losses and exposing animal and human consumers to serious health risks. Mycotoxins are not very soluble in water, are difficult to degrade by living organisms and are very stable to heat and acidity.

They are resistant to several technological processes that foodstuffs may undergo (roasting, extrusion and cooking) before ending up on the consumer's plate. They remain present in the food even when the fungal excretory agent is no longer present (FFSA, 2009). More than 400 mycotoxins have been identified, but only around ten pose food safety problems, as they have been reported to contaminate foodstuffs (Hellany *et al.*, 2023). Figure 1 depicts the harm caused by the most common food-borne mycotoxins to human organs.

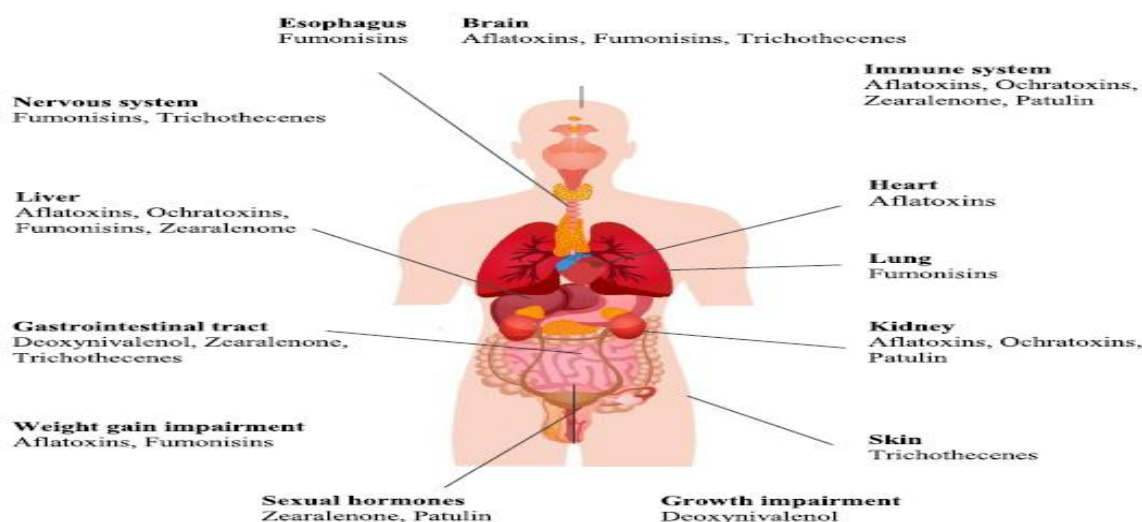


Figure 1. Harms caused by mycotoxins to human organs (Nasrollahzadeh *et al.*, 2022)

3.1. Aflatoxins (AFs)

They are the most common mycotoxin contaminants of groundnuts and other crops worldwide, which affect both food safety and agricultural economies. They were the first mould toxins to be discovered in poultry feed (made from groundnut meal) during the investigation of "turkey disease X", which hit England in the 1960s (FFSA, 2009). The emergence of today's mycotoxicology as a scientific discipline began with this outbreak. Aflatoxins are extremely strong, all-purpose natural poisons that are generated by certain fungi, particularly those in the *Aspergillus* genus, as secondary metabolites. Major aflatoxins consist of B1, B2, G1, and G2. Compared to the G forms, the B forms are 10–50 times more poisonous. Aflatoxin B1 (AFB1) is the most carcinogenic and poisonous substance. The metabolites AFB2 and AFB1 are the source of aflatoxins M1 (AFM1) and M2 (AFM2). This is the mycotoxin group that is the most well-known, researched and regulated (WHO, 2023). Aflatoxins are recognised for their cancer-causing potential, especially hepatocellular carcinoma and are immunosuppressive, mutagenic, and teratogenic.

The International Agency for Research on Cancer (IARC) has categorised them as the most potent and poisonous mycotoxins that cause cancer in people, placing them in group 1. When the hepatitis B virus (HBV) or other hepatotoxic variables, including alcohol, tobacco, and infectious agents, are present, aflatoxins' ability to induce liver cancer is greatly boosted (FFSA, 2009). Remember that there is a correlation between concurrent hepatitis B virus infection and liver cancer (HBV). The capacity of aflatoxins to cause liver cancer is significantly increased in the presence of infection by the hepatitis B virus (HBV) or other hepatotoxic factors such as alcohol, tobacco and infectious agents (FFSA, 2009).

Let's not forget to mention that there is a relationship between liver cancer and simultaneous infection with hepatitis B virus (HBV). HBV infection is thought to increase AF metabolism, decrease glutathione-S-transferase activity, and therefore detoxify harmful compounds. Aflatoxins have also been associated with reduced immunity and fertility, as well as possible stunted growth in children (Hellany *et al.*, 2023). With 821 million people suffering from chronic undernourishment worldwide and reports of the impact of aflatoxins on stunted growth in children in Africa, major losses of food and feed due to mycotoxin contamination are exacerbating the situation in developing countries, particularly in sub-Saharan Africa. Economic losses, a direct consequence of aflatoxin contamination of groundnuts, are almost impossible to estimate accurately. But the total cost of mycotoxin contamination, due to reduced yields, losses of food and feedstuffs, and increased inspection and analysis costs, can easily run into billions of euros a year (FAO, 2018).

