2017, Scienceline Publication
**World's Veterinary Journal**
***World Vet J,*** 7(4): 117-127, December 25, 2017
ISSN 2322-4568

# Biological Control of Mycotoxins: An Update

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**ABSTRACT**

Biological control, or rather the deployment of living organisms in an effort to arrest the growth and development of another organism, is a hot topic in mycotoxin studies. Confirmed environmental inclemency and increasing cases of resistance, brought about by the use of chemical applications have invited the development of natural and better alternatives. Many candidates from bacteria through yeasts to fungi have been exploited to control mycotoxin-producing fungi with appreciable success. This review takes a critical look at the development and harvest the reaction of crop and livestock farmers and other stakeholders and, concludes that the bio- control of mycotoxins is a field with a promising future, in spite of a few research gaps that have to be filled.

**Key words:** Biological control, Crop, Food safety, Mycotoxin

REVIEW ARTICLE
pii: S2322456817000015-7
Received: 09 Aug 2017
Accepted: 14 Sep 2017

## INTRODUCTION

Mycotoxins are toxic secondary metabolites that are produced by some pathogenic fungi on crops, whether raw or processed, in farm, transit or store, they are capable of inciting morbidities in man and animals under appropriate conditions. Targets of this contamination include cassava, maize, millet, groundnut, rice, sorghum, melon, wheat, soybean, beans, milk and a variety of spices and vended foods that are intended for human consumption (Kayode et al., 2013, Rubert et al., 2013, Anjorin et al., 2016, Abdi-Mohammed et al., 2016).

Mycotoxins can be classified according totheir structure and effect. On the basis of their chemical structure they can be grouped as Aflatoxins (*Aspergillus flavus*), Ochratoxin-OTA (*Aspergillus westerdijkiae),* Patulin (*Aspergillus* and *Penicillium*) and DON, ZEA, Fumonisin . However, by taking their health impact into consideration, it should be noted that they can be carcinogenic (Afl, OTA, Fumonisin,) neurotoxic (Fumonisin) nephrotoxic (OTA), dermatotoxic (Trichothecenes DON), immunosuppressive (AF, OTA, DON) and embryotoxic, teratogenic (AF, ZEA). The health impacts have been well documented among livestock (Table 1). Feedmillers and other animal care enthusiasts sometimes introduce binders and other interventions to reduce the adverse effect of mycotoxins on their stock. On the bases of surveillance, health impact as well as the need for interventions they are broadly categorized as **'**regulated**'** and **'**non-regulated'.

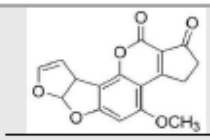
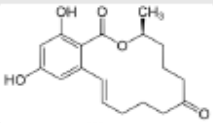
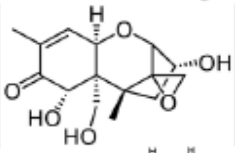
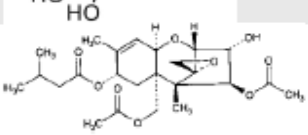
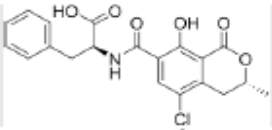
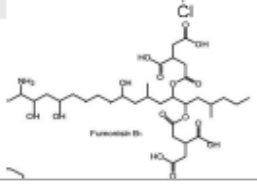
High level of broken grains and nuts, length of time stored, damage by insects and mites, degree of invasion before purchase and inadequate harvesting, drying and storage practices are some of the predisposing factors that make the grain susceptible to mycotoxigenic fungi. Contamination can occur at any given stage in the overall food and feed value chain – pre-harvest, at harvest, and in the storage. However, delivery of the fungus fighters can only be achieved in pre harvest and store. Dietary mycotoxin was found to incite oxidative stress in mice (Hou et al., 2013) and rat (Vasatkova et



To cite this paper: Fapohunda SO, Esan AO and Anjorin TS (2017). Biological Control of Mycotoxins: An Update. *World Vet. J.* 7(4): 117-127.
Journal homepage: www.wvj.science-line.com

al., 2009). In humans there is a suggested link between aflatoxin $B_1$ and viral load in HIV positive cases (Jolly et al., 2013).

