Biologically Based Methods for Control of Fumonisin-Producing Fusarium Species and Reduction of the Fumonisins

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Infection by the fumonisin-producing Fusarium spp. and subsequent fumonisin contamination of maize adversely affect international trade and economy with deleterious effects on human and animal health. In developed countries high standards of the major food suppliers and retailers are upheld and regulatory controls deter the importation and local marketing of fumonisin-contaminated food products. In developing countries regulatory measures are either lacking or poorly enforced, due to food insecurity, resulting in an increased mycotoxin exposure. The lack and poor accessibility of effective and environmentally safe control methods have led to an increased interest in practical and biological alternatives to reduce fumonisin intake. These include the application of natural resources, including plants, microbial cultures, genetic material thereof, or clay minerals pre- and post-harvest. Pre-harvest approaches include breeding for resistant maize cultivars, introduction of biocontrol microorganisms, application of phenolic plant extracts, and expression of antifungal proteins and fumonisin degrading enzymes in transgenic maize cultivars. Post-harvest approaches include the removal of fumonisins by natural clay adsorbents and enzymatic degradation of fumonisins through decarboxylation and deamination by recombinant carboxylesterase and aminotransferase enzymes. Although, the knowledge base on biological control methods has expanded, only a limited number of authorized decontamination products and methods are commercially available. As many studies detailed the use of natural compounds in vitro, concepts in reducing fumonisin contamination should be developed further for application in planta and in the field pre-harvest, post-harvest, and during storage and food-processing. In developed countries an integrated approach, involving good agricultural management practices, hazard analysis and critical control point (HACCP) production, and storage management, together with selected biologically based treatments, mild chemical and physical treatments could reduce fumonisin contamination effectively. In rural subsistence farming communities, simple, practical, and culturally acceptable hand-sorting, maize kernel washing, and dehulling intervention methods proved to be effective as a last line of defense for reducing fumonisin exposure. Biologically based methods for control of fumonisin-producing Fusarium spp. and
decontamination of the fumonisins could have potential commercial application, while simple and practical intervention strategies could also impact positively on food safety and security, especially in rural populations reliant on maize as a dietary staple.

Keywords: Fusarium, fumonisins, prevention, biological control, reduction, sub-Saharan countries

**INTRODUCTION**

Fusarium spp. are agriculturally important plant pathogenic fungi associated with disease and mycotoxin contamination of grain crops (Wild and Hall, 2000; Picot et al., 2011). Fusarium ear rot in maize is one of the major diseases affecting maize production worldwide and poses an enormous threat to the international trade of foods and feeds. Fungal species of Fusarium Section Liseola, including Fusarium verticillioides, Fusarium proliferatum, and Fusarium subglutinans are some of the most important causative fungal agents of Fusarium ear or kernel rot as well as symptomless infection of maize crops, leading to contamination with the fumonisin mycotoxins (Munkvold et al., 1997).

Fifteen Fusarium spp. have been reported to produce fumonisins. Eight species are from the Section Liseola, i.e., F. verticillioides, Fusarium sacchari, Fusarium fujikuroi, F. proliferatum, Fusarium trichothecioides, Fusarium anthophilum, and Fusarium globosum (Rheeder et al., 2002). Another five species fall within Section Dlaminia, i.e., Fusarium nygamai, Fusarium lini, and Fusarium napiforme. Trace amounts of fumonisins were detected in culture material of two species, i.e., Fusarium andiyazi and Fusarium pseudonygamai. The remaining two fumonisin-producing Fusarium spp. are one species in Section Elegans, i.e., Fusarium oxysporum and one in Section Arthrosporiella, i.e., Fusarium polyphialidicum. The fumonisins are associated with several diseases in humans, animals, poultry, and fish (Marasas, 2001; Marasas et al., 2004; Kimanya et al., 2010) and are classified as Group 2B carcinogens (IARC, 2002). Home-grown maize is a major dietary staple in southern Africa and known to be frequently contaminated with unacceptable levels of fumonisins, with fumonisin B1 (FB1) being the most prevalent natural occurring fumonisin (Marasas, 2001; Marasas et al., 2004; Shephard et al., 2007, 2013; Burger et al., 2010). The Eastern Cape Province of South Africa is one of the areas in the world where the highest levels of FB1 were recorded in home-grown maize. As a result exposure to FB1 in adults is more than four times above the provisional maximum tolerable daily intake (2 µg FB1/kg body weight/day) set by the Joint Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO) Expert Committee on Food Additives (Bolger et al., 2001).

The fumonisins comprise a group of 28 characterized analogs, which can be separated into four main groups: fumonisin A, B, C, and P (Rheeder et al., 2002). The fumonisin B (FB) analogs, which includes FB1, FB2, and FB3, are the most abundant naturally occurring fumonisins, with FB1 predominating and usually being found at the highest levels. Apart from FB, some of the other analogs may occur in naturally contaminated maize at relatively low levels. The complete fumonisin molecule plays an important role in toxic and cancer-initiating activities in vivo (Gelderblom et al., 1993). Studies evaluating the structure-activity relationship of fumonisin analogs, hydrolysis products and a monomethyl ester of FB1 in short-term carcinogenesis in rats and cytotoxicity assays in primary rat hepatocytes, indicated that the free amino group plays a pivotal role in the toxicological effects of the fumonisins in vitro and in vivo. It was suggested that the tricarballylic acid moiety is required for effective absorption of the fumonisins from the gut. The fumonisins disrupt sphingolipid biosynthesis by inhibiting the enzyme ceramide synthase (Wang et al., 1991), and the tricarballylic acid moiety is required for maximal effect (Van der Westhuizen et al., 1998).

