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Mycotoxin management through transformations – A review

Archana Negi*, Roopali Sharma, Nandani Shukla, Sourav Kumar Modak, Manjari Negi and Bhupesh Chandra Kabdwal

Department of Plant Pathology, College of Agriculture, G.B. Pant University of Agriculture and Technology, Pantnagar- 263145, U.S. Nagar, (Uttarakhand), INDIA

*Corresponding author. E-mail: archanagbpuat.patho@gmail.com

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Abstract: Mycotoxins are toxic secondary metabolic products of various fungi, mainly belonging to the genera *Fusarium* (Trichothecenes, Zearalenone), *Aspergillus* (Aflatoxin) and *Penicillium* (Ochratoxin) and can be found in almost 25% of the world's agricultural commodities. These compounds are toxic to humans, animals and plants and therefore, efforts should be made to avoid mycotoxin contamination in food and feed. It has been estimated that at least 300 of these fungal metabolites are potentially toxic to animals and humans. In India 50% losses of agricultural commodities are due to postharvest losses. A number of physical and chemical approaches have already been taken to reduce the effect of mycotoxins, but due to certain limitations of physical and chemical strategies prompted search for other solutions to the mycotoxin hazards. Thus, there is an increasing public pressure for a safer and eco-friendly alternative to control these organisms. Consequently, a new approach is applied for managing mycotoxins through transformations that offer specific, efficient and eco-friendly way for detoxification of mycotoxins. This review aims to bring about the up-to-date management strategies mainly through transformation (genetic and bio) to prevent or reduce post harvest damages to the crops caused by storage fungi and the contamination of food and feed by mycotoxins. It will make aware of the new technologies or management methods for mycotoxins through transformation. The transformation methods may become the technology of choice, as they offer a specific, irreversible, efficient and environment friendly way of detoxification that leaves neither toxic residues nor any undesirable by-products.

Keywords: *Aspergillus flavus*, Mycotoxin, Ochratoxin, Trichothecenes, Zearalenone

INTRODUCTION

Mycotoxins are a group of highly toxic secondary metabolites of the fungi mainly belonging to genera *Fusarium*, *Aspergillus* and *Penicillium*, produced under certain favourable environmental conditions, such as temperature, humidity, pH, atmospheric gasses, photoperiod light intensity and water availability. The temperature range usually reported for fungal growth is broad (10-35°C), with a few species capable of growth below or above this range. In general, the optimum temperature for mycotoxin production is below the optimum for growth. Moisture requirements of food-borne moulds are relatively low; most species grow at a 0.85 *aw* (water activity) or less, although yeasts generally require a higher water activity. 0.60 *aw* is considered the limit for cell growth, but spores of *Aspergillus* and *Penicillium* for example, are able to survive at lower *aw* for several years (Carlile and Watkinson, 1996). The acid/alkaline requirement for growth of all yeasts and moulds is quite broad, ranging from pH 3 to above pH 8, with optimum around pH 5, if nutrient requirements are satisfied. In general, *Aspergillus* species are more tolerant to alkaline pH while *Penicillium*

species appear to be more tolerant to acidic pH (Wheeler *et al.*, 1991).

According to ICAR (2012) 50% losses of agricultural commodities is due to post harvest losses in India. Among which maximum post harvest losses is for fruits (18%), for vegetables (12%), for oil seed (10%) and for pulses and cereals it is 6%. It has been estimated that at least 300 of these fungal metabolites are potentially toxic to animals and human. Human food can be contaminated with mycotoxins at various stages in the food chain (Bennett and Klich, 2003). Factors contributing to the presence or production of mycotoxins in foods or feeds include storage, environmental, and ecological conditions. Often times most factors are beyond human control (Hussein and Brasel, 2001). A wide range of commodities can be contaminated with mycotoxins both pre- and post-harvest (CAST, 2003). The wide range of effects and losses due to mycotoxins make their management and prevention of contamination in food products necessary. A number of physical and chemical approaches have already been taken to reduce the effect of mycotoxins, but due to certain limitations of physical and chemical strategies prompted a search for other solutions to the mycotoxin haz-

ards. Consequently, a new approach is applied for managing mycotoxins through transformations by using genetic engineering and biotechnological techniques (genetic transformation) and; microbes and enzymatic detoxification (bio-transformation) that offer specific, efficient and eco-friendly way for detoxification of mycotoxins that leaves neither toxic residue nor any undesirable by-product.

