Importance of Aspergillus, Penicillium, Fusarium Genera and Contamination Control Strategies

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Abstract
Contamination of plant substrates with micromycetes from the genera *Aspergillus, Penicillium* and *Fusarium* is favored by the existence of optimal environmental conditions for their development, with negative consequences on production, animal health and food safety. The development of micromycetes causes a great loss of nutritional value and the production of extremely toxic metabolites - mycotoxins. The situation is very complicated when it is found that there are a multitude of secondary fungal metabolites. There is a problem with global food and feed safety, so we live with a certain degree of risk. Although the research effort has been immense in trying to delineate several aspects of mycromicetes and mycotoxin contamination, many questions remain unanswered. It is essential to carry out several investigative studies on this scourge for consumer safety, therefore the role of each manufacturer, control bodies and regulators should be paramount in the current mycological and mycotoxin situation, in order to obtain favorable results to facilitate the improvement of the quality control system of plant substrates, food and this desideratum can be achieved only through certain control strategies to prevent contamination.

Keywords: contamination, fungi, micromycetes, secondary metabolites.

Introduction

Micromycetes, fungi or molds are terms with identical etymological meaning, which can be used alternatively to characterize eukaryotic microorganisms, which do not have chlorophyll, are immobile, unicellular and / or multicellular. From a phenotypic point of view, they may have a yeast or filamentous appearance [1]. Regarding the classification at present, the living things of the biosphere are grouped in five kingdoms, such as: Monera, Protista, Fungi, Plantae and Animalia. The topic of our study includes a special group - fungi from the kingdom of Fungi, with two branches: Pseudomycota, respectively Eumycota [2]. Filamentous fungi occur widely in the environment and contaminate soil, air, food and other substrates. Due to the wide spread of these micromycetes, there are medical and economic implications. Most filamentous fungi produce metabolites associated with a number of health risks to both humans and animals [3]. It is also important to note that species of the genera *Aspergillus* and *Penicillium* are saprophytes becoming opportunistic pathogens and contaminating production sometimes but without associated diseases, and the one that produces the most different toxins is the genus *Fusarium* [4,5].

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Influential factors of micromycetes

Crops are exposed to fungal contamination both in the field before harvest and during storage in unsuitable conditions for longer periods of time, being a favorable environment for the development of fungi [5,6]. Fungi are an integral part of the microflora of crops and silage feed, respectively stored, but the production of mycotoxins is highly dependent on certain factors, such as: the presence of micromycetes/fungi, agricultural practices, the chemical composition of the forages, but especially the harvest conditions, handling and storage [7,8]. Humidity, relative humidity, temperature and mechanical damage are classified as physical factors; substrate composition, carbon dioxide, oxygen, pesticides and fungicides are included in the category of chemical factors while insects and stress include biological factors [9]. Depending on these factors, the amount of toxin in plant substrates depends. For cereals, the most important conditions are the temperature and activity of the water (the available water content in the substrate) to facilitate the production of mycotoxins [10] (Figure 1).

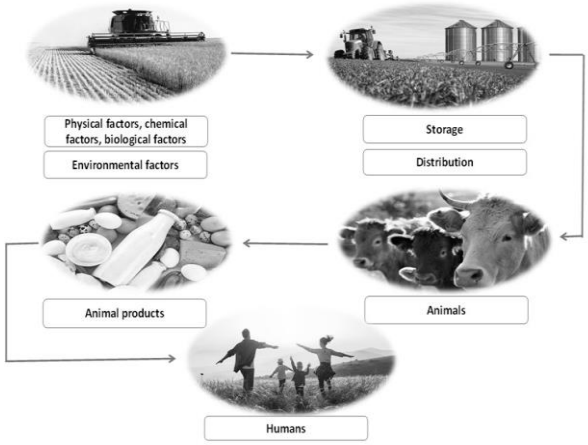

Figure 1. Factors influencing the feed and food chain

On the other hand, Teller R.S. et al. (2012) states that environmental factors include humidity, temperature, crop rotation, insect damage and some unapproved agronomic practices [11]. Plant stress is triggered by the above mentioned steps, which mainly favors the appearance of mold and contamination with mycotoxins [12]. Humidity is highly dependent on the water content of the grain at harvest, drying, aeration before or during storage, but also on the presence of insects and microorganisms [13]. Humidity does not increase if the cereals are dry before being stored. This increases if there is a high degree of condensation and leakage and thus would favor mold growth [14]. Also, high temperature, low rainfall and drought lead from a climatic point of view to aflatoxin formation [11]. However, the increased temperatures during the night would lead to mold growth and mycotoxin synthesis in the dark, as the plant has less resistance to fungal invasion, being deprived of energy [15,16].