3.2. Ochratoxin A (OTA)

The fungi *Aspergillus ochraceus* and *Penicillium verrucosum* are the primary producers of OTA, a common mycotoxin that is created when groundnuts, cereals, grain products, and other plant items like herbs, spices, and grapes are stored. It is a mycotoxin that is present in wine, dried fruit, grains, cereals, beans, and coffee. Due to the presence of mouldy fodder, it is also found in pork offal and flesh as well as in sausages made with pig's blood. Due to unfavourable elimination toxicokinetics, OTA is extremely persistent in humans when consumed as a food contaminant, with a blood half-life of 35 days following a single oral intake. Nephrotoxic, immunotoxic, teratogenic, and carcinogenic effects of OTA are well-documented (Peng *et al.*, 2022).

Several studies have reported the occurrence of OTA among the mycotoxins contaminating groundnuts around the world (Guchi, 2015). Although OTA is neither deposited nor stored in the body, its uneven dispersion throughout the body might seriously harm the kidneys. According to its classification, this toxin is probably harmful to humans (listed as a 2B carcinogen) (EFSA, 2009).

3.3. Fumonisin (Fs)

They are produced by *Fusarium moniliforme* (*verticillioides*) and *F. proliferatum*, frequently found in maize, wheat, barley and also in groundnuts. These moulds are thermotolerant. They develop in a temperature range of between 5 and 40°C, whereas the optimum temperature for toxin production is 20°C, with a minimum temperature of 4°C and a maximum of 35°C. The main mycotoxins produced are fumonisin B1, fumonisin B2, fumonisin B3 and fumonisin B4. The toxicity of fumonisins is linked to their ability to disrupt sphingolipid metabolism. The most frequently encountered and the most toxic is fumonisin B1 (FB1). FB1 is neurotoxic, hepatotoxic and nephrotoxic in animals, and has been classified as a possible human carcinogen (Group B) (Mulunda *et al.*, 2013).

3.4. Trichothecene

These are mycotoxins produced by several *Fusarium* species. One hundred and seventy trichothecenes have been identified and classified into different groups according to their chemical structure. Group A trichothecenes, namely Diacetoxyscirpenol (DAS), 15-acetoxyscirpenol, T-2 Toxin, HT-2 Tetraol, T-2 Triol and verrucarol, are produced by the following species: *F. graminearum*, *F. sporotrichoides*, *F. avenaceum*, *F. culmorum*, *F. langsethiae*, *F. tricinctum*, *F. Solani* and *F. equiseti*. Group B trichothecenes are deoxynivalenol (DON), 15-O-deoxy-nivalenol (15-DON), 3-acetyl deoxynivalenol (3-ADON), de-epoxy deoxynivalenol (DON-1), and nivalenol (NIV). These trichothecenes are produced by *F. graminearum*, *F. equiseti*, *F. poae*, *F. nivale*, *F. crookwellense*, *F. acuminatum* and *F. sambucinum*. Trichothecenes type A are five to ten times more toxic than DON; their toxicity ranking in this order is HT-2=T-2<DAS. The distribution of trichothecenes in nature has been reported as saprophytes and plant parasites in a wide range of countries. Information on their natural occurrence, mainly DON, NIV and certain type A trichothecenes, in cereals and other agricultural products is limited. However, the occurrence and worldwide contamination of cereals and agricultural products by trichothecenes have been observed. The frequent contamination of cereals and animal

feedstuffs by type A and type B trichothecenes is well known worldwide and has been reported in cattle feed, maize, rice, wheat, sorghum and groundnuts (Badmos *et al.*, 2023).

Mycological, chemical and toxicological studies have shown that some of these mycotoxins are closely associated with dietary diseases in humans and animals. Acute symptoms reported include skin irritation, dermatitis, vomiting, inflammation, nausea, refusal to eat, bloody diarrhoea, haemorrhage, haematological changes including leukopenia and anaemia, neuronal disorders, abortion, food toxic aleukia, stachybotryotoxicosis, mouldy maize toxicosis, anorexia and impaired immune function. At the molecular level, trichothecenes can inhibit DNA and protein synthesis (Manizan *et al.*, 2017).

3.5. Zearalenone (ZEA)

Zearalenone is an oestrogenic mycotoxin produced by several species of the genus *Fusarium* (*F. graminearum*, *F. culmorum*, *F. crookwellense*, *F. equiseti*, *F. oxysporum*). It is also produced by *Aspergillus oryzae*, *A. parasiticus* and *A. versicolor*. It is a regular contaminant of cereals such as maize, wheat, barley and soya, as well as vegetable and fruit crops. The natural presence of zearalenone has also been reported in sesame and groundnuts. The chemical structure of ZEA resembles that of natural oestrogens. This property determines its ability to bind to oestrogen receptors in cells and its bioaccumulation. This leads to disturbances in the body's hormonal balance, which can lead to numerous diseases of the reproductive system, such as prostate, ovarian, cervical or breast cancer (Hellany *et al.*, 2023).

3.6. Patulin

Patulin is a mycotoxin produced by various fungi, of which *Penicillium expansum* is the main producer. *P. expansum* can infect various fruits and vegetables but has a preference for apples, where it causes blue rot. It is one of the mycotoxins contaminating and found in wines. As a result, apples and apple-based food products are the main source of exposure to patulin for humans. Patulin has also been found in other grains such as rice, maize, cowpea, acha, millet, sorghum and groundnuts. Although classified as non-carcinogenic, patulin has been associated in recent decades with neurological, gastrointestinal and immunological adverse effects, mainly causing liver and kidney damage. For this reason, it has been withdrawn from use as a drug, although it was discovered to be an antibiotic (Peng *et al.*, 2022).