According to FAO 25% of the world's crops are affected by mycotoxins with annual loss of 1 billion metric tons of food and food products. In India 50% losses of agricultural commodities are due to post harvest losses.

Government of India, Ministry of food processing industries (2016): As a quarter of agricultural products loss is due to mycotoxin contamination and with the knowledge that mycotoxins can have serious effects on humans and animals has led many countries to establish regulations relating to mycotoxin in food to protect the consumer from harmful effects of these compounds. First time the limit is set for aflatoxin in late 1960's but by the end of 2003 more than 100 countries had developed specific limits for mycotoxins in food and feed. According to FAO (2013) 25% of the world's crops are affected by mycotoxins with annual loss of 1 billion metric tons of food and food products. In United States losses associated with deoxynivalenol were estimated at \$655 million/year, with the majority of the losses in wheat (APS, 2013). Losses due to aflatoxins in three Asian countries (Indonesia, Philippines, and Thailand) were estimated at \$900 million annually (APS, 2013). Diversity in the toxic effects and the synergistic properties of mycotoxins has made it mandatory to consider them as "risk factor" to humans and in livestock health (Yiannikouris and Jonany 2002; D'iaz-Llano and Smith 2006). Concerns over health effects of mycotoxins are being seriously considered worldwide. Mycotoxicosis is the general term given for the disease that is caused by mycotoxins (Nelson and others 1993).

Implications of mycotoxins: Mycotoxins produce adverse effects on agricultural commodities, humans and animals and also may have role in pathogenicity.

Contaminate agricultural commodities (crop plants, fruits, seed and grains): they may cause qualitative as well as quantitative loss.

Impact on humans and animals: mycotoxin infected agricultural commodities causes mycotoxicosis when consumed by animals and humans. A well known example of mycotoxicosis is ergotism that occurs due to consumption of mycotoxin (ergopeptine and clavine)

Table 1. The Post-harvest losses is as under.

Crops	Cumulative losses (%)
Cereals	4.65-5.99
Pulses	6.36-8.41
Oil seeds	3.08-9.96
Fruits and Vegetables	4.58-15.88

contaminated rye.

Mycotoxin may be phytotoxic: They may have potential role in pathogenesis. Aflatoxin produced from *Aspergillus flavus* and *A. parasiticus*, pathogenic on a variety of plant species are reported to be phytotoxin and may have potential role in pathogenesis. Trichothecens (deoxynivalenol) are produced by destructive pathogen *Fusarium* that attack wide range of plant species. There is acute phytotoxicity of trichothecens in plant tissues.

According to Joint Expert Committee on Food Additives (JECFA) which is a scientific advisory body of FAO and WHO (that provide mechanism for assessing the toxicity of food additive), mycotoxins which are currently considered most significant are- Aflatoxin, Ochratoxin A, Zearalenone, Fumonisin, Trichothecenes (deoxynivalenol), Patulin.

Aflatoxin: It is the first mycotoxin to be reported in 1960 when death of thousands of turkey occurs due to consumption of contaminated peanuts, meals (Turkey 'X' disease). These are produced by *Aspergillus* species especially *A. flavus* and *A. parasiticus*. Frequently affected cereals, oil-seeds, various spices.

There are 24 types of aflatoxin but only four are found in food stuff i.e. aflatoxin B₁, B₂, G₁, G₂. Aflatoxin may also be found in the milk of animals that feed on aflatoxin contaminated food (aflatoxin M₁ and M₂). Toxicity of aflatoxin is due to epoxide ring present at 8-9 carbon position in its chemical structure. These are genotoxic & carcinogenic and can cause liver cancer in humans, chronic hepatitis and jaundis.

The biosynthetic pathway of aflatoxin includes; a Type-I polyketide synthase (PKS) and a pair of specialized fatty acid synthase subunits (FAS-1 and FAS-2) to synthesize norsolorinic acid. Then a series of around 15 redox reactions mediated by cytochromes P450 (isozyme system) occurs that leads to the production of aflatoxins. The biosynthetic pathway progresses as under:

Norsolorinic acid (NOR) → Averantin (AVN) → 5'-hydroxyaverantin (HAVN) → Oxoaverantin (OAVN) → Averufanin (AVNN) → Averufin (AVF) → Versiconal hemiacetal acetate (VHA) → Versiconal (VAL) → Versicolorin B (VERB) → Versicolorin A (VERA) → Demethylsterigmatocystin (DMST) → Dihydrodemethylsterigmatocystin (DHDMST) → Sterigmatocystin (ST) → Dihydrosterigmatocystin (DHST) → O-Methylsterigmatocystin (OMST) → Dihydro-O-methylsterigmatocystin (DHOMST) → Aflatoxin B₁ (AFB₁) → Aflatoxin B₂ (AFB₂) → Aflatoxin G₁ (AFG₁) → Aflatoxin G₂ (AFG₂) (Yu, 2012)