The importance of the genus *Aspergillus* and secondary metabolites

The genus *Aspergillus* is the most studied genus of all and its widespread spores can contaminate nature, air, soil under certain conditions [17]. Rice, wheat, corn, barley, sorghum, soybeans, peanuts and black beans are among the areas susceptible to contamination with aflatoxin strains. Gruber-Dorninger et al. (2018) states that aflatoxins have frequently appeared in feed samples (maize, silage maize and cereals) in Africa in unsafe concentrations . Identified species such as *A. parasiticus, A. flavus, A. niger, A. ochraceus, A. oryzae* are those that would produce mycotoxins in these products [18,19] (Figure 2).

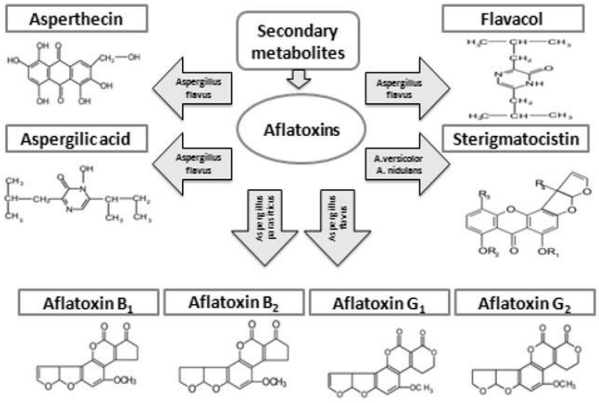

Figure 2. Chemical structures of mycotoxins produced by the genus *Aspergillus*

Aspergillus-specific micromycetes belonging to its taxonomy are of considerable importance both in ecosystems and in their pathogenicity. In terms of food, aflatoxin can be found in milk and ochratoxin in meat [20]. Aflatoxins M_1 are also transferred to milk from 1 to 6% of the food intake. These are the issues of greatest concern [12]. In the study by Gizachew D. et al. (2019), maximum AFB_1 production was observed at 27°C and 0.90 water activity in *A. flavus* and *A.*

parasiticus species. A study of feed administered to cattle in Belgium showed that certain strains of *Aspergillus* synthesize a mycotoxin called verruculogen [17]. Also, Belgium being a country where beer production plays a significant role, it must be remembered that the feed had malt residues. The fungal species was found to be *Aspergillus clavatus*. Traces of patulin were also detected in feed samples [21]. In Pakistan, several samples of both fresh and silage maize feed were subjected to mycological analysis. *Aspergillus flavus* and *Aspergillus niger* predominated the most, followed by *Aspergillus terreus* and *Aspergillus ochraceus*. In fresh fodder, aflatoxin B_1 and ochratoxin A were detected in a higher percentage and in a lower percentage in silage [22,23].