**Table 1.** Mycotoxins and their effects on livestock

| Mycotoxins | Chemical Structure | Productivity Loss | Immuno Toxicity | Frequently Related Clinical Signs | Main Affected Organ System | Animal Affected |
|---|---|---|---|---|---|---|
| Aflatoxins |  | +++++ | +++++ | Hepatitis, poor response to vaccination, unspecific infections, increased susceptibility to diseases | Liver, Kidney, immune system | Dairy cattle, poults, pigs |
| Zearalenone |  | +++++ | ++ | Hyperestrogenism, reproductive disorders | Reproductive tract -mainly female | Pigs, poults |
| Deoxynivalenol |  | +++++ | +++++ | Feed refusal, vomiting | Central nervous system, GUT epithelium, liver, immune system | Pigs, dairy, cattle |
| T-2 toxin |  | +++++ | +++++ | Oral and epithelial lesion, loss of appetite | GUT epithelium, liver, immune system | Horses, pigs, poults |
| Ochratoxin A |  | +++++ | +++++ | Nefritis (kidney damage- enlarged kidney) nepatitis | Kidney, liver, immune system | Poults, cattle, rats |
| fumonisins |  | +++++ | +++ | Porcine Pulmonary, Edema (PPE) Equine Leukoencephalomalacia (ELEM) | Lungs and heart (pig), central nervous system (horse), liver, immune system | Beef, cattles, pigs, horses |

Source-https://www.impextraco.com/products/animal-protection/power-protexion-power-mycotoxins-eliminator , as modified by authors.(accessed 19 August 2017).

Although some half-hearted success has been recorded in chemical control (Belli et al., 2007; Valero et al., 2007). The regularly observed and high number of fungal strains resistant to chemical agents and the noxious impact of chemicals in the control of mycotoxins make them increasingly unattractive. Recently, there has been a global call for the wholesale embrace of organic and green agriculture, a major component of which is non-chemical applications for soil improvement as well as pest and herb control. Although phytochemicals could hold a promise in fungal control in Africa (Anjorin et al., 2014) this product of bio -prospecting can at present can only attract a measured trust. This article hereby has attempted to review of the most regulated mycotoxins biocontrol agents and the possible challenges that are inherent in adopting these strategies.

**Mycotoxins control agents**

Mycotoxin biocontrol agent, was first employed in the US, with success on a million - acre of susceptible crops are treated annually (Bandyopadhyay et al., 2016) The major success of patented ones is the intervention done by Cotty (1994) on cotton seeds, Dorner et al. (1999) on maize and rice and Dorner et al. (2003) on peanuts. For example, the production of norsorolinic acid, a biosynthetic intermediate involved in an early step of the aflatoxin biosynthetic pathway, can be inhibited thereby reducing the mRNA level of *afIR*, a gene encoding a key regulatory protein necessary for the expression of aflaxoxin-biosynthetic enzymes (Jermnak et al., 2013).

Biological control strategies have been employed with some success in using fungi, bacteria and yeasts (Tsitsigiannis et al., 2012). Some of them are being marketed under different trade names (Table 2). Qualities expected in a biological control agent include: genetic stability, efficacy at low concentrations and against a wide range of pathogens, simple nutritional requirements, survival in adverse environmental conditions, growth on cheap substrates in fermenters, lack of pathogenicity for the host plant , no production of metabolites potentially toxic to humans, resistance to the most frequently used pesticides and compatibility with other chemical and physical treatments (Spadaro and Gullino, 2004). A criticism of fungus-based bio-pesticide is that they act slowly and, therefore, give limited protection to crops. Clearly, more aggressive strains of fungal BCAs can be sought i.e. those which work more quickly and require a lower inoculum. Factors that determine pathogen virulence (virulence determinants) should be identified and used in strain selection and quality control. Fortunately, some progress has been made in this area regarding enzymes and metabolites having been identified as important virulence or antagonistic determinants.

Applications of fungal bio-control agents are usually timed to coincide with frequent rainfall and high soil moisture. When drought conditions prevail after application, the active ingredient, the fungi, remain alive on the carrier grains and



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are expected to sporulate when the conditions are conducive. Sporulation has also been observed under drought conditions with low soil moisture on carrier grains that are lodged under the plant canopy with the canopy both protecting the carrier and providing humidity for night sporulation. Screening and natural selection are essential preliminary ingredients in biocontrol. This ensures that the fighter 'organism' has the requisite traits to gain the most effective result out of the screened lot.