Fusarium infect maize in the field with the highest levels of fumonisins present at harvest, concentrated in the pericarp and embryo of the maize kernel (Fandohan et al., 2006; Kimanya et al., 2008; Burger et al., 2013). Kinetics of Fusarium growth and mycotoxin production are mainly affected by water activity, temperature, and atmospheric composition, while nutritional factors such as kernel endosperm composition and nitrogen sources also play an important role (Chulze, 2010; Picot et al., 2011). Fumonisin production strongly depends on the kernel stage, and may be regulated by physicochemical factors that vary during ear ripening. Insect damage of maize by the European corn borer (Ostrinia nubilalis Hübner) and the corn earworm (Helicoverpa zea Boddie) further favors Fusarium infection (Betz et al., 2000).

**Methods for reduction** of fumonisins in maize are applied pre-harvest or during harvesting and processing (Wild and Gong, 2010). These include several existing strategies to reduce Fusarium growth and production of fumonisins in food sources, i.e., controlled agricultural practices, enabling strategies, breeding for insect and fungal resistance in maize cultivars, various physical-, chemical-, and biological treatment methods and genetic engineering approaches. Good agricultural management and hazard analysis and critical control point (HACCP) practices promote the general condition of crops, reducing but not eliminating fungal growth, and mycotoxin contamination, while resistance breeding strives to achieve a balance between developing resistant crops and maintaining high quality crop yield (Cleveland et al., 2003; Wild and Gong, 2010). However, optimization of agricultural management practices is not always possible due to high production costs, the geographical location or nature of the production systems, and challenging environmental conditions.

Several physical and chemical control methods for mycotoxins have been commercialized involving sorting and flotation, solvent extraction, chemical detoxification by alkalinization (e.g., ammonia, sodium hydroxide, and sulfur dioxide treatments), oxidation (e.g., ozone), and irradiation and pyrolysis (He and
There are, however, several limitations, challenges, and concerns with regards to physical and chemical control methods (Schatzmayr et al., 2006). Physical methods generally have low efficacy and less specificity, while chemical methods are not always effective, are considered expensive and may decrease the nutritional value of foods, affect the sensory quality, and could produce toxic derivatives (Alabouvette et al., 2009; He and Zhou, 2010). Furthermore, methods involving fungicides pose a potential health, safety, and environmental risk as certain antifungal chemical compounds are not biodegradable or have a long degradation period, could contaminate soil and water and their effect on food quality and human health is a concern (Larkin and Fravel, 1998; da Cruz Cabral et al., 2013). Prolonged chemical treatment of grains can lead to the development of resistance in fungal strains, a demand for higher concentrations, and an increase in toxic residues in food crops. Increasingly more stringent regulation is enforced with regards to the use of chemical control methods together with a strong consumer demand to reduce the use of potentially harmful chemicals in the food supply (Liu et al., 2013). There is also an ecological and societal movement toward safe and natural food, without chemical treatments and/or preservatives (Edlayne et al., 2009).

Research over the past 25 years indicates support for agricultural management practices and a renewed interest in practical and biological control methods as possible alternatives. In this regard several methods for controlling fungal growth and mycotoxin production pre- and post-harvest involving clay minerals, plant extracts and a variety of microbial taxa have been commercialized (He and Zhou, 2010). In rural subsistence farming communities a number of effective, practical, and culturally acceptable intervention methods have been developed (Kimanya et al., 2008; Van der Westhuizen et al., 2010). While the focus in the past was more on the most economically important mycotoxins, i.e., aflatoxin B₁ (AFB₁), much less information is available on other important mycotoxins such as FB₁, trichotheccenes, zearalenone, citrinin, and patulin (Kabak et al., 2006). This paper presents a comprehensive overview of recent research on biological- and practical-based approaches for control of fumonisin-producing Fusarium spp. and methods for reduction thereof during pre- and post-harvest conditions. Current information on the application of natural clay adsorbents, biocontrol organisms, antioxidants, essential oils, plant extracts, and molecular approaches are reviewed; as well as practical and culturally acceptable methods for reduction of fumonisin exposure in rural subsistence farming communities.

**PRE-HARVEST BIOLOGICALLY BASED CONTROL METHODS FOR FUMONISIN-PRODUCING FUSARIUM Spp.**

**Biocontrol Microorganisms**

This approach involves a three-way interaction between the host commodity, the pathogen and the antagonistic biocontrol microorganism together with dynamics such as competition for nutrients and space, parasitism of the pathogen, secretion of antifungal compounds, induction of systemic resistance (ISR), biofilm formation and involvement with reactive oxygen species in defense response (Larkin and Fravel, 1998; Alabouvette et al., 2009). Recent research also suggested that the aflatoxin biocontrol mechanism, employing atoxigenic strains of *Aspergillus flavus*, is triggered by physical contact or interaction between hyphae of the competing fungal strains (Damann, 2014). Essential criteria for effective biocontrol microorganisms include the ability to colonize the plant part infected by the pathogen organism, efficacy under the relevant environmental conditions and compatibility with other control methods that are applied (Bacon and Hinton, 2011; Liu et al., 2013). Niche overlap indices (NOIs) provide information on ecological similarity, coexistence, and competition between microorganisms in a specific niche and assists in identifying possible microbial antagonists against *F. verticillioides* colonization (Cavaglieri et al., 2004). Microorganisms naturally associated with and adapted to the vegetative parts of a specific plant, sharing the ecological niche with pathogen microorganisms, could hold advantages as biocontrol agents. One such a microorganism, *Bacillus subtilis* occupies the same ecological niche as *F. verticillioides* within the maize plant and effectively inhibits growth of the fungus, based on competitive exclusion (Bacon et al., 2001; Table 1). *B. subtilis* is considered generally regarded as safe (GRAS) by the United States Food and Drug Administration [US FDA, GRAS substances evaluated by the Select Committee on GRAS substances (SCOGS)], is easy to cultivate and manipulate genetically, and therefore suitable for industrial processes. A pre-harvest biological control system, involving *B. subtilis* RRC101, was developed on maize which reduces fumonisin accumulation during the endophytic growth phase of *F. verticillioides* (= *F. moniliforme*; Bacon et al., 2001). The endophytic phase of *F. verticillioides* is transferred vertically to the next generation through clonal infection of seeds. This phase is characterized by intercellular systemic infection of plants and seeds, which cannot be controlled with fungicides. Effective biocontrol has also been demonstrated with wild type and fusaric acid resistant mutant strains of the bacterial endophyte, *Bacillus mojavensis*, in *vitro* and *in planta* (Bacon and Hinton, 2011). Efficacy of these strains under field conditions could be influenced by fusaric acid produced by *F. verticillioides*. The mechanism of biocontrol by *B. mojavensis* is complex and still unclear, as indicated by broad differences in maize seedling protection by a range of strains evaluated.