4 DEFINITION AND HISTORY OF BACS

Biological control is the application of natural substances or live organisms by humans to stop or lessen the harm that is caused by dangerous organisms (food poisons, diseases, weeds, and animal pests). Microorganisms (plants, nematodes, and insects), microorganisms (viruses, bacteria, yeast, and fungi), and natural compounds derived from minerals, plants, or animals are the three types of BCAs. According to Ons *et al.* (2020), plant varieties that are resistant to insect attacks and other damaging agents are now regarded as biological control agents. Characteristics anticipated in a biological control agent encompass genetic stability, low-concentration effectiveness against a broad spectrum of pathogens, uncomplicated dietary needs, resilience in harsh environmental settings, cultivation on inexpensive substrates in fermenters, absence of pathogenicity for the host plant, and absence of potentially toxic metabolites for humans.

Chinese farmers first employed insects for biological control in 324 BC, when they employed ants to consume citrus pests. In the 18th century, exotic species were successfully introduced to combat the red locust in Mauritius. Then, in the United States (19th century), the cottony mealybug was spectacularly eradicated in California by a mealybug imported from Australia (Guchi, 2015).

Mycotoxin BCAs were first successfully employed the first time in the United States using atoxigenic strains of *Aspergillus flavus* to reduce aflatoxin levels in groundnuts, maize and cotton, and the technology was followed by Africa and Europe (Bandyopadhyay *et al.*, 2016). Today, biological agents such as Aflaguard and AF36, registered by the Environmental Protection Agency in the United States, and Aflasafe, registered in Nigeria and Kenya, are used to manage mycotoxins.

5. SOURCES OF BACS

BCAs can come from anywhere in nature, and the soil remains its natural reservoir. Thus, several preferred niches for the study and screening of these agents are seeds, fruits or other foodstuffs, warehouses, fields, orchards and rubbish dumps. They can also come from plants, animals or even wildlife. In groundnut, some BACs have been isolated from the surface of seeds, pods or the

rhizosphere, such as *Aspergillus* and *Trichoderma* genera, and have proved effective against soil-borne phytopathogenic fungi contaminating leaves and crops (Ons *et al.*, 2023).

6. DIFFERENT TYPES OF BACS

6.1. Fungi

Fungi form a kingdom in their own right: the Fungi or Eumycota kingdom. They are eukaryotic microorganisms, generally filamentous, saprophytic or parasitic, heterotrophic and dependent on a source of organic carbon. Whether or not they are mycotoxigenic, fungi generally follow the same life cycle through various processes, in particular, sporulation, spore germination, growth of the vegetative apparatus (mycelium), symbiotic associations and reproduction by sexual or asexual means. Spores give fungi their considerable power of contamination. Spores are dispersed by wind and insect vectors. As a result, they are found in the soil (primary habitat). The air we breathe, water, and most importantly, a vast array of plant and animal-based foods, including cereals (rice, wheat, corn, paprika, ginger), lentils, sunflower, soy, fresh or dried fruit, groundnuts, almonds, walnuts, pistachios, coffee, cottonseed, fruit juices, cider, pasture, feed for livestock and poultry, silage and fodder, milk and its derivatives, meat. In general, fungi flourish in typical conditions because they are mesophilic. Their growth is most excellent from 20 to 25°C, and it is frequently feeble between 5 and 35°C. Temperatures below -20°C do not kill conidia; conidia from mesophilic species remain viable for an extended period of time, but do not germinate below 5°C. Psychrotrophic moulds, which grow slowly at temperatures below 4°C, include *Penicillium expansum*, *P. verrucosum*, and *P. viridicatum*, among other hazardous moulds (Badmos *et al.*, 2023).

Aspergillus spp are ubiquitous in the environment but are more often associated with warmer climates. In nature, they represent 1 to 7% of environmental fungi and are widespread in decomposing organic matter in the soil or on the surface. It is the fearsome myco-contaminant of groundnuts, and certain species (*flavus*, *parasiticus*, spp) produce mycotoxins, in particular aflatoxins, which are known to cause cancer. Figure 2 describes the life cycle of *Aspergillus* in groundnut.