**Aflatoxins**

Aflatoxins are a notorious group on crops like grains, pulses and many others. They also attack animal products. The problem of aflatoxin lies particularly in the sub Saharan Africa is that is caused by a lack of awareness and economic challenges. These two factors combine effectively and result in many cases of unrecorded morbidities as well as rejections of export crops by the European Union. Morbidity can come as liver cancer and immuno-suppression. Hell and Mutegi (2011) reported that the leading self-sustaining, commercially effective, environment-friendly technology to reduce aflatoxin accumulation of crops is by using atoxigenic isolates of *A. flavus* as biocontrol agents for the displacement of aflatoxigenic fungi. This innovative technology is already widely used in the US, and field testing of the product in Nigeria has produced extremely positive results as aflatoxin contamination of maize and groundnut is reported to have been consistently reduced by 80-90% (Bandyopadhyay and Cotty, 2013). The success of bio-control products as bio-pesticides in the US has encouraged researchers of the International Institute of Tropical Agriculture (IITA) and the USDA-ARS to develop, adapt, and improve the bio-control approach for African agro-ecosystems. Collaboration among IITA, USDA-ARS, and several partners has resulted in both successful adaptation of the bio-control technology for use on maize and groundnuts in various African nations and the development of several bio-control products under the trade name 'Aflasafe' (Atehnkeng et al., 2014).

Afla-Guard based on the nontoxigenic strain *A. flavus* NRRL21882 for aflatoxin control in the USA, on corn (field, sweet, and popcorn) and peanuts (Isakeit, 2012) has been very effective. In Nigeria, Aflasafe TM with an indigenous non-toxigenic strain *A flavus* to act as the bio-pesticide formalize is the attractive equivalent. Successful attempts at reducing aflatoxin through non toxigenic strains were also recently carried out (Alaniz Zanon et al., 2016; Mallikarjunaiah et al., 2016).

*Bacillus, Pseudomonas* and *Bulkholderia* strains completely inhibit *A. flavus* growth (Palumbo et al., 2006). Full-faeces-sourced *Stenotrophomonas maltophilia*, and a few other microbes were also able to confer remediation strategies. Specifically, Shifa et al. (2016) had also succeeded in reducing the aflatoxin load when there was challenge with *Bacillus subtilis* in a groundnut matrix The multiple genetic mechanisms which incite aflatoxin production (Kelly et al., 2014) are often the target of attack.

Crop specificity is a significant factorin determining a candidate biopesticide. For example, there are no known cotton varieties that demonstrate enhanced resistance to *A. flavus* infection and aflatoxin contamination. Therefore, transgenic approaches are being undertaken in cotton fields that utilize genes encoding antifungal/anti-aflatoxin factors from maize and other sources to counter fungal infection and toxin production (Cary et al., 2011).

The timing and environment of release are a few factors that need to be observed before determining the efficacy of a bio-control candidate. Garber and Cotty (2006) had reported that spore production of AF36 had reduced significantly after AF36 product was exposed to six herbicides, Buctril, Bueno, Caparol, Gramoxone, Prowl and Roundup, at the recommended use rates, which indicated that non-toxigenic strains should be applied, post herbicide administration. The same year, Pitt and Hocking (2006) recommended that the application of non-toxigenic strains to soil should be delayed until soil temperature reaches at least 20$^{o}$C. All these factors are aimed at ensuring that the non-toxigenic strains reach high population levels when the threat of crop infection is at its greatest (Yin et al., 2008).

It has been reported that aflasafe products provide excellent protection from aflatoxin accumulation both before and after harvest and throughout the value chain (Bandyopadhyay et al., 2016). To date, fungal bio-control products have been registered for use in Nigeria as aflasafe in 2014, in Kenya as aflasafe KE01 in 2015, and Senegal/The Gambia as aflasafe SN01 in 2016. Research is currently underway to secure registration of tailor-made aflasafe products in Burkina Faso, Burundi, Ghana, Malawi, Mozambique, Rwanda, Tanzania, Uganda, and Zambia (Grace et al., 2015). Most of the popular biological control products on mycotoxins are pre-harvest interventions. In Nigeria and sub-Saharan Africa, aflatoxin is still considered and reported as a postharvest challenge.

**Fumonisin**

This *Fusarium* mycotoxin is dangerous as it incites hepatotoxicity and nephrotoxicity in most animals it's in contact with. It is associated with leucoencephalomalacy in horses, pulmonary edema in pigs and can be related to oesophageal cancer in humans. The biological control of fumonisin has involved an extensive use of bacteria and fungi.