The importance of the genus *Penicillium* and secondary metabolites

Various studies have shown that the genera *Penicillium, Cladosporium* and *Aspergillus* were the most isolated. Elizondo-Zertuche et al. (2019) stated that indoor mushrooms greatly influence food contamination, so they assessed the fungal density of 20 food units, where 100 l of air were taken using Air Test Omega, the genus *Penicillium* being present in each sampled area [9]. Another study involved 124 farms in Sweden and Norway, where feed bales were examined. An analysis of the chemical composition of the fodder was ordered in each farm, but also the data regarding the production and storage system were important. The most common fungal species on the surface of the bale was *Penicillium roqueforti* [24]. Magan R.C. et al. (2015) studied the effects of some diseases (anorexia, rumen damage, posterior paralysis) on a number of 17 cattle, which were fed low concentrated feed. Laboratory samples grown in PDA environment, through their macroscopic and microscopic aspects led to the conclusion that the identified fungal species were *Penicillium citrininum, Aspergillus versicolor* and *Trichoderma harzianum* [25,26]. These authors believe that the reason for these diseases is mycotoxicosis caused by the aforementioned genera. Among the secondary metabolites of this genus are antibiotics and pharmaceuticals, a likely example being that of penicillin, an antibiotic widely used worldwide. Penicillin is produced by *Penicillium chryogenum*, and another equally important drug in the world is statins, which in turn are produced by *Penicillium citrinum*, their target being a lower level of cholesterol [27]. These *Penicillium* strains have the ability to produce chemical, toxic metabolites such as cyclopiazonic acid, penicillic acid, patulin, citrinin and especially ochratoxin A [28] (Figure 3).

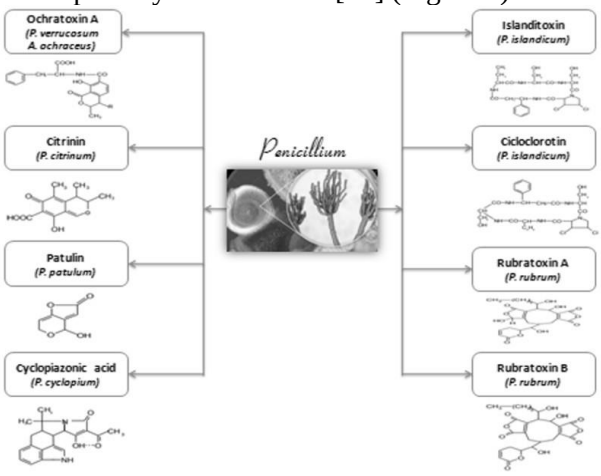

Figure 3. Chemical structures of mycotoxins produced by the genus *Penicillium*

Patulin is mainly found in rotten apples, but also in vegetables, fruits. It is mainly produced by *Penicillium* and *Aspergillus*, being stable at high temperatures, which suggests that it is thermally resistant. Patulin is known as an antibiotic against gram-positive and gram-negative bacteria, but it has also been found to be toxic and therefore no longer used as an antibiotic [29]. Fungi of the genus *Penicillium* are known as antibacterial agents against antifungals, many authors reporting the activity of *Penicillium* secondary metabolites against pathogens such as *Fusarium* [30].

The diversity and roles of the genus *Penicillium*

The diversity of the genus *Penicillium* is fundamental to the fungal lifestyle, and the members of this genus are among the most important groups of fungi studied [24,30]. *Penicillium* produces bioactive metabolites with antibacterial, antifungal, immunosuppressive properties, cholesterol lowering properties, with applicability in various sectors such as human health, industry and biotechnology [31]. *Penicillium* is a genus of ascomycete fungi, with an important role in various natural processes that are manifested in a wide and ubiquitous presence. *Penicillium* is one of the most common fungi that

occur in various environments such as soil, air, but especially temperature, salinity, water deficiency and pH [3]. *Penicillium* plays an important role in almost all environments, including the marine environment. The genus *Penicillium* is also commonly isolated from various terrestrial environments [31]. In one study, 96 species of *Penicillium* were detected in mud and sand in Korea, and *Penicillium* proved to be a decomposer of marine organisms, playing an important role in nutrient recycling and pollutant degradation [2]. In terms of *Penicillium* diversity, it was significantly higher in winter than in summer. In the winter season, the genus *Penicillium* may manifest as spores or may grow slowly. Due to this fact, only one species can no longer be dominant, which is hindered by this factor. On the other hand, nutrient deficiency can occur in winter [12]. *Penicillium* species in various environments produce a variety of enzymes such as alginase, cellulose, chitinase and protease [32].