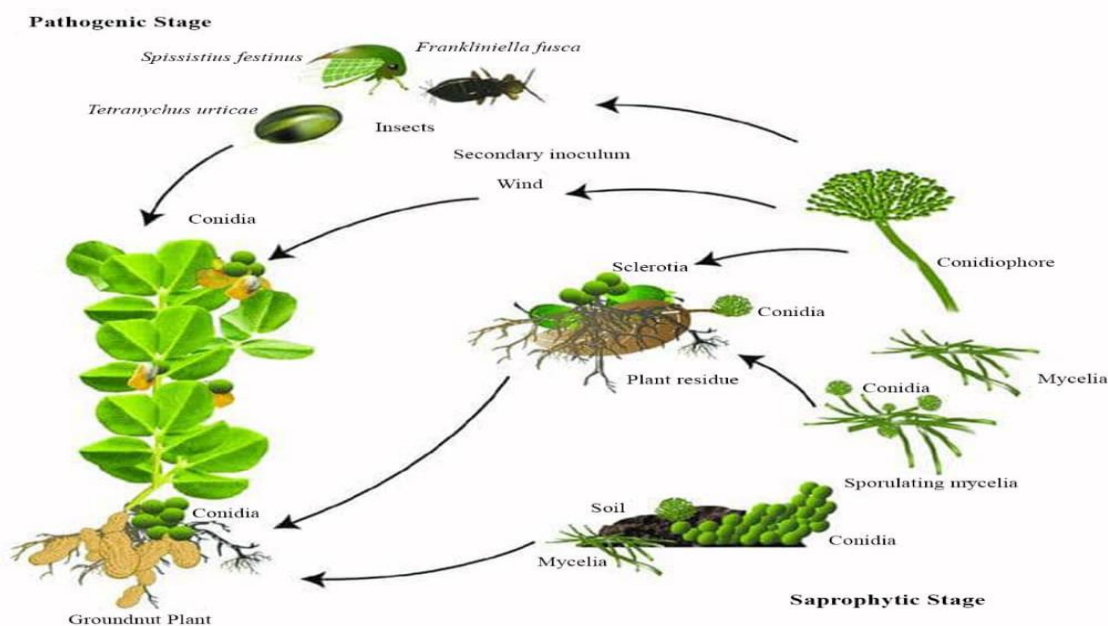


Figure 2. Life cycle of *Aspergillus* in groundnut (Alam *et al.*, 2020)

Both *Aspergillus flavus* and *Aspergillus parasiticus* are common in crops and can secrete aflatoxins. They can contaminate produce when it is in the field. A subset of fungi known as thermophilic and xerotolerant fungi may thrive in drier environments and at greater temperatures than the majority of other fungi or microbes that infect crop plants. *Aspergillus* can be divided into two groups: S and L strains, according to the phenotyping of the toxigenic strains. *Aspergillus parasiticus* produces aflatoxins B1 (very dangerous) and G1 and G2 (less toxic), but *A. flavus* can only create AFB1. The S strains produce significant quantities of aflatoxins, while L strains produce huge sclerotia but less aflatoxin (Alam *et al.*, 2020).

The use of non-toxic fungal strains to control contamination by the toxigenic strain is a biological control method for mycotoxin contamination of groundnuts in the field. Thus, the atoxigenic strain does not have the inherent ability to synthesise the mycotoxin, which gives it potential as BACs for controlling mycotoxin contamination. Research has shown that treatment of groundnut, cotton or maize crops with atoxigenic strains of *A. flavus* can significantly reduce 70-90% of aflatoxins. According to regression analysis, the existence of non-toxic strains in groundnuts and the reduction of aflatoxin were found to be strongly and significantly correlated. *Aspergillus flavus*, a mycotoxigenic fungus, is antagonistic to the atoxigenic *A. flavus*. Non-toxic strains can either restrict harmful strains through receptor competition on the host plant or trigger the host plant's defensive mechanisms. Today, soil treatment techniques using non-toxic fungal strains have been developed in several countries. The results

showed that this treatment had a lasting protective effect on oilseed and cereal crops against contamination by mycotoxigenic fungi, even during storage (Cotty, 1994).

Trichoderma spp is a facultative multiparasite saprophyte, a filamentous fungus frequently found in soil. These fungi are mostly asexually reproducing, including facultative plant symbionts that colonise the rhizosphere and are very effective soil colonisers with strong biodegradation potential. Many studies have shown that *Trichoderma*, through many interaction mechanisms, including colonisation, antibiotics, and mycoparasitism, is one of the most efficient biocontrol agents against soil, foliar, and postharvest phytopathogenic fungi in crops. Srilakshmi *et al.* (2013) looked into the bioactivity of small molecules or secondary metabolites made by *Trichoderma* spp. and how well they worked to prevent aflatoxin contamination of groundnuts. The findings showed that 48 strains of *Trichoderma* were very successful in inhibiting the *A. flavus* isolate (AF11-4) and so lowering the amount of aflatoxin produced in groundnuts.

According to Thakur *et al.* (2003), a notable decrease in the *A. flavus* population in the groundnut rhizosphere indicated that *Trichoderma* species were effective BCAs that offered more effective protection against groundnut seed infection caused by *A. flavus* strain Af 11-4 than other seed contamination. *Trichoderma* species have been shown by Gachomo and Kotchoni (2008) to exhibit a strong inhibitory impact against *Fusarium* spp., *A. flavus*, *A. parasiticus*, *A. niger*, and *A. ochraceus*-induced groundnut infections.

These bacteria's ability to eradicate other harmful bacteria from the environment has been related to their extracellular enzymatic activities, including lipolytic, chitinolytic, pectinolytic, amylolytic, and proteolytic activities. Many other fungi, including *Streptomyces yanglinensis* 3–10, are promising in groundnut cultivation as BACs. This is because of its antifungal active substances, which effectively inhibit the growth and aflatoxin AFB1 production by *Aspergillus flavus* on groundnut seeds, both in vitro and in.

Armando *et al.* (2011) also report that *Saccharomyces cerevisiae* strains RC008 and RC016 have been shown to reduce aflatoxin B1 production and *Aspergillus parasiticus* growth in culture, and that their application in groundnut cultivation may be promising.

6.2. Bacteria

Bacteria are helpful biological agents for mycotoxin detoxification, as demonstrated by numerous studies. beneficial biological agents for mycotoxin detoxification. Mycotoxins can be removed by bacteria by biosorption or biodegradation.

Gram-positive bacilli

The *Bacillus* represent a significant group of BACs within this family. These bacteria, known as endospores, develop spores and inhabit the rhizosphere and soil of plants. They are known as Plant Growth-Promoting Rhizobacteria (PGPR), and they shield the plants from dangerous or phytopathogenic microorganisms. Their endospores are resistant to heat, chemicals, and sunlight and can survive for extended periods. They are found in nature in large quantities, mostly in the soil where they infiltrate dust particles. In vitro, *Bacillus* grows in aerobic or anaerobic conditions and thrives on ordinary culture media. On agar, they form medium-sized circular colonies with a regular or irregular surface and a whitish colour (Hassan *et al.*, 2021). Some of these strains have long been known to have PGPR activity. They are potentially useful as biological control agents and are capable of solubilising phosphate, producing indole acetic acid, a siderophore and an antifungal agent, and playing a role in reducing mycotoxins. For this reason, bacilli are an important family of bacteria that have the potential to degrade aflatoxins. Selected from the rhizosphere of several plants, *Bacillus subtilis* is a diazotrophic species in the genus with a concentration of above 10⁷ bacteria per gram of rhizospheric soil.