For example, maize seed treatment with *Bacillus amyloliquefaciens* and *Enterobacter hormaechei* may improve the quality of maize grains obtained at harvest by reducing their toxin content (Perreira et al., 2010). Rhizobacteria, particularly those belonging to the *Pseudomonas* and *Bacillus* species were reported to produce significant reduction in *Fusarium verticilloides* and fumonisin B production (Cavaglleri et al., 2004). The use of *Bacillus amyloliquefaciens* and *Microbacterium oleavarans* to reduce fumonisin in maize was reported (Sartori et al., 2013) just as *Trichoderma harzianum* T16 and T23 on *F. moniliforme* and Fumonism B1 and B2 (Altinook, 2009). Also, the inhibitory effect of *Trichoderma* species on fumonisin–producing *Fusarium* has been traced to a combination of antibiosis through production of volatile compounds, extracellular enzymes and antibiotics (Alberts et al., 2016). Many *Trichoderma* species are also known to produce Microbe-Associated Molecular Patterns (MAMPs) which respond by reflex to the presence of foreign body by expressing anti-microbials. Through these mechanisms, many Generally Recognized as



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Safe, GRAS, species like *T. viride* effectively reduce *F. verticillioides* growth and fumonisin production both *in vitro* and *in planta* (Yates et al., 1999).

Microbial transformation of fumonisin to less toxic derivatives have been carried out by using organisms like *Exophiala spinifera* ATCC 74269, *Rhinocladiella atrovirens* ATCC 74270, bacterium ATCC 55552, and *Sphingopyxis macrogoltabida* MTA144 (Heinl et al.,2010). The bacterium, Serenade, (*B. subtilis*), has been successfully used to reduce fumonisin and aflatoxin (Formenti et al., 2012).

In the rural subsistence farming clusters, simple, practical, and culturally acceptable methods like hand-sorting, washing, and de-hulling are very effective in fumonisin reduction (Alberts et al., 2016). It is on record that while mechanical de-hulling is effective, mechanical shelling is not; in fact it leads to more *Fusarium* invasion and enhance fumonisin contamination (Fandohan et al., 2006). A consortium of mycoparasitic bacteria and some fungi was used to challenge *Fusarium verticillioides* and fumonisin production and was found to have been successful in toxin reduction (Samsudin and Magan, 2015).

**Ochratoxin**

Ochratoxin A is another important mycotoxin present in coffee, spices, and cocoa. *Aspergillus westerdjikiae* and *A. ochraceus* are usual sources. OTA affects rat mid brain (Wilk-Zasadna and Minta, 2009) and reproductive disorders due to mycotoxin effects are often reported in farm animal species. Health impacts on humans and livestock include nephrotoxicity and general reduction in productivity. The total removal of OTA from foods and feeds is not attainable till date. Kapetanakou et al. (2012) reported the importance of yeasts and bacteria in OTA degradation. Experimental *Beauveria bassiana* ITEM-1559 was reported to be a valid bio-insecticide against the moth *L. botrana* and that grape moth bio-control is a strategy to reduce OTA contamination (Cozzi et al., 2013).

Yeast isolates, *Issatchenkia arientalis, Metschnikowia pulcherrima, Issatchenkiaterricola* and *Candida incommninis*have been observed to reduce the *A. carbonarium* and *A. niger* colonization on grape berry (Bleve et al., 2006). This is due principally to the biology and no -toxin properties of yeasts (Ponsone et al., 2012). These two fungi were earlier successfully bio-controlled by the use of yeast strains (Ponsone et al., 2011). Strains of *Aureobasidium pullulans* could drastically reduce the OTA production in wine grapes (de Felice et al., 2008). Some mycotoxins have a combination effect which needs to be studied in order to serve as guide in their control. For example, there is the possible synergistic effect between Ochratoxin A (OTA) and other mycotoxins, such as Penicillic Acid (PeA) and Fumonisin B1 ($FB_1$), contributing to this nephropathy (Stoev, 2008). Shi et al. (2014) had reported that *B. subtilis* CW 14. had inhibited the growth of the OTA-producing species *Aspergillus achraceus* 3.4412 and *Aspergillus carbonarius*. The bacterium according to the researchers was able to both prevent OTA contamination and degrade OTA in crops. Out of many wild yeasts tested for OTA reduction, *Pichia anomala* CCMA0148 and *Saccharomyces cerevisiae* CCMA0159 provided the greatest inhibitory influence on toxin producing strains (de Souza et al., 2017).