*Pediococcus pentosaceus*, a lactic acid bacterial isolate from maize, inhibits *F. verticillioides* and *F. proliferatum* growth *in vitro* (Dalé et al., 2010; Table 1). Antifungal activity in *P. pentosaceus* culture supernatant was observed toward the end of the exponential phase of growth and was pH dependent. The antifungal metabolites produced proved to be heat stable and resistant to proteolytic enzymes. Culture fractions exhibiting antifungal activity contained compounds with molecular masses ranging from 500 to 1400 Da. *P. pentosaceus* has GRAS status, has been widely used in the fermentation of a variety of foods and could be suitable as biocontrol organism to improve the quality of ensilage. *Clonostachys rosea*, a fungal isolate from straw, stubble, seed surfaces, and the phyllosphere or roots of cereal crops, effectively reduced sporulation of *F. verticillioides* and *F. proliferatum* on maize stalks *in vitro* and in field trials.
TABLE 1 | Current information on reduction of fumonisin-producing *Fusarium* spp. by biocontrol microorganisms *in vitro*, *in planta*, and in field trials.

| Biocontrol microorganism | *Fusarium* spp. studied | Test system with details of experimental model | Reduction criteria | Application | Reference(s) |
|--------------------------|-------------------------|-----------------------------------------------|-------------------|-------------|--------------|
| **Trichoderma spp.** | *F. verticillioides* (= *F. moniliforme*) | *In vitro* studies on the potential for biological control of *Aspergillus flavus* and *F. moniliforme* by *Trichoderma* spp.; a study of the production of extracellular metabolites by *Trichoderma* spp. | *In vitro*: Effect of carbon source on antifungal properties of *Trichoderma* spp.; *Trichoderma* spp. inhibited *F. verticillioides* growth on growth medium with glucose as carbon source; no inhibition with sucrose as carbon source; inhibition observed with L-alanine as nitrogen source; *T. harzianum* T2 and *T. viride* T5 exhibited the strongest inhibitory effect; Antifungal activities of *Trichoderma* sp. culture filtrates: general inhibition of *F. verticillioides* growth; culture filtrates of *T. harzianum* T2 and *T. viride* T5 resulted in pronounced morphological alterations; Production of volatiles: the presence of volatile compounds of *T. harzianum* T2 and *T. viride* T5 and T6 were able to suppress *F. verticillioides* growth; Production of extracellular enzymes: amylase and cellulase activity exhibited by all four strains; lysozyme activity exhibited by *T. harzianum* T1, T2 and *T. viride* T5; proteolytic activity exhibited by *T. harzianum* T2 and *T. viride* T5; extracellular pectinolytic activity exhibited by *T. harzianum* T1 and *T. viride* T5; *T. viride* produced the widest spectrum of extracellular enzymes; Evaluation of osmotic potential: enzyme production decreased with increasing osmotic potential; Production of antibiotics: *T. viride* T5 exhibited the greatest inhibitory effect on *E. coli* and *S. aureus*, suggesting production of antibiotics | *Trichoderma* spp. exhibited potential for biocontrol against mycotoxin-producing fungi; the lack of osmotolerance in air-dried seed could be a disadvantage | Calistru et al., 1997 |
| **T. viride UPS101** | *F. verticillioides* (= *F. moniliforme*) | *In vitro*: *T. viride* suppressed radial extension of *F. verticillioides* colonies (46% reduction after 6 days; 90% after 14 days); *Effect on FB*$_1$ levels: single and co-cultivation on *maize* kernels; 85% reduction in *FB*$_1$ levels when the *T. viride* and *F. verticillioides* were inoculated simultaneously; 72% reduction in *FB*$_1$ levels when *T. viride* was inoculated 7 days after *F. verticillioides* | *Trichoderma* spp. mainly applied to soil as biocontrol agents; *T. viride* could be applied to inhibit *F. verticillioides* growth pre-harvest, to prevent disease during plant development, postharvest during storage or to suppress *FB*$_1$ accumulation in inadequately dried maize kernels; applicable for *FB*$_1$ reduction in maize kernels intended for animal feed | Yates et al., 1999 |
B. subtilis could be applied as B. subtilis under seed treatment to act as biocontrol agent during the growth of maize plants; evaluation of field conditions needed.