In the field and greenhouse with *A. flavus* infecting groundnuts, the BCA activity of *Bacillus subtilis* strain G1 was assessed. The findings demonstrated that *A. flavus* growth was suppressed by 93–100% in the two groundnut crops that were evaluated

when compared to different isolates of *A. flavus* that contaminated groundnuts in vitro. According to these findings, *B. subtilis* strain G1 considerably reduces the amount of *A. flavus* in the soil, the *A. flavus* infection, and the amount of aflatoxin B1 in the kernels while boosting pod yield when administered to groundnuts as a seed treatment and as a soil application. These investigations demonstrate the potential of *B. subtilis* strain G1 as a biocontrol agent to prevent aflatoxin contamination of groundnuts.

Numerous studies around the world have reported on the effect of bacilli on mycotoxins and the inhibition of *Aspergillus* in food chains. *Bacillus subtilis* UTBSP1, isolated from Iranian pistachios, was shown by Farzaneh *et al.* (2012) to degrade AFB1 by an estimated 78.4–95.0%, but another *B. subtilis* ANSB060, isolated from fish intestines, was able to break down 81.5% of AFB1 in 72 hours. Furthermore, it was demonstrated that additional *Bacillus* species, including *B. licheniformis* CFR1, *Bacillus velezensis* DY3108, *Bacillus subtilis* JSW-1, and *Bacillus shackletonii* L7, could digest AFB1 at 94.7% and 67.2 per cent respectively.

The term "*Eubacterium*" refers to gram-positive, pleomorphic, homogeneous, non-spore-forming bacteria that are chemoorganotrophic and anaerobic by necessity. This genus contains both saccharolytic and non-saccharolytic species that can move or remain motionless. These bacteria do not produce lactic acid alone, propionic acid as the primary acid, greater amounts of acetic acid than lactic acid with or without the formation of formic acid, lactic and succinic acid with small amounts of acetic acid or formic acid, or mixtures of organic acids from carbohydrates or peptone that may include large quantities of butyric, acetic, and formic acids. The first and only microbe approved in Europe to lessen feed contamination by deoxynivalenol as a technical ingredient for animal feed was *Eubacterium* BBSH 797 (BIOMIN BBSH 797). This bacterium produces a specific enzyme, called deepoxidase, which biotransforms the toxic deoxynivalenol in the animal's gastrointestinal tract into an atoxigenic metabolite. Through enzymatic deactivation, biotransformation provides a targeted, permanent, and eco-friendly detoxification process that eliminates any harmful residues or unwanted byproducts (EFSA, 2017).

The genus *Lactobacillus* contains gram-positive, non-flagellate, non-spore-forming bacteria that are either heterofermentative, producing ethanol, CO₂, lactic acid, and acetic acid in equimolar amounts or homofermentative, producing over 85% of the lactic acid from glucose. Following the groundbreaking in vitro research by El-Nazami *et al.* (1998) on the binding characteristics of

mycotoxins by lactic acid bacteria, a methodical search was conducted to identify microorganisms with particular qualities for the adsorption of mycotoxins by lactic acid bacteria. Due to their ability to preserve food by acidifying it and/or enhancing its flavour, texture, and nutritional value, many *Lactobacillus* species are linked to the food manufacturing industry. Their natural habitat is quite diverse, encompassing everything from food, plants, and wastewater to the gastrointestinal, genital, and oral tracts of both people and animals.

Some *Lactobacillus* species found in the gastrointestinal tract have been linked to health advantages, which has led to their classification as "probiotic micro-organisms" according to the FAO/WHO (2002), which defines them as "live micro-organisms which, when administered in adequate quantities, confer a benefit to humans." Due to their excellent safety record in food applications, *Lactobacillus*, also known as lactic acid bacteria, is the top microorganism for the breakdown of mycotoxins. Because they naturally thrive in the human gut and can effectively eliminate mycotoxins, they are preferred above other microorganisms for use in food. Additionally, many strains of these microorganisms are easy to culture and maintain (Nasrollahzadeh *et al.*, 2022).

There are two ways *Lactobacillus* detoxifies mycotoxins found in food. *Lactobacillus* strains that produce certain enzymes can be used with the microorganisms' live cells to detoxify food. It has been used for a long time to protect food from fungi and other organisms that cause spoilage. Numerous bioactive metabolites produced by *Lactobacillus* can inhibit fungal development and stop the synthesis of mycotoxins in food. Acids, carbon dioxide, hydrogen peroxide, phenyl-lactic acid, and low molecular weight bioactive peptides are examples of *Lactobacillus* bioactive molecules. A wide variety of proteolytic enzymes, such as cell wall-bound proteinase, which hydrolyses proteins into polypeptides, peptide transporters, which convey peptides into cells, and a profusion of intracellular peptidases, which break down transferred peptides into amino acids, are produced by *Lactobacillus*. The most significant role that *Lactobacillus* proteolytic enzymes perform.

