Review paper
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CURRENT DEVELOPMENTS IN REMOVAL OF MYCOTOXINS BY BIOLOGICAL METHODS AND CHEMICAL ADSORBENTS

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Abstract

Mycotoxins are one of the greatest and significant problems that must be taken under control in order to provide food and feed safety. One of the methods to be followed in controlling mycotoxins by microorganism is to provide mould strain, not a toxin producer, to replace with mould, natural toxin producer. Other method is to remove mycotoxins adding some specific microorganisms or chemicals such as in the last few years, most studies related to the alleviation of mycotoxicosis by the use of adsorbents are focused on aluminosilicates (mainly zeolites, hydrated sodium calcium aluminosilicates (HSCAS), and aluminosilicate-containing clays), and esterified glucomannan (EGM) derived from cell wall of *Saccharomyces cerevisiae* and calcium montmorillonite into product. Biotransformation of mycotoxins by live microbes and their enzymes and bioprotection are two new strategies for mycotoxins removal.

***Key words**: Mycotoxin, biological control, Flavobacterium, Lactobacillus.*

1. Introduction

Mycotoxins are secondary metabolites produced by fungi. They are produced under favourable environmental conditions. Mycotoxins are chemically stable toxins, resistant against high temperature and pressure and resist feed and food processing conditions and many factors such as: weather conditions, moisture, humidity, farming, shipping conditions and processing.

Mycotoxicoses in animal and human can occur with toxin concentrations below detection limits. There are also masked mycotoxins that have synergistic effects. They have immune suppression, hematopoietic, hepatotoxic, nephrotoxic, carciogenic, pathological and gastro-intestinal effects in animals and humans. The most hazardous mycotoxins are aflatoxin (AF), deoxynivalenol (DON), fumonisin, ochratoxin, trichothecenes and zearalenon (ZEN). We should worry about aflatoxin B_1 in animal feeds because it is carried over aflatoxin M_1 into the milk and has possible carcinogenic effect in human. Mycotoxins are also carryed-over into blood and muscless which leads to their presence in animal products as residues. Vegetable, fruits, food, feed and drinks are exposed to mycotoxin formation in the event of appropriate conditions during whole period from harvest to consumption. Consumers do not have a remarkable role in mycotoxin formation except in several circumstances.

Mycotoxins are "secondary metabolites" that moulds, biological factors, are to generate in product as a result of their own development; so, they are classified as "chemical threat". Natural mycotoxin formation depends on that weather the mycotoxigenic mould will contaminate product and then develops just before the harvest. Since it is too hard to protect against this kind of mould contamination, natural mycotoxins are considered to be "unavoidable threats".

Important issues which should be taken into consideration in choosing the method to be followed to control and protect mycotoxins are that this method/s should:

- Be economically and technically appropriate and applicable;
- Not cause remarkable change in nutritional value of food material and to generate more toxic compounds;
- Not release toxically and healthily hazardous residues.

In this paper are presented effects of microorganisms which are keeping mycotoxins under control and the current developments related to mycotoxin bonder chemicals. According Köhl *et al.* [8], the use of many of the available physical and chemical methods for the detoxification of agricultural products contaminated

with mycotoxins is restricted due to problems concerning safety issues, possible losses in the nutritional quality of treated commodities, coupled with limited efficacy and cost implications. Two different methods are followed to take mycotoxins under control using microorganisms. In the first one, toxin formation especially just before harvest is controlled by vaccinating mould strains, not toxin producer, into soil or dead plant pieces. In the second one, some specific microorganisms are vaccinated into product within mycotoxin; that way the mycotoxins in product are removed.

2. Controlling Mycotoxins by Non-Toxic Mould Strains

The fact that mycotoxins are to be taken under control by "bio-control based on biological competition" method finds application area for itself on products such as peanut, cotton and corn in which mycotoxin formation has been monitored especially just before harvest. Mycotoxin contamination of seeds is mostly caused by inadequate storage conditions of harvested crops However pre-harvest contamination of the seeds can also occur especially with *Fusarium* spp. producing zearalenone, trichothecenes and fumonisins, while other fungal contamination can produce ergot alkaloids, tremorgen mycotoxins and aflatoxins. Pre-harvest interventions include production of genetically enhanced resistant crop, application of good agronomic practices, harvesting crop at the optimum stage of development, biocontrol methods (e.g. use of atoxigenic *Aspergillus flavus*) and chemical methods.

For example, one method is application *Aspergillus parasiticus* strain in soil. This strain from one hand is non-toxin producer and from other hand is very competitive. That way, the mould becomes dominant soil microflora, and takes the place of natural aflatoxin generator. The same applies for *A. flavus and A. parasiticus* strain. Thus, peanut exposed to drought stress at the end of season is exposed to attack of dominant competitive mould. On the other hand, aflatoxin does not occur in product or occurs in a acceptable levels, because added mould does not form toxin. Similarly, non-toxic *Aspergillus niger* liquid and ochratoxin as solid nutrition decompose A (OA). *A. niger* releases carboxypeptidase and it decomposes OA into ochratoxin ά and phenylalanine [12]. It has been determined that this method is most applicable for removing OA in solid materials such as green coffee beans and grains. It has also been determined that there has been a decrease from 86% to 0% in *Fusarium* infection by using competitive moulds such as *Geotrichum candidum* as starter culture during beer manufacturing.