**TABLE 1 | Continued**

| Biocontrol microorganism | Fusarium spp. studied | Test system with details of experimental model | Reduction criteria | Application | Reference(s) |
|--------------------------|-----------------------|---------------------------------------------|-------------------|-------------|--------------|
| **Bacillus subtilis strains isolated from maize in northern Italy:** | F. verticillioides (≡ moniliforme) | Biological control of F. moniliforme in maize. | **In planta:** Young and vigorous maize seedlings: B. subtilis exhibited a protective effect on maize seedling growth and percentage seedling root infection; B. subtilis reduced F. verticillioides colonization of soils; FB₁ was significantly reduced (50%) by all bacterial antagonists; **Field trials:** Mature maize plants: B. subtilis exhibited protection in matured plants at treatments, especially under drought stress; | Application of antagonists on flowering maize ears: promising results in preliminary field trials; further experiments under disease conducive conditions needed; several antagonists exhibited potential to control Fusarium spp. in wheat and maize crop residues postharvest, and at the flowering ear stages | Bacon et al., 2001 |
| B. subtilis RRC101 (wild type) (Patent 5, 994, 117), B. subtilis RRC26es and B. subtilis RRC24wf (rifampicin resistant mutants); | Wild type strains: MRC826, RRC410, RRC408, RRC408; F. verticillioides transformed ecological marker strains: MRC826gus, RRC408gus | Potential of fungal antagonists for bio-control of Fusarium spp. in wheat and maize through competition in crop debris. | In vitro: Reduction of Fusarium spp. conidia formation (wheat straw bioassay); sporulation of F. culmorum and F. graminearum on straw: overall reduction (~80%) by antagonistic isolates; Sporulation of F. culmorum on C. cladosporioides rosea-treated straw: 85-99% reduction; sporulation of F. graminearum on C. rosea-treated straw: 91-100% reduction; Highly effective fungal antagonists: C. rosea, F. equiseti, C. cladosporioides gossypii and Epicoccum nigrum; non-pathogenic Fusarium spp. exhibited moderate antagonism; Yeasts were weak competitors; Reduction of Fusarium spp. conidia formation (maize stubble bioassay): less effective reduction in sporulation than reported for wheat straw; strongest antagonist: C. rosea; | Application of antagonists on flowering maize ears: promising results in preliminary field trials; further experiments under disease conducive conditions needed; several antagonists exhibited potential to control Fusarium spp. in wheat and maize crop residues postharvest, and at the flowering ear stages | Luongo et al., 2005 |
| A large variety of potential antagonistic bacterial and fungal strains isolated from straw, stubble, seed surfaces, and the phyllosphere or roots of cereal crops; Additional isolates: Cladosporium spp., Fusarium equiseti | Fusarium isolates from infected wheat grains in The Netherlands: Fusarium culmorum, Fusarium graminearum, Fusarium proliferatum, F. verticillioides | Potential of fungal antagonists for bio-control of Fusarium spp. in wheat and maize through competition in crop debris. | Field trials: Maize stalks: determination of antagonism; pre-inoculation of stalks with potential antagonists; subsequent inoculation of stalks with F. verticillioides, F. proliferatum and F. graminearum; plots inoculated with strips containing stalk pieces; culture of harvested stalks on modified PDA; identification of F. verticillioides, F. proliferatum and F. graminearum; colony morphology and microscopic examination; | (Continued) |
TABLE 1 | Continued

| Biocontrol microorganism | Fusarium spp. studied | Test system with details of experimental model | Reduction criteria | Application | Reference(s) |
|--------------------------|----------------------|------------------------------------------------|-------------------|-------------|--------------|
| Lactic acid bacterial isolates from maize tissues collected in maize fields (66 isolates); *Pediococcus pentosaceus* L006 | A variety of *F. verticillioides* and *F. proliferatum* strains from the INRA MycSA collection | Potential of *P. pentosaceus* (L006) isolated from maize leaf to suppress fumonisin-producing fungal growth. In vitro: Antifungal activity against *F. verticillioides* and *F. proliferatum*: Overlay MRS agar plate method; selection of the most efficient isolate; Identification of the most efficient antifungal lactic acid bacterial isolate: biochemical and physiological characterization (API 50 CHL test); 16S rRNA gene sequencing; Antifungal spectrum of *P. pentosaceus* L006 on solid medium: *P. pentosaceus* L006 inhibited the growth of all fungal strains tested; Production of active antifungal metabolites by *P. pentosaceus* L006: antifungal activity increased with incubation time; antifungal substances are possibly secondary metabolites; pH decreased (pH 6.5 to 3.8) during incubation; Characterization of *P. pentosaceus* L006 cell-free culture supernatant: antifungal activity was not reduced by heat and proteolytic enzyme treatments; antifungal compounds not proteinaceous; antifungal activity lost at pH 7; antifungal activity was ascribed to the presence of organic acids, excluding lactic acid | Application of *P. pentosaceus* L006 can possibly improve silage quality; results obtained in vitro need to be extended to *in planta* studies and field trials | Dalie et al., 2010 |
| Biocontrol microorganism          | Fusarium spp. studied                                                                 | Test system with details of experimental model                                                                 | Reduction criteria                                                                 | Application                                                                 | Reference(s) |
|----------------------------------|--------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|-------------------------------------------------------------------------------|----------------|
| Bacillus mojavensis strains RCC101 (ATCC55732) (patented); NRRL B14699; NRRL B14701; NRRL B14703 to NRRL B14706; NRRL B14708 to NRRL B14712; B. mojavensis rifampin mutant RRC112rif; B. mojavensis fusaric acid resistant mutant RRC112fa | F. verticilloides strains: MRC 826 (symptomless endophytic strain), “Patgus”; NRRL B14699 (virulent strain); 408 (virulent wild type strain), and UV28 (non-fusaric acid producing mutant strain) | In planta reduction of maize seedling stalk lesions by the bacterial endophyte B. mojavensis. In planta: B. mojavensis (strains RCC101; NRRL B14699; NRRL B14701; NRRL B14703 to NRRL B14706; NRRL B14708 to NRRL B14712) inoculated Zea mays “Early Sunglow” seeds were cultivated for 35 days in a plant growth light room and inoculated with a spore suspension of F. verticilloides “Patgus”; B. mojavensis RRC112fa inoculated Zea mays “Pioneer 3140” seeds were cultivated and inoculated as described above with F. verticilloides strains MRC 826, 408 and UV28; B. mojavensis RRC112fa inoculated Zea mays “Early Sunglow” seeds were cultivated as described above and inoculated with F. verticilloides strains “Patgus” and UV28; Determination of stalk lesion development; Measurement of the length of necrotic lesions and stalk diameters | In planta: Range of B. mojavensis strains + F. verticilloides “Patgus” ("Early Sunglow" maize): 24-58% reduction in stalk lesion length; large differences in the ability to reduce lesions; B. mojavensis RRC101 exhibited 58% reduction; B. mojavensis RRC112fa + F. verticilloides strains MRC 826, 408 and UV28 ("Pioneer 3140" maize): 30-41% reduction in stalk lesion length; B. mojavensis RRC112fa significantly (P = 0.05) reduced stalk lesion lengths caused by F. verticilloides “Patgus” on "Early Sunglow" maize (54% reduction); B. mojavensis RRC112fa: 70% reduction in stalk lesion length; reduction not significantly different from results for the RRC101 wild type strain and rifampin mutant strain (RRC112rif); Significant (P ≤ 0.05) reduction in stalk lesion length by the bacterium regardless of its ability to tolerate fusaric acid; F. verticilloides UV28 significantly (P ≤ 0.05) reduced maize stalk diameter; no enhanced effect when the fungus was co-inoculated with B. mojavensis | Application of B. mojavensis for suppression of seedling disease in maize: to prove the efficacy of B. mojavensis as biocontrol agent additional studies should be performed in vitro and in the field utilizing mutants and wild-type strains of bacteria and non-fumonisin producing fungi; more pathological factors should also be evaluated | Bacon and Hinton, 2011 |