Another theory for how mycotoxins is removed from some foods is that they are adsorbable and are absorbed by the cell walls of *Lactobacillus* strains. The presence of polysaccharides, proteins, and peptidoglycans in the cell walls of *Lactobacillus* strains is related to this function. Wang and colleagues (2015) observed a decrease in patulin levels in culture media as a result of specific *Lactobacillus* binding activity. Because these strains have the most binding sites per unit area and cell wall volume, binding activity was enhanced by

utilising these strains. The author proposed that polysaccharides and proteins were the primary functional elements involved in patulin adsorption.

Mycotoxin starting concentration, *Lactobacillus* strain, number of lactobacilli, dietary complexity and pH, and incubation temperature are some of the variables that affect *Lactobacillus* cells' ability to bind mycotoxin. Reuterin, low-molecular-weight bioactive peptides, fatty acids, phenolic compounds, and fatty acids are examples of metabolites produced by *Lactobacillus* strains that interact with mycotoxins to reduce their levels in food. According to Nasrollahzadeh *et al.* (2022), these metabolites can bind to mycotoxins and lessen their toxicity. Many explanations have been put forth, including the interaction between mycotoxins and *Lactobacillus* metabolites, the degradation of mycotoxins by *Lactobacillus* enzymes, the adsorption of *Lactobacillus* cells, and the degradation activity of proteolytic enzymes and the binding of specific metabolites to mycotoxins.

Gram-negative bacilli

This family generally includes enterobacteria, which live commensally in the intestines of humans and animals and are also found in the environment, soil and water. Certain species are of interest as BCAs for mitigating mycotoxins in foodstuffs and groundnuts in particular.

Among all the Gram-negative soil bacteria, *Pseudomonas* spp. Is the most prevalent genus of Gram-negative soil bacteria in the rhizosphere. The name *Pseudomonas* (derived from the Greek words *pseuds*, meaning "false," and *monas*, meaning "a single unit" or "a false unit") encompasses one of the most diverse and ecologically adapted groups of bacteria on the planet, whose members are collectively referred to by the generic term *Pseudomonads*. Yang *et al.* (2017) demonstrated for the first time that *Pseudomonas fluorescens* strain 3JW1, which possesses broad-spectrum antibiotic activity, decreased the amount of aflatoxin B1 produced by *A. flavus* in groundnuts by up to 99%, as well as the amount of *A. flavus* growth, aflatoxin synthesis, and aflatoxin destruction. Other research has shown that *Pseudomonas stutzeri* YM6 isolated from marine sediments prevents mycofungal infection of groundnuts and maize during storage.

This *Pseudomonas* generates numerous volatile chemicals that can stop *A. flavus* conidia from germinating and growing mycelially. The inhibition rate was 100%. *Pseudomonas stutzeri* YM6 could therefore be considered an effective biocontrol agent for future applications. Other *Pseudomonas* isolated from the groundnut rhizosphere also showed aflatoxin and *A. flavus* destructor inhibitor activity in vitro and in the field, such as *Pseudomonas fluorescens* Pf7 and *Pseudomonas*

fluorescens CI, which have antifungal properties that can be used during groundnut preservation. It should be noted that other enterobacteria may behave as BACs of mycofungal due to the abundance and diversity of bacteria in this family (Chulze *et al.*, 2015).

Enzymes

An enzyme (protein nature) is a biological catalyst. It can intervene multiple times, staying intact after each event, and speeds up the particular metabolic reaction occurring in the cell. There are thousands of distinct kinds of enzyme molecules in a cell, each of which is unique to a certain biochemical reaction. With great specificity, enzymes are essential for the biotransformation of mycotoxins into innocuous products and, in mild circumstances, preserve the environment and the integrity of living things, even for the full detoxification of the toxin (Li *et al.*, 2020). Degradation mechanism optimisation and the development of mycotoxin reduction techniques therefore depend on the identification and characterisation of a degradation enzyme (DE). Compared to the employment of live microorganisms, enzymes provide predictable and constant performance, are easy to handle, do not pose a risk of contamination, and pose no safety concerns for the operators. Both in vitro and in vivo mycotoxin contamination is eliminated or reduced by a variety of enzymes.

However, their application is still quite limited because of the lack of knowledge on the possible hazardous effects of the products generated and their impact on the nutritional quality of food and feed (Boudergue *et al.*, 2009). A few commercial mycotoxin-degrading enzyme products are listed by Loi *et al.* (2017): Mycofix®, FUMzyme®, Biomin® BBSH 797, and Biomin® MTV (Biomin Holding GmbH, Getzersdorf, Austria). Of these, only FUMzyme® employs an esterase, a purified enzyme, to break down fumonisins. If used properly, enzymatic detoxification may be a useful method for most mycotoxins. Two criteria can be used to assess the effectiveness of the biodegradation process: (i) finding and identifying the transformed products, and (ii) testing the metabolites and intermediates produced by the process for toxicity. Moreover, because the enzyme's genetic sequence is known, molecular engineers can alter the enzyme's active region to increase its selectivity and catalytic efficiency. To help reduce the effects of mycotoxins, all of these perceptions need to be considered. By concentrating on the isolation of particular microorganisms, enhancing their growth, and establishing environments that support the mass synthesis of enzymes, the promising technique of biological degradation of mycotoxins can be further refined.