The suppressing effect of *Streptomyces aureofaciens* on OTA producing *A. niger* in grapeis on record (Haggag and Abdal, 2012). Also, Ponsone et al. (2012) investigated two epiphytics strains of yeast *Lanchancea thermotolerans* that were able to control the growth and OTA accumulation of ochratoxigenic fungi both "*in vitro*" and "*in situ*". Yeasts are noted fungicides. This toxin is also known to be controlled by the yeast *Trichosporon mycatoxinivorans*, a species that can also detoxify the zearalenone (Vekiru et al., 2010). Environmental factors like temperature and humidity can affect the efficacy of bio-control agents on *Aspergillus carbonarius* and *A. niger* (Leong et al., 2006) and particularly when yeast *Metschnikowia pulcherrima* LS16 and two strains of the yeast-like fungus *Aureobasidium pullulans* LS30 and AU34-2-were investigated by De-Curtis et al. (2012) against infection by *A. carbonarius* and OTA accumulation in wine grape berries. *Saccharomyces cerevisiae* was also successful on *A. ochraceus* and OTA in coffee (Velmourougane et al., 2011). The yeast bullet impact was also tested on *Penicillium nordicum* and OTA production when a consortium of yeasts belonging to *D.hansenii, D. maramus, C. famata, C. zeylanoides* and *H. burtonii* species, was individually screened for antagonistic activity against a toxigenic strain of *P.nordicum* and inhibition of OTA biosynthesis (Virgili et al., 2012) *C. zeylanoides* and *H. burtonii* were the most effective and had their activity was enhanced by the presence of sodium chloride.

A trip into the mechanism of action of OTA degradation by biological means was taken by Shi et al. (2014). Using the 16SrRNA gene sequence it was revealed that *B. subtilis* CW 14 could inhibit the growth of the OTA-producing species *Aspergillus ochraceus* 3.4412 and *Aspergillus carbonarius*, with inhibition rates of 33.0 and 33.3% respectively. An interesting dimension was introduced when it was observed that, using high-performance liquid chromatography, the cell-free supernatant degraded 97.6% of OTA after 24h or incubation at 30 $^{o}$C and no degradation products were produced. It could only suggest that OTA was an ingredient of survival and growth for this bacterium.

Yeasts are considered one of the most potent biocontrol agents due to their biology and non-toxic properties. Epiphytic yeasts are the major component of the microbial community on the surface of grape berries and they are evolutionarily adapted to this ecological niche. Nowadays, several yeast species included in different genera are considered as potential bio-control agents to control both growth of ochratoxigenic *Aspergillus* species and OTA accumulation (Ponsone et al., 2012).

**Deoxynivalenol (DON)**

DON, sometimes thought to be a temperate contaminant has its presence also in African countries. Since this notorious field mycotoxin, is distributed throughout the kernels, with higher content in the outer skin, milling can also be effective in reducing the DON levels of wheat-based foods, if bran and shorts are removed before thermal cooking. Field conditions that guarantee the moisture level of 22 and 25 percent such as delayed harvesting will lead to *Fusarium* growth and toxin contamination. DON is water-soluble and cooking with larger amounts of water lowers DON content



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in products such as spaghetti and noodles. During baking or heating, DON is partially degraded to DON-related chemicals, (Kushiro, 2008). There has been a confirmation of antibody mediated reduction in *Fusarium* mycotoxins (Hu et al., 2008).

**Patulin (PAT)**

The mycotoxin, patulin (4-hydroxy-4H-furo (3,2c]pyran-2[6H]-one) was first isolated from *Aspergillus clavatus*. It is prevalent and well disseminated in apple juice and pome fruits generally. The level 50ppb has been set at the USA, the world largest consumer of apple juice its mechanism of action, though not well known it is believed to be linked with yeast growth inhibition (Iwahashi et al., 2006). The combination of *Rhodosporidum kratochvilovae* LS11 (originally named *Rhodotorula glutinis*) and *Cryptococcus laurentii* LS28 and some low dose fungicides reduced the level of patulin in apples (Lima et al., 2011). Biocontrol yeasts *Rhodasphoridium kratochvilovae* strain LS11 (Castoria et al., 2011); *Rhodotorula glutinis* (Wright et al., 2008) are ready candidates of patulin reduction. Ianiri et al. (2013) while working on *Sporobolomces sp.* strain IAM 13481, a basidiomycetes yeast, investigated the genetic approach to patulin reduction and linked this to the mechanism of resistance of the mycotoxin. Manning et al. (2013) believed that clean grounded corn DON fed at high dose of 5ppm (5mg DON/Kg) to catfish had actually conferred protection fromexposure to the pathogenic bacterium *Edwardsiella ictaluri.* It strengthens their immune system and allows alternative use of such contaminated corn instead of discard.