The importance of the genus *Fusarium* and secondary metabolites

From a taxonomic point of view, species belonging to the genus *Fusarium* have always been a controversial topic. The genus *Fusarium* comprises a very large number of micromycetes, over 20 species, which have adapted to the most diverse pedoclimatic conditions [33]. The species of micromycetes belonging to this genus are ubiquitous microorganisms, being found in abundance in almost all environments [1]. In northern Italy, more precisely in the Emilia Romagna region, 51 maize fields that were cultivated in 2014 were subjected to research in terms of climate variability. The presence of the genus *Fusarium* was up to 46%, while the presence of the genus *Aspergillus* was up to 6.3%. Farmers face this problem - climate variability, which defines a strong impact on the appearance of fungi [33,34]. The most important fungal species that contaminate corn belong to the genera *Fusarium Discolour* and Liseola. The genus *Fusarium* is very common in regions with cold and humid climate, unlike the genus *Discolour* which predominates in warmer and drier areas [35]. Reyneri A. (2006) states that the cold season temperatures and high humidity favor the development of micromycete species belonging to the genus *Fusarium*. *Fusarium* species that are closely consistent with these contaminations are *Fusarium graminearum, F. sporotrichoides, F. poae, F. avenaceum, F. culmorum, F.accuminatum, F. verticillioides, F. proliferatum, F. oxysporum* and *F. paranaense*. *Fusarium graminearum* is the most common species when it comes to contamination [36]. The most common diseases in plant substrates are *Fusarium* Head Blight - FHB which causes enormous yield losses and low crop quality [37]. Toxic molds belonging to the genus *Fusarium* synthesize secondary metabolites, such as *Fusarium oxysporum, F. culmorum, F. roseum* and *F. graminearum*. Fumonisins, zearalenone, trichothecenes, deoxynivalenol and nivalenol are the most common groups of mycotoxins that belong to the category of the genus *Fusarium* [38]. Different fungal species generate fumonisins. Currently, 28 types of fumonisins have been identified, which are divided into four, fumonisins A, B, C and fumonisins P, but the most important group of fumonisins is group B, which contains fumonisins B_1, B_2, B_3, [39,40] (Figure 4).

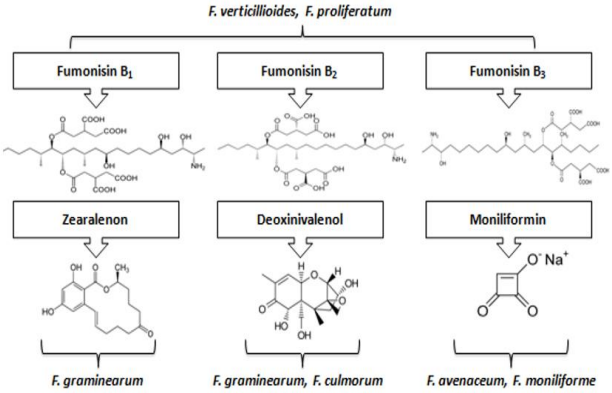

Figure 4. Chemical structures of mycotoxins produced by the genus *Fusarium*

Fumonisins are produced by *Fusarium verticillioides* and *Fusarium proliferatum. Fusarium verticilioides* grows in both vegetative and reproductive tissues of maize for example, but often without certain symptoms. Depending on the weather conditions, the presence of damaged insects, the genetics of fungi, plants, defects in seedlings and rot can occur [41]. Trichothecenes are also classified into four types from A to D. Types A and type B are the most common and occur widely in cereals. They are produced by several fungal genera, such as *Fusarium, Trichoderma, Myrothecium, Trichothecium, Stachybotrys* and *Cephalogensporium*.

Trichothecenes have been identified in Africa, where they have been present in feed. They are found in feed such as corn, wheat and oats. They are known to inhibit protein synthesis and are resistant to heat [42]. Deoxynivalenol (DON), also called vomitoxin, is the most detected trichothecenes in wheat, barley, oats, rye and corn [43]. In several countries around the world, several metabolites belonging to the genus *Fusarium* have often appeared in medium concentrations in feed samples (corn, silage corn, cereals), such as moniliformin, aurofusarin or in higher concentrations - fusaproliferin. These secondary fungal metabolites were determined using a method based on mass spectrometry in tandem with liquid chromatography. *Fusarium* mycotoxins have appeared in concentrations that exceed the limits of European legislation [44].