6.3. Mycotoxin Resistant Groundnut Variety

Resistance in groundnuts initially occurs at two barrier levels before the fungus reaches the cotyledons and draws its subsistence: the pod wall and the seed tegument, which are physical barriers (Pandey *et al.*, 2019). Microscopic fissures in the seed coat can arise due to heat stress and moisture during pod formation. On the other hand, fungal diseases can enter growing pods in the soil through the pod wall and seed coat when insects and nematodes cause damage to them. In response to fungal attacks, groundnuts produce stilbenic phytoalexins which inhibit mycofungi and other phytopathogens. Quantitative trait locus (QTL) mapping is one common technique for examining complex traits' genetic foundation. Many QTLs have been found in groundnuts recently for several significant features, including rust resistance, bacterial wilt, late leaf spot, drought tolerance, plant height, pod shape, and seed shape (Chen *et al.*, 2021).

As a component of marker-assisted selection, molecular markers that are strongly associated with QTLs have been found, verified, and used. A total of 12 QTLs for aflatoxin accumulation were detected by unconditional analysis, and four of them (qAFB1A07 and qAFB1B06.1 for AFB1, qAFB2A07 and qAFB2B06 for AFB2) exhibited major and stable effects across multiple environments with 9.32–21.02% PVE. Still, limited efforts have been made to identify QTLs for complex groundnut traits, such as aflatoxin contamination. Six QTLs for resistance to invasion by *A. flavus* have been detected in three independent recombinant inbred line populations (Yu *et al.*, 2019). These promising findings complement many extensive trait mapping projects being carried out at eminent research institutions like ICRISAT-India, the University of Georgia in the United States, the USDA in the United States, and the Oil Crops Research Institute of the CAAS in China. These studies use a panel of association mapping, two- and multi-parent genetic populations, and transcriptomics. Future improvements in groundnut resistance to mycotoxigenic fungi and other biotic and abiotic stressors may be greatly aided by recent developments in molecular biology and genomics of the plant. Table 3 shows some groundnut varieties with a level of resistance against toxigenic *Aspergillus*. Finally, the genetic resistance of groundnuts cannot by itself eliminate the problem of contamination of groundnuts by mycotoxins; it must be accompanied by good agricultural practices.

6.3 Antifungal Plant products

Unlike primary metabolites like proteins, carbs, and lipids, plants also contain "secondary" metabolites. These substances play a part in the interaction between the plant and the surrounding living things, even though their precise functions are yet unknown. These chemicals vary from species to species. As pollinators and disseminators as well as co-evolving species like diseases, parasites, and predators, they are most likely crucial components. Classified as terpenoids, alkaloids and phenolic compounds, and estimated at over 200,000, some secondary metabolite molecules have proven antifungal and antimycotoxic properties (Hussain *et al.*, 2012). Research has shown that essential oils of certain plants and bioactive compounds with antimycotoxic and antifungal properties in their extracts. Their use in agriculture inhibits the production of mycotoxins and toxin-producing fungi, with no harmful effect on the environment or consumers. Boldo, poleo, clove, aniseed, and thyme oils were shown to be possible antifungal candidates in screening tests using 41 aqueous and ethanolic extracts and 22 essential oils against strains of *Aspergillus* section Flavi (Perczak *et al.*, 2019). Other research, however, has demonstrated that smaller molecules, like monoterpenes, work better as volatiles in the headspace (Avila-Sosa *et al.*, 2011). Because of this property, essential oils are intriguing candidates to use as fumigants to preserve groundnuts and other agricultural products that are stored.

The antifungal impact of clove oil (50-100 µl/mL) on groundnuts was established by Duke *et al.* (2003). They showed that within three days, there was no new growth and that the *A. flavus* population was significantly reduced. Eugenol (89.8%), caryophyllen (4.7%), and vanillin (2.9%), the three components of clove oil, were found. Groundnuts' microbiological safety can be raised by using clove oil suspensions. The main ingredient in clove oil, eugenol, has also been the subject of extensive research to combat mycotoxigenic fungi and mycotoxins in rice grains contaminated with *A. flavus*. According to Reddy *et al.* (2010), 2.4 mg of eugenol per gram of rice completely inhibited the moulds and, consequently, the biosynthesis of mycotoxins. Garlic extract has been demonstrated in antifungal studies to be effective against toxic species of *Aspergillus* and *Penicillium* that are spread by oilseeds. Garlic allicin decomposes into many useful compounds that function as antimicrobial agents, including diallyl sulphide, allyl methyl trisulphide, dithiols, and E. Z-ajoene. Garlic may be useful in the fight against fungi that cause food to deteriorate because of its inhibitory effects on *Aspergillus* and *Penicillium* fungi. Water-based juices can also be used as an alternative to chemical solvents, which in some

cases might be harmful. To prevent fungal infections in stored groundnuts, garlic juice can inhibit fungal growth and be utilised as a source of antifungal compounds. Naseer *et al.* (2014) conducted a study on *Aspergillus* infection of groundnuts and found that neem, garlic, ginger, and onion leaf aqueous and alcoholic extracts were efficient in preventing *A. flavus*, which is spread by seeds. Betel leaf extract has been reported to significantly inhibit the growth of *Aspergillus flavus* at a concentration of 10,000 ppm. Likewise, essential oils derived from various species of *Ocimum basilicum* and *O. gratissimum* have demonstrated effective inhibitory activity against *Aspergillus*, *Penicillium*, and *Fusarium* species, while also reducing fumonisin production.

A global ethnobotanical study revealed that around 3,000 plant essential oils are recognised, of which 300 are commercially significant for the fragrance industry. Anise, calamus, camphor, cedarwood, cinnamon, citronella, clove, eucalyptus, lavender, lemon, lemongrass, lime, mint, nutmeg, orange, palmarosa, rosemary, basil, vetiver, onion, tea tree, vanilla, neem, garlic, ginger, green oregano, galangal, and wintergreen are a few of the essential oils that are frequently derived from plant materials. Across the world, they have historically been employed for a variety of purposes (Van de Braak and Leijten, 1999).