**ZEARALENONE (ZEA)**

This *Fusarium* toxin was noted to incite a drastic reduction in productivity of livestock has been controlled by the use of *Aspergillus niger* (Sun et al., 2014). According to them, rats administered with contaminated corn steep liquor treated with the strain FS10 culture filtrate had shownto have entailed a significantly less severe liver and kidney damage, and organ index values were comparable to the non-ZEN-exposed control.

Many *Rhizopus* strains, including *R. stolonifer, R. oryzae* and *R. microspores* were found to completely degrade ZEN (Varga et al., 2005). A substantial biotransformation of zearalenone by the two fungal genera *Aspergillus* and *Rhizopus* was reported by Brodehl et al. (2014). Biotransformation normally could result in less toxic derivatives as in identification and characterization of a lactonohydrolase enzyme in fungus *Clonostachys rosea* which converts ZEN to a less estrogenic compound (Takahashi-Ando et al., 2005; Kosawang et al., 2013). The metabolism of zearalenone was also recorded by using *Gliocladium roseum* which gave 80-90% yields in less toxic residue (Saleh and Yusuf, 1988). Bacteria also have a role to play as *Lactobacillus acidophilus* had the ability to protect the liver, kidney, and uterus from the toxicity of zearalenone in albino rats (Ali et al., 2015).

**Table 2.** Biocontrol products for mycotoxins

| Product/Trade name | Microbial agent | Food commodity | Manufacturer/distributor |
|---|---|---|---|
| **AF36** | *Aspergillus flavus* AF36 | Corn and cotton | Arizona Cotton Research and protection Council USA |
| **Afla-guard** | *A flavus* Strain NRRL21882 | Peanuts and corn | Syngenta Crop Protection, USA |
| **AQ-10 biofungicide** | *Ampelomyces quisqualts* Cesah ex Schlechtendahl | Apple, grapes, strawberries, tomatoes and cucurbitus | Ecogen. Inc. USA |
| **Aspire** | *Candida olephila* strain 1-182 | Apple, pear and citrus | Ecogen. Inc. USA |
| **Biosave 10LP. 110** | *Pseudomonas syringae* (stain 10 LP, 110) | Apples, pear and citrus, cherries and potatoes | Eco Science Corporation, USA |
| **Blight Ban A 506** | *Pseudomonas fluorescence* A. 506 | Apple, pear, strawberries and potatoes | Nu Farm lnc. USA |
| **Contrans WG, Intercept WG** | *Coniothytrium minitans* Campbell | Onion | Prohyta Biologischer, Germany |
| **Messenger Rhio-plus** | *Erwinia amylovora* (Burnll) Winslow et al *Bacillus subtilis* FZB 24 | Vegetables Potatoes and other vegetables | EDEN Bioscience Corporation, USA KFZB Biotechnick, Germany |
| **Serenade** | B subtilis | Apple, pear, grapes and vegetables | AGRO Quess lnc. USA |
| **Aflasafe** | Mixture of four *Aspergillus flavus* Atoxigenic VCGs La3279, Kal6127, Og0222 and La3304 | Maize and groundnut | IITA Business Incubation Platform, Ibadan Nigeria |

Source: (Sharma et al., 2009; Bandyophadyay 2015, Personal communication)



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**Challenges of commercializing fungal Biocontrol Agents (BCAs)**

Successful usage and adoption of these bio- control strategies are often faced with some challenges. There is still restricted adoption of biocontrol agents (BCAs) in crop protection in developing countries. There are no strong incentives to develop these agents and/or discourage chemical pesticides.

The infrastructure which facilitates the transfer of new technologies and research knowledge to the "end user" (i.e. farmer) in most developing countries is either absent or has does not function.