FB1, Fumonisin B1; CFU, Colony forming units; PDA, Potato dextrose agar; MRS broth/agar; de Man, Rogosa and Sharpe broth/agar.
Plant disease severity is (Luongo et al., 2005). C. rosae exhibited potential to control *Fusarium* spp. in maize at the flowering ear stages and in crop residues post-harvest. Food-grade yeasts are also considered ideal biocontrol microorganisms, as they are generally genetically stable, effective at low concentrations, easy to cultivate, capable to survive under adverse environmental conditions, compatible with commercial processing, and resistant to pesticides.

**Trichoderma spp.**

*Trichoderma* spp. are considered effective biocontrol agents because of their repertoire of extracellular lytic enzymes that cause necrotrophic action through lysis of fungal cell walls as well as the role they play in ISR in plants (Bacon et al., 2001; Hermosa et al., 2012). *Trichoderma* mainly colonizes the rhizosphere and intercellular root areas of plants, and maintains interactions by promoting plant growth and providing protection against infections, while utilizing plant sucrose to facilitate root colonization (Hermosa et al., 2012). Plant disease severity is reduced in the presence of *Trichoderma* by inhibition of a wide range of plant pathogens through antagonistic and mycoparasitic action; ISR or induction of localized resistance. *Trichoderma* is also able to withstand toxic metabolites that are produced by the plant in response to invasion. Plants are able to detect pathogen- or microbe associated molecular patterns (MAMPs), which leads to activation of defense mechanisms and eventually synthesis of antimicrobial compounds. Certain *Trichoderma* strains produce a variety of MAMPs, contributing to activation of plant defense responses. Salicylic acid, jasmonic acid and ethylene play a key role in plant immunity and hormone-signaling pathways as well as defense response pathways of the hormones abscisic acid, indole-3-acetic acid, and gibberellin (Piterse et al., 2009). Indole-3-acetic acid produced by *Trichoderma* contributes to ethylene biosynthesis, which in turn stimulates abscisic acid biosynthesis. Depending on *Trichoderma* stimuli, phytohormone homeostasis will control plant development and immune responses. *Trichoderma* chitinases also release fungal chitin oligosaccharides, and elicit ISR by jasmonic acid/ethylene dependent pathways, thereby triggering defense responses in plants. A polyketide synthase/non-ribosomal peptide synthetase hybrid enzyme of *Trichoderma virens* is involved in plant interactions and was shown to induce plant defense responses (Mukherjee et al., 2012). Several *Trichoderma* spp. with GRAS status, including *Trichoderma viride* and *Trichoderma harzianum*, are capable of effectively reducing *F. verticillioides* (= *F. moniliforme*) growth and fumonisins production *in vitro* and *in planta* (Calistru et al., 1997; Larkin and Fravel, 1998; Yates et al., 1999; Table 1). The inhibitory effect on *F. verticillioides* growth when co-cultured with *Trichoderma* spp. can be attributed to antibiotic through production of volatile compounds, extracellular enzymes and antibiotics. The antagonistic fungal species *T. viride* is widely used in biofertilizers for biological control of soil borne plant-pathogenic fungi in crops.

**Non-Pathogenic Biocontrol Strains**

Non-pathogenic strains of pathogenic species are often applied for biocontrol (Liu et al., 2013). In this regard, moderate suppression of toxigenic *F. verticillioides* and *F. proliferatum* strains by non-pathogenic *Fusarium* strains was demonstrated by Luongo et al. (2005; Table 1).

The development of *Fusarium* biocontrol strains with reduced mycotoxin production ability through RNA silencing technology may be a useful tool for reducing mycotoxin contamination in agricultural products (McDonald et al., 2005). Transformation of *F. graminearum* with inverted repeat transgenes (IRT) containing sequences of mycotoxin-specific regulatory genes results in suppression of mycotoxin production. Other gene silencing techniques involving deletion of ZFRI of *F. verticillioides*, which regulates sugar transporter genes and in turn affect fumonisin biosynthesis during kernel colonization, resulted in significantly less growth on maize kernel endosperm tissue (Bluhm et al., 2008).