A study conducted in Nigeria yielded results from the collection of 280 plant species, of which 100 exhibited antifungal activity that directly impacted the growth of fungi or the generation of mycotoxins. Plant secondary metabolites are described by Duke *et al.* (2003) as a massive reservoir of bioactive phytochemicals, and their potential as biopesticides is still largely untapped. This demonstrates how effective plant use is turning out to be in mitigating the effects of mycotoxins globally, particularly in sub-Saharan Africa.

7. STRATEGIES OF BIOCONTROL

It is important to note that environmental conditions are the primary factors in mould contamination and mycotoxin production. Zhang *et al.* (2024) have shown that drought and hydric stress in plants are very favourable to mycotoxigenesis and that groundnut and corn are very sensitive to this toxigenesis. To reduce the extent of contamination by mycofungal species and protect the health of groundnut consumers, biological mycotoxin control strategies can be used at different stages of the agricultural production process. These include pre-harvest strategies or prevention strategies and post-harvest strategies or contamination strategies.

7.1. Pre-harvest strategies

Considering that groundnut is a geocarpic plant whose pods develop and ripen in the soil, a natural environment conducive to moulds, several studies have shown the presence of contaminating soil fungi in both healthy and rotten groundnut seeds (Ilunga *et al.*, 2022). Good agricultural practices (GAP) are frequently used in preventive pre-harvest measures. These practices include adequate watering, the use of groundnut cultivar mycotoxin-resistant or healthy seeds, and, in certain situations, the administration of biofungicides. Other important pre-harvest practices include crop rotation, using resistant varieties, managing irrigation and fertilisation, preventing insect damage, preventing overwintering, harvesting early, having enough moisture, and clearing away debris from previous harvests to stop the growth of fungi and prevent mycotoxins (Mishra and Das, 2003).

Preventive pre-harvest strategies also include the application of BCAs such as the non-toxicogenic *Aspergillus flavus* strains NRRL 21882, 18543 and 30797, which compete with toxigenic strains by preventing them from accessing the various receptor sites in the groundnuts. This is the principle behind the action of aflasafe (GH01) and Asafe (GH02), which are BCAs based on non-toxicogenic aflatoxin strains. Other BCA bacteria, apart from their ability to detoxify mycotoxins, can induce the plant's resistance mechanism to colonisation by mycotoxigenic fungi from certain bacterial molecules that trigger this mechanism at the plant level.

7.2 Post-harvest strategies

Fungal growth and activity are influenced by post-harvest storage conditions, which are crucial for controlling mycotoxin production. Mycotoxin generation and fungus development are facilitated by two main storage factors: high temperature and humidity. Reducing fungal growth and mycotoxin accumulation can be achieved through temperature control, ventilation, humidity management, and packaging procedures.

In this way, stored crops form part of the ecology created by man, encapsulating grains and preventing harmful insects, rodents and contaminating fungi. The use of essential oils, in particular cinnamon oil, clove oil and bay leaf oil, is effective in preventing the production of certain mycotoxins. Other microbial BCAs producing volatile antifungal compounds can be used. Disinfection with a biodesinfectant is required to eliminate insects and other parasites. The strategy can go as far as modified atmosphere storage, which has proved effective in controlling the proliferation of insects and moulds in stored groundnuts and cereals (Guchi, 2015).

Post-harvest, mitigating insect damage, processing, transportation, sufficient storage, drying right away after harvest, and appropriate handling are crucial. It is recommended that crops are dried in an environment with a humidity level of less than 14% to avoid the accumulation of mycotoxins. A humidity level of less than 14% is recommended to avoid the accumulation of mycotoxins (Mishra and Das, 2003). Enzymes are used to neutralise mycotoxins in foodstuffs during the manufacturing process. These applications are common in animal feed and are a promising way of preventing a health threat. The European Union has currently approved FUMzyme 32159. This is a purified enzyme for the biotransformation of fumonisins. The Mycofix 5. E range, used to decontaminate mycotoxins in feed for pigs and poultry, acts by adsorption or biotransformation of these toxins or by protecting the animal by stimulating its defence mechanisms against mycotoxins. BBSH 797 is added directly to cattle feed at a dose of 0.5 to 2 kg/t of finished feed (EFSA, 2017).

8. CONCLUSION

Mycotoxins are toxic secondary metabolites produced by fungi that pose significant threats to human and animal health while causing substantial economic losses in agriculture. Groundnuts and many other food commodities are highly vulnerable to mycotoxin contamination. Biological control offers a sustainable, cost-effective, and environmentally friendly alternative to synthetic chemicals for reducing mycotoxin contamination, particularly in developing countries where the burden is greatest. However, its successful implementation requires a better understanding of the interactions between biological control agents, host crops, and mycotoxigenic fungi. As biological control alone cannot eliminate contamination, it should be integrated with good agricultural practices (GAPs), effective monitoring systems, and farmer awareness programmes. Strengthened research, supportive policies, and coordinated actions by governments and development partners are essential to improve mycotoxin management and enhance food safety.

Future research should focus on identifying and validating effective indigenous biological control agents against mycotoxigenic fungi in groundnuts and other staple food crops. Further studies are needed to elucidate their mechanisms of action and evaluate their long-term efficacy under field and storage conditions. Integrating biological control with good agricultural practices, improved post-harvest management, and modern biotechnological tools will be essential for developing sustainable strategies to reduce mycotoxin contamination and improve food safety.

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