Mitigation of mycotoxin contamination

All stored foods are a substrate suitable for mold development and mycotoxin production. If there is a type of mycotoxin in a feed, it is difficult to reduce and focus on maintaining compound stability. Therefore, it is essential to have care policies in place to allow the use of pollutants. The solution is an obvious dilution of uncontaminated feed. The detection of the degree of contamination, the dilution obtained and its availability for an uncontaminated food, however, addresses the progress [45]. This authorization is used in a variety of countries.The appearance and impact of mycotoxins are in close accordance with crop biology, fungal ecology, harvesting methods, storage conditions, feed processing and control strategies. The following general strategies for reducing mycotoxin contamination in the human food chain also relate to the food chain: genetic engineering of fungi and crops, stages of agronomic and biological control, climate modeling to predict mycotoxin risk, storage management, food processing, detoxification, integrated mycotoxin management, human involvement [46]. At present, there are several methods, modern strategies for combating mycotoxins, such as the use of gold or silver nanoparticles in plants [47], chitosan-coated nanoparticles, but also essential oils [48]. At EU level, the Rapid Alert System for Food and Feed (RASFF) plays a very important role, as it monitors the contamination of food and feed by mycotoxins on a weekly basis. With the help of RASFF, all European Member States are informed about the measures that can be taken to ensure the safety of food and feed [49].

Control strategies

Good agricultural, manufacturing, hygiene and storage practices should be closely aligned with the development of a HACCP program. A first step could be to improve plant cultivation, limit insecticides and fungicides, irrigate to prevent drought and last but not least harvest at maturity. Genetic resistance also plays an important role in resisting the plant to fungal attack [50]. It is recommended that good agricultural practices (GAP) be applied at the same time as good manufacturing practices (BPM) to facilitate the proper performance of critical control points (HACCP) by specialized personnel [51]. Among those listed above, we also mention good storage practices (GSP) in mitigating mycotoxin contamination [48]. Mycotoxin contamination could be reduced in the production chain sector through integrated management, which involves the application of critical control points (HACCP). HACCP is a food surveillance system in which safety comes first, through an analysis, control and monitoring of physical, chemical and biological hazards from the production, supply and handling of raw materials to the production, distribution and consumption of the finished product. The HACCP system is based on 7 principles, among which we mention: identification and analysis of hazards, determination of critical control points, establishment of critical limits for each critical point, establishment of a monitoring procedure, establishment of corrective actions, establishment of HACCP plan verification procedures, establishment documentation and record keeping [50,51] (Figure 5).