**Rhizobacteria**

*Fusarium verticillioides* is the most prevalent *Fusarium* spp. present in the rhizoplane and endorhizospheric areas of maize, while *Arthrobaeter* and *Azotobacter* are the predominant bacterial genera (Cavaglieri et al., 2005a). Pathogens germinate and colonize roots within a few days of planting, while biocontrol rhizobacteria could be metabolically active during this period. A number of rhizobacterial isolates of maize plants sampled from a commercial maize field and exhibiting high NOIs with *F. verticillioides*, including *Arthrobacter globiformis*, *Azotobacter armeniacus*, *Pseudomonas solanacearum*, *B. subtilis*, *Enterobacter cloacae*, and *Microbacterium eoleovorans* exhibited antifungal activity *in vitro* by effectively reducing *F. verticillioides* growth and FB1 production on maize meal extract agar (Cavaglieri et al., 2004, 2005a,b,c) (Table 2). Maize seeds pre-treated with *A. armeniacus* RC2, *A. globiformis* RC5, *E. cloacae*, *M. eoleovorans*, and *Bacillus* sp. CE1 and evaluated in planta, resulted in effective reduction of *F. verticillioides* growth in the rhizoplane and endorhizospheric areas. A good correlation was observed between results obtained from *in vitro* and *in planta* studies (Cavaglieri et al., 2005c). *Enterobacter cloacae* exhibited potential for biocontrol of root colonization by *F. verticillioides*. Inducible Type 1 fimbrae of *E. cloacae* may play a role in the colonization of roots (Hinton and Bacon, 1995). Rhizobacterial strains could have potential application as seed inoculants to reduce *F. verticillioides* colonization on root level, in the rhizoplane and endorhizospheric areas (Cavaglieri et al., 2005c). Effectiveness of a biocontrol organism to colonize the rhizosphere and its value as biocontrol agent could, however, be influenced by environmental conditions and the initial cell concentrations of the biocontrol organism and the pathogen.

**Antioxidants, Phenolic Compounds, and Essential Oils**

Several natural phenolic compounds derived from plants are strong antioxidants and exhibit antimicrobial activity by inhibiting the activity of key fungal enzymes, and are applied as preservatives in the cosmetic, food and drug industries (Table 3). These compounds are also considered promising antifungal agents for controlling fungal growth and associated mycotoxin production in agricultural crops pre-harvest, post-harvest, and during storage.
### TABLE 2 | Current information on reduction of *Fusarium verticillioides* growth and fumonisin B₁ production by rhizobacteria *in vitro* and *in planta*.

| Rhizobacterial microorganism | *Fusarium* sp. studied | Water activity (aw) | Test system with details of experimental model | Reduction criteria | Application | Reference(s) |
|-----------------------------|-------------------------|--------------------|------------------------------------------------|-------------------|-------------|--------------|
| Rhizobacterial isolates from maize plants in Italy: *Enterobacter cloacae* | *F. verticillioides* (= *F. moniliforme*): isolates from maize: MRC 826, RRC 374, RRC 408, isolates from rice: RRC 410 | N/A | *E. cloacae* is an endophytic symbiont of corn. Reduction of *F. verticillioides* root colonization of maize seedlings by *E. cloacae*: *In planta*: Distribution of *E. cloacae* root colonization: sterile maize seed inoculated with *E. cloacae* and cultured in tubes with soil; cultured in plant growth rooms under light; microscopic examination of root colonization; *In vitro*: Cultivation of inoculated seeds on PDA, damp filter paper or sterile soil; microscopic examination of root colonization; Determination of antagonism: co-cultivation on PDA; examination of zones of inhibition; microscopic examination of seedling roots: light microscopy; transmission electron microscopy; scanning electron microscopy | *E. cloacae* root colonization: *E. cloacae* biologically associated with maize seedling roots; observed internally and in the rhizoplane areas; on maize seedlings *E. cloacae* was distributed over the epidermis and internally in several locations of the cortex; no bacteria observed in the endodermis, but intercellular within the outer margin of the pericycle; *E. cloacae* not observed in the pith area; present in stems and leaves; *E. cloacae* distributed externally along the secondary and primary seedling roots as well as the root cap of the primary root; a matrix-like capsule observed surrounding the bacterial cells on the external surface of the primary root; *E. cloacae* no damage to host cells; no reduction in percentage germination or time of germination; Determination of antagonism: all bacterial isolates inhibited growth of *F. verticillioides* strains | | Hinton and Bacon, 1995 |
| Rhizobacterial isolates from maize roots, sampled from a commercial maize field: *Arthrobacter globiformis, Azotobacter armeniacus, Pseudomonas solanacearum, B. subtilis* | *F. verticillioides* isolates from maize roots sampled from a commercial maize field | 0.937; 0.955; 0.982 | Screening procedures for selecting rhizobacterial strains with biocontrol effects upon *F. verticillioides* growth and FB₁ production: | *In vitro*: Determination of NOIs: utilization of 17 compounds in maize as sole carbon source; selection of isolates with the highest NOIs; Antibiosis and antifungal activity of selected isolates: 2% MMEA; adjustment of aw levels; inoculation and incubation; measurement of zones of inhibition and colony diameters; FB₁ levels in MMEA cultures: HPLC analyses | | Cavagliere et al., 2004 |

(Continued)
A. armeniacus RC2 exhibited potential as maize seed inoculant for reduction of F. verticillioides root colonization.

**Antibiosis and effect on fungal growth rate:**

**In vitro:**
- F. verticillioides isolates paired with each bacterial strain in dual culture;
- Antibiosis and effect on fungal growth rate: MMEA; inoculation and incubation; measurement of zones of inhibition; measurement of colony diameters;
- FB₁ levels in MMEA cultures: HPLC analyses;

**Greenhouse studies:**
- Effect of separate and combined bacterial treatments on F. verticillioides root colonization in the rhizoplane and endorhizosphere areas; inoculation of seeds with rhizobacterial strains; modified tube assay; determination of F. verticillioides CFU counts in the rhizoplane and endorhizosphere areas.