Work on the biological control of citrinin received a boost when Abd Allah et al. (2005) had observed a successful control using *Trichoderma harmatum*. The matrix was rice. A genetic approach was earlier exploited by Ammar et al. (2000), when one special *pet-ts* mutant had been identified that exhibited a high sensitivity against citrinin. The genetic system of yeast allowed the isolation of the respective wild-type gene. Generally, the genetic influence of yeast *Pichia* on mycotoxin producing species of *Penicillium* had reported interactions among mould species under stress conditions. The yeast *Pichia anomala* (J121) is handy here as it inhibited growth of *P. roqueforti* in grain stored in malfunctioning airtight storage systems (Boysen et al., 2000).

**Econo-ecological factors and biological control**

Farmers in developing countries are hardly rewarded for guaranteeing reduced mycotoxins in their crop produce.. The visible detection of mould is a preliminary but unreliable indicator of mycotoxin contamination. For rural farmers, many incidents of mycotoxins on crops go un-noticed and therefore undetected by some cultural practices. Biological control will have to rest on the confirmation of the occurrence and the access to available intervention strategies. The cost of the available chemical control is unaffordable by these set of crop and livestock producers. In large scale commercial production toxin reduction cost can also build up thus adding to the operational cost of production which will be passed on to the consumers. The organic farming option is also gaining more ground in Africa with many NGOs willing to partner with producers of bio-pesticides for mycotoxin control, the two might be the cheap twin –option that pull wholesome consumption along the food value chain One principle on biological control is the fungus-fight, where a fungus known to be genic is brought to challenge a toxigenic one in a manner that the latter overcrowds the former and hinder its growth and possible mycotoxin production.

Generally, in carrying out the function of 'fungus-fight', bio-pesticides still need to respond to ecological factors A strain of mould with the genetic potential to produce a particular mycotoxin may not do so under all circumstances. There must be enough nutrients to encourage sustained mould growth and the level of mycotoxin production would in part be influenced by the nutrients available to the mould. Substrate effect is critical to toxin production, e.g., there is a high proportion of toxin-producing strains of *A. flavus* isolated from peanuts and cottonseed than from rice or sorghum. It has also been found that strains of ochratoxin and citrinin-producing *P. viridicatum* isolated from meat were more unstable than those isolated from grain and rapidly lost toxin-producing ability. Field fungi like *Fusarium* and *Alternaria* contaminate grains before or during harvest. The storage fungi (e.g. *Penicillium* and *Aspergillus*) are capable of growing at lower water content than the field fungi and they tend to contaminate the grains in silos and other storage places. It is known that aflatoxin - production is favoured by the prolonged end of season drought and associated elevated temperatures (Kabaluk et al., 2010).

The subject of biological control continues to wallow in academic scrutiny, as a few questions keep returning to the surface. The issues are however, to assist in making bio- control as effective and reliable as initially thought out. What is the mode of action of biopesticides with reference to the chemical state of the mycotoxins? This question is apt for all intervention steps. However, does a biological control agent have an advantage whether the mycotoxin is in free or modified state? Modification can come from diverse sources e.g. thermal as in DON (Beyer et al., 2009), hydrolysed fumonisin, UV- induced in OTA and citrinin (Schmidt-Heydt et al., 2012) or treatment with sodium bisulphite (Beyer et al., 2010). As mycotoxicology is grappling with the issue of 'modified' (formerly 'masked' as proposed by Rychlik et al., 2014) mycotoxin, it is expected that bio-control will play a key role in addressing this group. Climate change with the attendant possibilities on mutation is also critical to the success of bio-control (Battilani et al., 2016).

If the overall intention of bio-control is to achieve bioremediation and bio- recovery of the matrix, then the basic assumption will be that the latter is confirmed toxin-laden *ab initio*. But what happens if this was not true after all? For post-harvest—(storage and processing) and feed- bound bio-pesticides, the suspicion of mycotoxin presence can be confirmed

Therefore, pre-application assessment of the target crop and environment may be critical, in determining the efficacy, just as sample preparation and sampling are to laboratory analysis of mycotoxins. Questions like 'was there any incidence of deoxynivalenone, fumonisin, aflatoxin or ochratoxin A in that particular field?'; was the experience of farmers traceable to a mycotoxin?'.