Ochratoxin: These are particularly produced by *Aspergillus* sp. (*A. ochraceus*, *A. parasiticus*, *A. niger*) and *Penicillium* sp.. Ochratoxin are of three types- Ochratoxin A, B, and C. Among these ochratoxin A is most important; it contaminates commodities like, ce-

Table 2 . Some human diseases in which mycotoxins have been implicated.

Disease	Mycotoxin source	Fungus
Akakabio-byo	Wheat, barley, oat, rice	<i>Fusarium</i> spp.
Alimentary toxic aleukia	Cereal grains (toxic bread)	<i>Fusarium</i> spp.
Balkan nephropathy	Cereal grains	<i>Penicillium</i> spp.
Cardiac beriberi	Rice	<i>Aspergillus</i> spp., <i>Penicillium</i> spp.
Celery harvester's disease	Celery (Pink rot)	<i>Sclerotinia</i>
Ergotism	Rye, cereal grains	<i>Claviceps purpurea</i>
Hepatocarcinoma	Cereal grains, peanuts	<i>Aspergillus flavus</i> , <i>A. parasiticus</i>
Kwashiorkor	Cereal grains	<i>Aspergillus flavus</i> , <i>A. parasiticus</i>
Neural tube defects	Maize	<i>Fusarium verticillioides</i> , <i>F. proliferatum</i>
Oesophageal tumors	Corn	<i>Fusarium verticillioides</i> , <i>F. proliferatum</i>
Onyalai	Millet	<i>Phoma sorghina</i>
Reye's syndrome	Cereal grains (grain dust)	<i>Aspergillus</i>
Stachybotryotoxicosis	Cereal grains (grain dust)	<i>Stachybotrys atra</i>

Source Bryden, W. L. (2007)

reals, coffee, grape juices and spices. Ochratoxin cause kidney damage in swine and are carcinogenic to humans. Ochratoxin-A (OTA) is a very important mycotoxin for food security and human health, but very little is known about the genetic background of OTA biosynthesis as compared to the other important mycotoxins such as aflatoxins, fumonisins and zearalenones (37). L-Phenylalanine was recognized as a precursor of OTA synthesis (Wang *et al.*, 2016). OTA biosynthetic pathway had been proposed as a scheme by Huff and Hamilton. They hypothesized that mellein (dihydroisocoumarin, a phenolic compound produced by *Aspergillus ochraceus*), catalyzed by Polyketide Synthase PKS, was oxidized to OT β and then transformed to OT α by a halogenase/chloroperoxidase. Subsequently, OT α was esterified to ochratoxin C via link with the ethyl ester, and finally biosynthesized to OTA by a deesterification reaction (Huff *et al.*, 1979).

Zearalenone and Zearalenol: These are produced by *Fusarium* sp. particularly *F. Gramminearum* mainly in maize. It is estrogenic mycotoxin occurring in *Fusarium*-infected cereals, causing hormonal effects (Michlmayr *et al.*, 2017). It is also associated with the ear or stalk rot of corn, but its role in pathogenesis is not yet defined.

Trichothecenes: These are one of the most recognised groups of mycotoxins, these includes over 60 compounds but the most effective are deoxynivalenol (DON), T-2, HT-2, nivalenol, diacetoxyscirpenol (DAS). These are associated with commodities like wheat, maize, rice, oats, rye, barley, mainly produced by *Fusarium* sp. The toxicity is due to epoxy group present at 12-13 carbon position. They cause acute and chronic mycotoxicosis in farm animals and humans. These mycotoxins may also have role in pathogenesis and causes ear rot in corn and scab & head blight in wheat, barley, oats and rye.

Biosynthesis of *Fusarium* trichothecenes begins with the cyclization of farnesyl pyrophosphate (primary metabolic intermediate) to form trichodiene. The enzyme terpene cyclase trichodiene synthase (Tri5) catalyzes this reaction (Hohn *et al.*, 1986). Trichodiene undergoes a series of oxygenations catalyzed by a cytochrome P450 monooxygenase. The four oxygens were added at C-2, C-3, C-11, and the C-12, C-13-epoxide to form the intermediate isotrichotriol. Isotrichotriol then, undergoes a non-enzymatic isomerization and cyclization to form isotrichodermol (=3 α -hydroxy EPT) (Mc Cormick *et al.*, 1990). During this process, the oxygen at the C-2 position becomes the

Table 3. Important mycotoxins produced by different fungi.