2.1 Removal of Mycotoxins by Microorganisms

Toxin is removed from a product by microorganisms such as bacteria, yeast, mould and a protozoan, in which mycotoxin has been formed. Remarkable developments and successful results have been obtained related to this method in recent years and it has gained value as an applicable and promising method in removal of mycotoxins. Mechanisms in removal of mycotoxins by microorganisms are still investigated, and it has been determined that effective parameters are: microorganism type (cell and components) and concentration, acidic or basic characteristics of the product and mycotoxin characteristics [6, 11 and 12]. Moreover, yeast supplementation can inhibit pathogenic bacteria and increase the number of anaerobic and cellulotic bacteria [1] and in addition, Celik *et al.*, [1, 2] reported that yeast culture (*Saccharomyces cerevisiae*) additives reduce the toxic effects of alfatoxin.

A variety of chemical, biological and physical strategies have been developed to: control the mycotoxigenic pathogens, minimize mycotoxin production at pre- or post - harvest level, contribute to decontamination and / or detoxification of mycotoxins from contaminated foods and feeds, or to inhibit mycotoxin absorption in the gastrointestinal tract.

Biological control using microbial antagonists either alone or as part of an integrated control strategy to reduce pesticide inputs, has emerged as a promising approach for control of mycotoxins in crops, both pre- and post-harvest [3]. The first microorganism is *Flavobacterium aurantiacum* [11] which is reported to remove aflatoxin from a solution. *F. aurantiacum* (NRRL B - 184) can decompose aflatoxin B_1 in both solution and various products such as: corn and corn oil, peanut cream and soybean [10]. Bacteria metabolize aflatoxin and can transform it into decomposition products which can be dissolved in water and chloroform, and CO_2. Some researchers like Smiley and Draughon [11], have stated that dead bacteria cells can also bond some aflatoxin but can not decompose it in a more advanced level. Researchers have also stated that number of bacteria must be almost $1x10^{10}$ CFU in one millilitre since an effective decomposition may occur. It has also been considered that cell proteins of *F. aurantiogriseum* bond AFB_1 and so mechanism may also be enzymatic [4]. Some lactic acid bacteria can also remove mycotoxin from liquid medium. For example, according Lahtinen *et al.* [9], *Lactobacillus rhamnosus* GG (LGG) strain has been determined to be the most effective microorganism to remove AFB_1 and zearalenon from liquid medium (it has also been reported that bonding of AFB_1 has occurred outside the cell physically on a study executed within viable and heat-treated bacteria). Treated within acid medium, inner cell bonding has occurred. AFB_1 bonding feature

of viable LGG strain has been analyzed and it has been reported that bonding is physical and peptidoglycan or elements covalent bonding to peptidoglycan have remarkable role in bonding AFB_1. It has been declared that carbohydrates such as teichoic acid on cell wall, exopolisaccaharides, and proteins such as Ca^{+2} or Mg^{+2} do not have a role in aflatoxin bonding.

Aflatoxin is not the unique mycotoxin removed from agricultural products by microorganisms. For example, trichothecenes are also removed by *Lactobacillus* and *Propionibacterium* [5]. However, researchers have stated that in vitro trichothecen bacteria bonding have remarkable differences.

There are many studies related that microbial flora in digestive system (rumen also included) of mammals such as sheep and cow, and caecum in rats and microorganisms in large intestine can decompose OA [7]. Similarly, intestine microflora of humans also decomposes OA partially.

Yeasts are another microorganisms absorbing mycotoxin especially from *Saccharomyces cerevisiae* medium. *S. cerevisiae* cell wall fractions are in 13.3 - 25% of total cell dry weight and it contains various substances such as glucan, mannan and chitin. It has been determined that mannan does not have a role in complex (the one bonding toxin) formation. The main molecule among elements forming cell wall, providing ZEN absorption is β- D glucan. The fact that clay content is high, limits absorption of ZEN by β- D glucans. Complex formation mechanism in ZEN is related to weak non-covalent bonds. Therefore, chemical relation between β- D glucans and ZEN is in absorption type rather than bonding. β- D glucans in yeast cell and their alkali-extractable fractions are the most suitable structures, increasing ZEN absorption efficiency [10]. Various bonding agents bond aflatoxin being added in feeds, thus aflatoxin amount absorbed by body declines. Calcium montmorillonite clay (HSCAS) is a powerful agent bonding AFB_1 and it does not create a negative effect that it is added in chicken feed in maximum 0.5% w/w level.

3. Conclusions

- Many bio-control agents have been tested in laboratory and field experiments to effectively reduce colonization and mycotoxin contamination by *Aspergillus*, *Fusarium* and *Penicillium* species in the hot and humid regions where with significant problems with mycotoxins in several crops. Therfore, atoxigenic fungal strains are being widely used to prevent pre-harvest especially for AF contamination of crops such as: maize, cottonseed, peanuts, paprika by *Aspergillus* spp. in hot rainy parts of the world.
- Recent advancements in the use of biocontrol strategies involving atoxigenic strains has led to registration of commercial products with increased practical applications for the benefit of growers. Molecular approaches and genetic, aimed at preventing mycotoxin biosynthesis have not yet reached commercial application in the field and require substantial further development in agricultural sector.
- More researches are required to evaluate the potential efficacy of various biological agents, including studies focusing on the dose, formulation and timing of the applications as well as molecular studies that elucidate impacts of the biocontrol agents. On the other hand, all the biocontrol agents or GMOs that are planned to be developed for the control of mycotoxigenic fungi have to pass all the necessary safety tests and stages in order to be safe for the human and livestocks.
- Prevention strategies at the post-harvest stage can only be effective for those mycotoxins that are formed during this stage of the food and feed production. Natural fungal contamination that occurs pre-harvest can only be minimized post-harvest by application of processing techniques which will minimize subsequent entry into the food and feed chain where possible by inhibition, detoxification or degradation of the mycotoxin.
- We can say that feed munufacturers and food precessors have to develop their mycotoxin risk mananagement plans and strategies that involve routine screening, guidelines and using mycotoxin preventitators and binders in feeds.

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