**Endorhizosphere bacterial isolates from maize roots, sampled from a commercial maize field:**

**Bacterial mixture 1:**
- E. cloacae, Microbacterium eoleovorans

**Bacterial mixture 2:**
- P. solanacearum, B. subtilis

**Rhizobacterial microorganism**

| Rhizobacterial microorganism | Fusarium sp. studied | Water activity (a_w) | Test system with details of experimental model | Reduction criteria | Application | Reference(s) |
|-----------------------------|----------------------|---------------------|-----------------------------------------------|-------------------|------------|--------------|
| Predominant bacterial isolates colonizing the maize endorhizosphere and isolated from maize roots, sampled from a commercial maize field: | A. globiformis, A. armeniacus | 0.937; 0.955; 0.982 | Rhizobacteria and their potential to control F. verticillioides: effect of maize bacterization and inoculum density; | **In vitro:** | Antibiosis and effect on fungal growth rate: effective inhibition of fungal growth at a_w 0.955 and 0.982; A. armeniacus RC2 and RC3 inhibited fungal growth of 60-100%; F. verticillioides strains at a_w 0.955-0.982; A. globiformis RC4 and RC5 inhibited fungal growth of 69-80% of F. verticillioides strains at a_w 0.955-0.982; A. armeniacus RC2 reduced (56-75%) FB₁ accumulation at a_w 0.955; A. globiformis RC4 and RC5 reduced (20-96%) FB₁ accumulation at a_w 0.955 and 0.982; | A. armeniacus RC2 exhibited potential as maize seed inoculant for reduction of F. verticillioides root colonization | Cavaglieri et al., 2005a |
| Predominant bacterial isolates colonizing the maize endorhizosphere and isolated from maize roots, sampled from a commercial maize field: | | | **Rhizobacteria and their potential to control F. verticillioides:** | | | |
| | | | **Effect of seeds treatment on maize root colonization:** | | | |
| | | | **In vitro:** | | | |
| | | | Antibiosis: MMEA; adjustment of a_w levels; F. verticillioides isolates paired with each bacterial mixture in dual culture; different bacterial inoculum sizes evaluated (10⁷, 10⁸ and 10¹⁰ cells/ml); measurement of zones of inhibition; | | | |
| | | | Antifungal activity: MMEA; adjustment of a_w levels; pour-plate method; inoculation with F. verticillioides isolates; measurement of colony diameters; FB₁ levels in MMEA cultures: HPLC analyses; | | | |
| | | | Greenhouse studies; Effect of combined bacterial seed treatments on F. verticillioides root colonization in the rhizoplane and | | | |
| | | | and endorhizosphere areas | | | |
| | | | Reducing criteria | Application | Reference(s) |

* TABLE 2 | Continued
| Rhizobacterial microorganism | Fusarium sp. studied | Water activity (aw) | Test system with details of experimental model | Reduction criteria | Application | Reference(s) |
|-----------------------------|----------------------|---------------------|-----------------------------------------------|-------------------|------------|--------------|
| Bacillus isolates from maize rhizoplane, sampled from a commercial maize field (10 isolates) | Toxigenic F. verticilloides isolated from maize in Argentina | N/A | Biocontrol of B. subtilis against F. verticilloides in vitro and at the maize root level: In vitro: MMEA cultures: Antibiosis: dual cultures of F. verticilloides and Bacillus sp. isolates; measurement of zones of inhibition; FB1 levels: HPLC analyses Maize kernel cultures: Effect of Bacillus sp. isolates on F. verticilloides ergosterol content, FB1 accumulation and CFU counts; dual cultures of F. verticilloides and Bacillus sp. isolates; ergosterol analyses as an indicator of fungal growth; determination of F. verticilloides CFU counts; FB1 levels: HPLC analyses; Greenhouse studies: Bacillus sp. CE1 inoculum sizes evaluated: 10^6, 10^7 and 10^8 cells/ml; Effect of Bacillus sp. CE1 treatments on F. verticilloides root colonization: inoculation of seeds with different Bacillus sp. CE1 inoculum sizes; modified tube assay; determination of F. verticilloides CFU counts in the rhizoplane and endorhizosphere areas | In planta: Bacterial mixture 1 (10^9 cells/ml) completely inhibited F. verticilloides growth; Bacterial mixture 2: inhibition of F. verticilloides growth not consistent | Potential biocontrol agent against F. verticilloides infection at root level; ability to reduce F. verticilloides colonization of maize rhizoplane and endorhizosphere areas | Cavaglieri et al., 2005c |

N/A, Not applicable; FB1, Fumonisin B1; NOI, Niche overlapping indice; MMEA, Maize meal extract agar; PDA, Potato dextrose agar; CFU, Colony forming units.
# TABLE 3 | Current information on reduction of fumonisin-producing *Fusarium* spp. and fumonisin production *in vitro* by antioxidants/phenolic compounds and essential oils extracted from plants.