Fungus	Mycotoxin
<i>Aspergillus flavus</i> and <i>A. parasiticus</i>	Aflatoxin
<i>A. Fumigatus</i>	Fumagillin
<i>A. versicolor</i> , <i>A. nidulans</i> , and <i>A. rugulosus</i>	Sterigmatocystin
<i>A. Ochraceus</i> , <i>Penicillium viridicatum</i>	Ochratoxin
<i>P. verrucosum</i>	Ochratoxin
<i>P. citrinum</i> , <i>P. viridicatum</i>	Citrinin
<i>P. patulum</i> , <i>P. expansum</i>	Patulin
<i>P. puberulum</i> , <i>P. purpurogenum</i>	Penicillic acid
<i>Fusarium</i> sp.	Trichothecenes and Zearalenone
<i>F. tricinatum</i> , <i>F. graminearum</i> , <i>F. equiseti</i> , <i>F. sporotrichioides</i> , <i>F. poae</i> and <i>F. lateritium</i>	T-2 toxin, HT-2 and Diacetoxyscirpenol(DAS)
<i>F. graminearum</i> , <i>F. tricinatum</i>	Zearalenone and Zearalenol
<i>F. graminearum</i>	Deoxynivalenol(DON) or Vomitoxin
<i>F. moniliforme</i>	Fumonisin
<i>Claviceps purpurea</i>	Ergot alkaloides(ergopeptine and clavine)

Table 4. Different physical and chemical methods for mycotoxin management.

S. N.	Physical approaches	Chemical approaches
1.	Washing	Treatment of contaminated feed with ammonia
2.	Polishing	
3.	Mechanical sorting and separation	Treatment of agricultural commodities by oxidising and reducing agents, acids, bases, salts and chlorinating substances have been tested for their ability to degrade mycotoxins
4.	Density segregator	
5.	Floatation	
6.	Autoclaving	
7.	Roasting and microwave heating	
8.	UV irradiation	
9.	Ultrasound treatment	
10.	Solvent extraction	

Source : Heidler (2003)

Table 5. Some of microorganisms that are to detoxify mycotoxin.

Microorganism	Mycotoxin
<i>Agrobacterium sp. and Eubacterium sp.</i>	Deoxynivalenol
<i>Saccharomyces cerevisiae and Paecilomyces sp.</i>	Patulin
<i>Flavobacterium aurantiacum and Aspergillus flavus</i>	Aflatoxin
<i>Exophiala pinifera, Rhinocladiella atrovirens and Bacterium ATCC 55552</i>	Fumonisin
<i>Gliocladium roseum NRRL 1859, Rhodococcus etythropolis</i>	Zearalenone
<i>Curtobacterium sp., Butyvirbio fibrisolvens and Selenomonas ruminantium</i>	T-2 toxin

pyran ring oxygen and the hydroxyl group at C-11 is lost. More complex Type A trichothecenes are built by modifying isotrichodermol through a series of paired hydroxylation (–OH) and acetylation or acylation (–OR) steps. Isotrichodermol (C-3 –OH) is converted to isotrichodermin (C-3 –OR) by an acetyltransferase. A second hydroxyl group is added to C-15. A third hydroxyl group is added at C-4. A fourth hydroxyl group is added to C-8 followed by the addition of an isovaleryl moiety. Finally, the acetyl group is removed from the C-3 position by an esterase to produce trichothecene (Mc Cormick *et al.*, 2011)

Fumonisin: There are various groups of fumonisins. Most important are A1, A2, B1, B2. These are produced from *Fusarium sp.* (*F. Moniliformae*, *F. Verticilloides*, *F. proliferate*). Corn is the mostly affected commodities. These are carcinogenic to humans & may cause oesophageal cancer. It causes liver & kidney toxicity in animals. These may also play a role in pathogenesis. It's been found that more than 95% concentration of fumonisins produced by *Gibberella fuskuroi* increases virulence on maize seedling. The Fumonisin (FB1) backbone is structurally very similar to sphinganine, a fatty acid-derived intermediate in sphingolipid metabolism (Merrill *et al.*, 1997). However, it is not clear whether fumonisins are derived from a fatty acid or polyketide because both types of compounds are derived from acetate.