Unlike in the EU, harmonized regulations for the adoption of biological control are not implemented in developing countries. The large number of countries with porous borders and the weak development of quarantine facilities on this continent pose particular challenges for implementing Africa-wide biological control programs. With an increase in the application of BCAs for pest and disease control, many countries are now adopting regulations for the registration and release of agents. A common set of regulations could play a dramatic role in enhancing the use of BCAs and eco-friendly pest and disease control especially in developing countries. Though strict observation of guidelines on Environmental Risk Assessment and national regulations may delay implementation of biological control Guidelines, these are necessary in order to enhance the existing standard and promote quality.

Mycotoxin-producing fungi are ubiquitous in soil and in crop produce throughout developing countries especially in tropical Africa (Fapohunda et al., 2012; Probst et al., 2014). The large majority of farmers, rural and urban dwellers consume the crop produce as foods without the possibility of monitoring for mycotoxin due to the absence of monitoring



To cite this paper: Fapohunda SO, Esan AO and Anjorin TS (2017). Biological Control of Mycotoxins: An Update. *World Vet. J.* 7(4): 117-127.
Journal homepage: www.wvj.science-line.com

mechanisms (Shephard, 2008) leading to high chances of human exposure. It is recalled that the European Union (EU) generally seems to be having issues with some Nigerian agricultural commodities like melon, beans, peanuts and *ogbono* due to their mycotoxin levels that are above permissible limits. During the 2006 outbreak investigation in Kenya, a portable screening tool was adapted for rapid assessment of aflatoxin contamination in maize in the rural village setting. This tool was used to identify households with contaminated maize, a key step in the maize-replacement effort (Saha, 2009).

The high investment profile in bio-control for relatively poor farmers, possibility of time –related mutation and subsequent pathogenic tendencies, coupled with inconclusive environmental impact report may invite suspicion of its overall safety and economics. The influence of environmental and cultural practices the survival of *Aspergillus* or any mycotoxigenic fungus cannot also be over looked (Jaime and Cotty, 2010) Recently, the post-release environmental fate of atoxigenic *A .flavus* is attracting research attention in the USA, it is believed that the outcome will resolve the fear earlier expressed (Abbas and Weaver, 2011).

Farmers in developing countries are hardly rewarded for guaranteeing reduced mycotoxins in their crop produce. The organic farming option is also gaining ground in Africa with many NGOs willing to partners with producers of biopesticides for mycotoxin control, the two might be the cheap twin –option that pulls wholesome consumption along the food value chain.

## CONCLUSION

Biological control agents should be made affordable to farmers and compounded in a manner that makes products easy and safe to handle. The efficacy of microbe-based control agents may be enhanced by selection of more efficient strains, gene manipulations, combination of a number of strains of microorganism and combination of synergistically acting other bio-products. At the global produce market, the demand for safe and productive crop is high.

The combination of Good Agricultural Practices (GAP), physical, chemical and biological control, and strong emphasis on awareness still remains the best panacea. Simple cultural practices like good storage practices, proper ventilation, washing and picking out/separating of contaminated seeds are still the accessible cheap attractions to an average farmer and housewife processors and vendors of agrochemicals who have informal education with zero knowledge of toxigenic mould infestations and mycotoxins. Much of the mycotoxin challenge can be solved through awareness and cultural practices. It is advised that, on a scale of expenditure, these affordable interventions should have about 70% of all funds released by donor or investors. This is the only way a genuine result can be attained at the lower level of production chain, characterized by people who are economically challenged and lack the required knowledge. Biological control is still viewed with caution and circumspection, particularly with the environmental impact assessment over time being regarded as inconclusive. It will be a 'hit-breakthrough' for humanity if any pre-harvest intervention confers protection on postharvest/stored crop irrespective of where or how it is stored or processed.

Certainly, some departments in the biological control project may need further scrutiny. In spite of that, the biological control of mycotoxins is an intervention with a bright future.

**Acknowledgments**

The authors appreciate Dr. Ranajit Bandyophadyay (IITA) for supplying some information during the compilation of the work.

**Author`s contribution**

Fapohunda initiated the review, and actively participated in the write up. He is the corresponding author. Esan contributed immensely to the writing of the manuscript through fresh suggestions. Anjorin wrote extensively and was involved at every stage of the review. Financing of the publication was by all authors.

**Competing interest**

The authors have no competing interest in preparing the manuscript

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To cite this paper: Fapohunda SO, Esan AO and Anjorin TS (2017). Biological Control of Mycotoxins: An Update. *World Vet. J.* 7(4): 117-127.
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