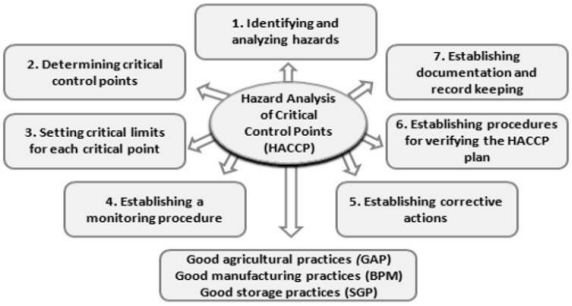

Figure 5. Control strategies in the production chain sector

Biological control of decontamination

"Biological decontamination" means the enzymatic transformation of mycotoxin into a less toxic compound. This type of decontamination seeks the degradation of natural mycotoxin with various microorganisms or enzymes. Identifying the degradation potential of microorganisms is the first step in developing processes [52]. This decontamination activity should be transferable to products contaminated with mycotoxins. Degradation of ochratoxin A by biological methods was observed during the silage process. Ochratoxin A present in barley can be degraded by existing microbial flora. OTA can also be degraded by bacteria present in wheat eaten by cattle, making these animals more resistant to mycotoxins than monogastric animals [53]. Ochratoxin A is hydrolyzed into ochratoxin and phenylalanine or esterified into ochratoxin A. Natural enzymes also have the ability to degrade ochratoxin A. Degradation of ochratoxin A in various unidentified products has been shown in wheat, barley or maize suspensions. These enzymatic transformations involve hydrolysis, methylation reactions that sometimes contribute to the loss of the toxic potential of molecules [54]. Moreover, the use of antagonistic fungi that can be considered biological control agents is an important strategy that is applied before harvest to prevent mycotoxin contamination in cereals [52]. Bacteria, yeasts and fungi are microorganisms that would promote the degradation of mycotoxins in food and feed being widely used. For example, among biological control agents against aflatoxin contamination are strains that do not form aflatoxin of *Aspergillus flavus* plus other nonoxygenic molds [51]. The use of biocontrol agents has emerged as a pre-harvest method, leading to a steady reduction in aflatoxin contamination from 70% to 90%, both in laboratory and field studies [50]. The use of non-toxic strains of *A. flavus* and *A. parasiticus* (fungi) has been very successful in reducing aflatoxin contamination, also micromycetes of the genera *Aspergillus, Rhizopus, Trichoderma, Clonostachys* and *Penicillium* have shown highly effective abilities to detoxify mycotoxin [53]. It was found that bacteria with lactic acid, propionic acid can inhibit the increase in the production of fungi and mycotoxins [55].

Physical control of decontamination

Physical methods of decontamination can be classified into two categories: methods that lead to the elimination of altered fractions and methods that cause the denaturation of toxins [56]. Regarding the elimination of the altered fractions, the altered parts of the grains are removed. These methods, often very simple, are important because the distribution of toxins in cereals is not homogeneous, being usually located only in a fraction of the crop [57]. Grinding, polishing, vacuuming of light fractions are methods applicable especially to cereals and are effective methods because mycotoxins grow mainly on the surface of cereals [58]. Separation by flotation and washing by water treatment can eliminate the content of aflatoxin in the proportion of 39-42% (30-38% is found in bran, 13-17% in gluten, 6-10% in germs and 1% in starch) [42]. Toxin denaturation processes refer to the physical treatment by which the complex mechanisms of degradation are based on the dehydration of mycotoxins or the intervention of radical reactions. These methods have certain disadvantages, as they leave degradation products in the raw material, which can be extremely toxic, leading to changes in the nutritional value of the raw material [55]. Heat treatments applied to mycotoxins have different efficacy depending on the nature of the mycotoxin. Thus, aflatoxins are resistant to thermal degradation and are not completely destroyed by hot water treatment, autoclaving or other thermal processes applied to the products. In contrast, ochratoxin A is partially sensitive to heat treatments performed without water [57]. It has also been found that exposure of aflatoxin-contaminated peanut oil to UV radiation reduces its contamination. Instead, exposure to X-rays to reduce mycotoxin contamination destroys food quality [51]. In conclusion, physical distortion of toxins cannot be proposed as a systematic method of decontamination. However, different treatments (especially heat) are applied to the raw material or food during storage or manufacturing process. In terms of physical treatment, drying, washing, cleaning, boiling, frying, irradiating and peeling are used for mycotoxin decontamination [39]. Physical control requires thorough cleaning to remove general dirt. It may also involve washing with sodium carbonate solution or water. These treatments may

partially reduce the toxicity of the products, but should in no case be considered as "restoration" techniques of the contaminated raw material. [58].