Management of mycotoxins: Due to harmful effects of mycotoxins to animals, plant and humans it is necessary to manage mycotoxin contamination in agricultural commodities. A number of physical and chemical approaches have already been taken to reduce the ef-

fect of mycotoxins. The efficiency of these techniques depends on the level of contamination and the distribution of mycotoxins in produce. Therefore, the results obtained are uncertain and often connected with high product losses. Moreover, some of these treatments are relatively costly and may remove or destroy essential nutrients in food and feed. The limitation of physical and chemical strategies prompted a search for other solutions to the mycotoxin hazard. Consequently, a new approach is applied for managing mycotoxins is through transformations by using genetic engineering and biotechnological techniques (genetic transformation) and; microbes and enzymatic detoxification (bio-transformation) that offer specific, efficient and eco-friendly way for detoxification of mycotoxins that leaves neither toxic residue nor any undesirable by-product.

However, the efficiency of these techniques depends on the level of contamination and the distribution of mycotoxins throughout the grain. Subsequently the results obtained are uncertain and often connected with high product losses. Moreover, some of these physical treatments are relatively costly and may remove or destroy essential nutrients in feed. Chemical methods require not only suitable reaction facilities but also additional treatments (drying, cleaning) that can make them time consuming and expensive. Only a limited number of these are effective without diminishing the feed's nutritional value or palatability (Heidler, 2003)

Genetic transformation: There are three strategies through which genetic transformation can be done

Reducing infection by the pathogen: In this strategy a novel gene is inserted that expresses antifungal pro-

teins or secondary metabolites such as – hydroxamic acid and phenolics. It induce existing plant defence pathway in warding off infection.

Inserting genes capable of degrading the toxin: Fumonisin is greatly detoxified by this strategy. Gene for Fumonisin esterase is been isolated & cloned from *Exophiala spinifera* which is capable of metabolizing Fumonisin. Then these genes are exposed in maize plants by using biotechnological tools. Then expression of these genes on Fumonisin level kernels is being evaluated.

Reducing mycotoxin accumulation by interfering with the biosynthetic pathway: This strategy involves engineering plants to produce proteins or compounds that interfere with mycotoxin biosynthesis, or altering plant genome, so it fails to produce signalling compounds involved in mycotoxin biosynthesis, for example- in case of aflatoxin, α -amylase helps in its synthesis and α -amylase inhibitor proteins can inhibit aflatoxin synthesis identified from legume *Lablab purpureus*. So genetically altered maize plant can be formed having α -amylase inhibitor.

Different gene transfer method: There are various gene transfer methods through which desirable gene is transmitted to plant. There is direct DNA gene transfer method and vector mediated gene transfer method. Among direct method there are some physical and chemical methods, while vector mediated transfer method is through *Agrobacterium* mediated method.

Physical methods

Electroporation: In this method there is use of electric impulse to produce pores in plasma membrane, thereby allowing macromolecules into the cells for the uptake of DNA. Here plant material is incubated in buffer containing target DNA then subjected to high voltage electric impulse.

Particle bombardment: It uses accelerated microprojectiles to deliver DNA into intact tissues & cells. For this purpose gene guns are been devised that literally fires DNA into target cells. The DNA which is to be transformed into the cells is coated with tungsten or gold particle. The coated particles are loaded into the firing chamber of the gene guns which is accelerated to high speed towards the target cell. The projectile penetrates the plant cell walls and membrane.

Microinjection: In this method needles or micropipettes are used for injecting DNA. This method is tried in mainly cereals.

Silicon carbide fibre mediated transformation: DNA coated with silicon carbide fibres are vortexed with plant material. DNA adhered with the fibres enters the cells & get integrated with host genome.

Chemical methods

Polythene glycol mediated method: In this method isolated protoplast of the plant cell is kept in a solution containing 40% polyethylene glycol (PEG), calcium nitrate/CaCl and mannitol/Sucrose also present as os-

motric buffering agent into the solution. Then carrier DNA is added into the solution. After exposure of DNA to protoplast PEG is allowed to remove.

Calcium phosphate Co-precipitation mediated method: In this method isolated DNA is mixed with solution of Calcium chloride & Potassium phosphate buffer under a condition which allow to form Calcium phosphate precipitate having DNA. Plant cell are incubated with precipitated DNA either in solution or in culture dish. Fraction of cell will take up the DNA precipitate by endocytosis.

Vector mediated gene transfer method: *Agrobacterium tumefaciens* has been widely applied as a vector for plant transformation. *Agrobacterium tumefaciens* is a plant pathogenic bacteria causing crown gall disease. This bacterium is having Ti plasmid that contains tumour genes that alter host plant metabolism in favour of pathogens. This bacterium is considered as nature's little plant transformer and has been exploited to mediate the introduction of gene of interest into the target plant cells with the help of T-DNA from Ti plasmid.

Ti plasmid contains three components- virulence gene region, T-DNA region and opine catabolism region. T-DNA region having tumour inducing genes or genes for biosynthesis of auxin and cytokinin and opine. Virulence genes region having genes that are responsible for transfer of T-DNA. It codes for protein involved in T-DNA transfer. At least nine vir genes operons has been identified these are virA, virG, virB, virC1, virD1, virD2, virD4, virE1, virE2. Opine catabolism region codes for protein involved in the uptake & metabolism of opines.

In this method firstly the gene of interest is inserted into the T-DNA region. Wounded plant cell produce phenolic defence compound which can triggers the expression of the bacterium's vir genes. It encoded for vir protein that process the T-DNA region from Ti plasmid and form T-strand. After the bacterium attack plant cell, the T-strand and several vir proteins are transformed to the plant through a transport channel. Inside the plant cell, the vir proteins interact with the T-strand, forming a T-complex. This T-complex targets the nucleus, allowing the T-DNA to integrate into the plant genome and results in the expression of the encoded genes.

Previously many studies have shown that genetic transformations can be used to manage mycotoxins by using specific gene responsible to detoxify a specific mycotoxin. For instance, detoxification of deoxynivalenol (DON) and Zearalenone (ZEN) were achieved by expression of acetyltransferase gene (Tri101) and lactonohydrolase gene (Zh101) respectively (Okubura *et al.*, 2002; Takahashi *et al.*, 2004). Thus with more knowledge in molecular plant pathology and biotechnology, problem of mycotoxin in agricultural commodities can be solved by inserting genes that can detoxify the mycotoxins.

(B) Bio-transformation: Bio-transformation was demonstrated first time by Binder *et al.* (2000). Bacterial strain having detoxification activity for Trichothecenes by selective cleavage at 12-13 epoxy groups was isolated from bovine rumen. Mycotoxin deactivating feed additive based on this bacterial isolate was developed and was named as BBSH. Several microbes have been identified having mycotoxin degradation activities. Detoxification of Ochratoxin A (OTA) and Patulin (PAT) by Lactic Acid bacteria Fuchs *et al.* (2007). Alberts *et al.* (2009) studied the enzymatic degradation of aflatoxin B1 by rot fungi in different liquid medium. They isolated different white fungi at different media and tested for their laccase activity and degradation in all medium. It was found that *Pleurotus ostreatus* and *Peniophora sp.* shows maximum laccase activity and thus maximum degradation of mycotoxin. Islam *et al.*, 2012 studied and describes a novel bacterial culture isolated from agricultural soil that was capable of transforming (deoxynivalenol) DON to de-epoxy DON under aerobic and anaerobic conditions at moderate temperatures. Twenty-three yeast species, including four *Trichomonascus* species and 19 anamorphic species were tested for their ability to convert the trichothecene T-2 toxin to less-toxic products (Mc Cormick, *et al.*, 2012). The Human Fecal Microbiota Metabolizes Deoxynivalenol and Deoxynivalenol-3-Glucoside (Gratz, *et al.*, 2013).

Conclusion

Mycotoxins have huge significant in post harvest loss and managing it is not an easy task. Transformation is a new approach to manage mycotoxin affected commodities by using biotechnological & genetic engineering techniques and enzymatic or microbial degradation that offers a specific, efficient, environmental friendly way of detoxification of mycotoxin and leaves neither toxic residue nor any undesirable by product. But since this approach is in its infant stage so further work into enzymology, genetic modification, residual toxicity and identification of gene for detoxification are required to harness the potential of these microbes that is used as feed additive or from which gene is extracted.

Future prospects: With more knowledge in molecular plant pathology and use of biotechnology techniques GM crops may offer a possible contribution to manage mycotoxin.

To determine whether the ability of the product (microorganism) to biologically degrade mycotoxin under *in vivo* or storage condition.

In case of bio-transformation, the responsible detoxification system (genes involved in process) remains to be identified.

The environmental safety of genetically engineered (GE) crops and foods, just as with those created by classical breeding and mutation and grown conven-

tionally or organically, must be evaluated on a case-by-case basis to perform meaningful risk assessments

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