Chemical control of decontamination

Like physical methods, chemical methods can leave mycotoxin residues in the treated products and their non-toxicity must be carefully checked. The method used depends on the specific structure of the degradation compound and the mycotoxin family [59]. Aflatoxins can be degraded by treatment with strong acids or bases, but the disadvantage of these methods is that acids or bases cannot be used directly in plant products. An additional effect can be obtained by combining these treatments with alkaline or acidic pressure and temperature, a technique called ammonification and nixtamalization [60]. Treatment with oxidizing or reducing agents involves the use of sodium or potassium bisulphite, as well as hydrogen peroxide, which leads to lower concentrations of aflatoxin [61]. The use of ammonia can be done in the plant for the decontamination with ammonia of mycotoxins using the process of high temperature/high pressure, the mobile unit for decontamination with ammonia of mycotoxins being able to process on site using the process of high temperature/high pressure. Also, under conditions of ambient temperature/atmospheric pressure, ammonia can be used to decontaminate mycotoxins. It is in the form of aqueous ammonia which is sprayed into the bags in which the grains are stored. These methods reduce the content of mycotoxins, but have the disadvantage that they are degradation products whose toxicity must be assessed, and the treatments applied can alter the nutritional value of cereals [57, 62]. Also, nixtamalization is the process by which dried corn grains are soaked and boiled in an alkaline solution, usually water with calcium hydroxide, to separate the transparent outer shell, the pericarp, from the grain. This process has several advantages, including the fact that the grains are better ground, increase the content of proteins and vitamins, improving the aroma and reducing mycotoxins [56]. Nixtamalization significantly reduces (by 90-94%) the mycotoxicity caused by Fusarium verticillioides and Fusarium proliferatum, molds that infect corn and whose toxins are potential carcinogens. The addition of oxidizing agents, such as hydrogen peroxide, has been shown to be an effective aid in the nixtamalization process. These chemicals degrade aflatoxins and fumonisins, reducing their toxicity [59,61]. Sulfur dioxide (SO_2) is one of the oldest food additives and has a long history as a disinfectant by burning elemental sulfur and using the resulting smoke. It is commonly used as a fungal inhibitor. Treatment of wet corn (24% m^3) with 0.3% SO_2 led to a significant decrease in microbial colonization and virtually no effect of grain quality change. Studies have shown that for a medium-term storage of cereals for 5 months, at least 4.4% SO_2 g / kg-1 is required for efficient storage [62]. Some researchers have shown that much higher concentrations may be required due to adsorption and binding of SO_2 to wheat grains, reducing antifungal activity. Wheat moisture has been shown to influence the decontamination process, while between 20-30% SO_2 gas binds to cereals during treatment. Thus, at an intermediate humidity of cereals (15-19%) the treatment may be more effective than in the case of wet grains (>20%), because a lower level of treatment will be bound to the substrate [63].

Mechanical control of decontamination

Mechanical control it involves the use of crop rotation, cultivation and harvesting techniques at the right time and in the right seasons and reducing plant stress. To prevent the harmful effects of mycotoxins on animals and human health, the prevention of fungus in the field is paramount. Weeds or agricultural residues should be greatly reduced to avoid contamination [57]. The growth of micromycetes is also affected by certain factors during storage. Controlled storage in all respects (ventilation, humidity, temperature, packaging) would greatly reduce fungal growth and mycotoxin production [61]. Successful control strategies should include approaches that control toxins throughout the production chain both before harvesting, on the farm, and after harvesting and storage, traceability being extremely important [64].

Conclusions

Micromycetes of the genera *Aspergillus*, *Penicillium* and *Fusarium*, respectively metabolic products have always been a significant risk in

terms of health and welfare of humans, animals and a significant problem for food safety. The situation is greatly complicated when it is found that there are a multitude of secondary fungal metabolites. Most metabolites are not tested for toxicity associated with disease outbreaks or reduced animal productivity. However, animal producers and the feed industry produce good, safe products, with continuous supervision. In addition, food safety management systems such as HACCP, GAP and Good Manufacturing Practices (GMP) should be an integral part of all stages of production, transport and storage, in order to minimize contamination in the industry grocery shop. In conclusion, it can be emphasized that physical or chemical decontamination techniques and the total eradication of mycotoxin contamination through these pathways are difficult and the effectiveness of the method varies from case to case. To date, we can say that the ammonia treatment of raw materials with or without the use of a group of solid soluble aluminosilicates has a particularly important role in the decontamination of aflatoxins. As for the other category of mycotoxins, no physical or chemical method can guarantee a complete decontamination. The elimination of mycotoxins by physical methods, by purifying and separating the raw material, is of particular interest. The most effective means of reducing contamination with trichothecene, zearalenone, ochratoxin and patulin remain the preventive methods, namely the reduction of humidity in storage spaces and in the field. Proper storage (humidity, temperature and insect control) and the addition of antifungal agents can reduce fungal growth, but cannot detoxify contaminated samples.

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