| Biocontrol compound | *Fusarium* spp. studied | Water activity (a_w) | Test system with details of experimental model | Reduction criteria | Application | Reference(s) |
|---------------------|-------------------------|----------------------|--------------------------------------------|-------------------|------------|--------------|
| **ANTIOXIDANTS/PHENOLIC COMPOUNDS** | | | | | | |
| BHA, BHT, THBP, and PP | *Fusarium verticillioides* strains RC2000, M7075, ITEM2424; *Fusarium proliferatum* strains ITEM 2443, ITEM 2444, M7089, RC2056 | 0.93; 0.95; 0.98; 0.995 | In vitro control of growth and fumonisin production by *F. verticillioides* and *F. proliferatum* using antioxidants under different water availability and temperature regimes. | In vitro: Efficacy of antioxidants: Control without antioxidants; increase in the lag phase of fungal growth with decreasing a_w and temperature; in the presence of antioxidants: increase in the lag phases of growth; no growth detected at antioxidants concentrations of 10-20 mmol.L^{-1}; *F. verticillioides* and *F. proliferatum* more tolerant of THBP and BHT than PP and BHA; BHA (20 mmol.L^{-1}) and PP (10 mmol.L^{-1}) completely inhibited growth of both fungal species at all a_w levels evaluated; FB_{1}, FB_{2} and FB_{3} levels in MMEA cultures: HPLC analyses | Food-grade preservatives BHA and PP exhibited potential for preventing mycotoxigenic fungi and their toxins entering the food chain | Elcheverry et al., 2002 |
| BHA, BHT, THBP, and PP | *F. verticillioides* RC2000, *F. proliferatum* ITEM2443 | 0.95; 0.98; 0.955 | Efficacy of antioxidant mixtures on growth, fumonisin production and hydrolytic enzyme production by *F. verticillioides* and *F. proliferatum* *in vitro* on maize-based media. | **In vitro:** Effect of antioxidant mixtures on lag phases and fungal growth rate: Significant (P < 0.001) increase in the lag phase growth of both fungal strains with BHA+PP treatment at all a_w levels evaluated; PP alone and in combination with BHA (0.5 and 1 mM) reduced growth rates (>85%) of both fungal species at all a_w levels evaluated; PP+BHT and PP+THBP treatments were less effective; FB_{1}, FB_{2} and FB_{3} levels in MMEA cultures: fumonisin levels produced by both fungal species significantly (P < 0.05) reduced with BHA+PP treatments at a_w 0.98 and 0.955; At 0.5 mM some antioxidant treatments resulted in stimulation of fumonisin production; Hydrolytic enzyme activity: All antioxidant treatments alone and in combination resulted in significant (P < 0.001) reduction in total enzyme activity at all a_w levels evaluated | BHA and PP are permitted by the US FDA for use as antimicrobial agents in foods; BHA and PP are considered GRAS; Efficacy of BHA+PP mixtures for biocontrol of *Fusarium* spp. should be evaluated *in planta* | Reynoso et al., 2002 |
### TABLE 3 | Continued

| Biocontrol compound | Fusarium spp. studied | Water activity (a<sub>w</sub>) | Test system with details of experimental model | Reduction criteria | Application | Reference(s) |
|---------------------|-----------------------|-------------------------------|-----------------------------------------------|--------------------|-------------|--------------|
| Commercial phenoic compounds: Benzoic acid, caffeic acid, ferulic acid; vanillic acid; Phenols extracted from plants: Chlorophorin, iroko, and maakianin | F. verticillioides MRC 826 | N/A | Naturally occurring phenols: a detoxification strategy for FB<sub>1</sub>. In vitro: MIC of each compound: Seed ed agar well diffusion technique; Effect on FB<sub>1</sub> production: Alberts' broth supplemented with the respective phenolic compounds (chlorophorin at 0.45, 0.8 and 1 µmol.ml<sup>-1</sup>, all the other compounds at 1 µmol.ml<sup>-1</sup>); determination of FB<sub>1</sub> levels: HPLC analyses | In vitro: MIC of each compound: chlorophorin, iroko, maakianin, vanillic acid and caffeic acid inhibited F. verticillioides growth; maakianin lowest MIC (3 µmol.ml<sup>-1</sup>) and therefore the most effective compound; benzoic acid and ferulic acid had no effect on fungal growth; Effect on FB<sub>1</sub> production: all the compounds, except benzoic acid, reduced FB<sub>1</sub> production (88-94% reduction) | Chlorophorin, iroko, maakianin vanillic acid and caffeic acid are effective in the inhibition of F. verticillioides growth and reduction of FB<sub>1</sub> | Beekrum et al., 2003 |
| BHA and PP | F. verticillioides RC2000, F. proliferatum ITEM2443 | 0.95; 0.98; 0.955 | Potential use of antioxidants for control of growth and fumonisin production by F. verticillioides and F. proliferatum on whole maize grain. In vitro: Fungal growth: rehydrated maize kernels (a<sub>w</sub> 0.95, 0.98 and 0.955); antioxidants incorporated to 100, 200 and 500 µg.g<sup>-1</sup> of maize; maize kernels dispensed as a monolayer in Petri dishes (a<sub>w</sub> 0.95, 0.98 and 0.955); inoculation with mycelial disc; fungal colonization of grains: colony diameters; FB<sub>1</sub>, FB<sub>2</sub> and FB<sub>3</sub> levels in maize kernel cultures: HPLC analyses | In vitro: Fungal growth: combinations of 500 µg.g<sup>-1</sup> of either BHA or PP at a<sub>w</sub> 0.95 resulted in extended lag phases of fungal growth for both species; effective inhibition of growth of both fungal species by BHA and PP at 500 µg.g<sup>-1</sup> at a<sub>w</sub> 0.95; PP more effective than BHA; FB<sub>1</sub>, FB<sub>2</sub> and FB<sub>3</sub> levels in maize kernel cultures: fumonisin production reduced (94-98%) by BHA and PP (500 µg.g<sup>-1</sup>) at a<sub>w</sub> 0.98; Antioxidant treatments less effective at a<sub>w</sub> 0.995 | BHA and PP are considered GRAS; BHA and PP effective in controlling F. verticillioides and F. proliferatum growth and fumonisin production on maize kernels; higher concentrations needed for an effect on whole maize kernels than on MMEA | Torres et al., 2003 |
| 6,7-Dimethoxycoumarin, isolated from Citrus sinensis cultivar Valencia (Valencia orange) | F. verticillioides | N/A | Biocontrol of aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>, and FB<sub>1</sub> with 6,7-dimethoxycoumarin, a phytoalexin from Citrus sinensis. In vitro: Induction of 6,7-dimethoxycoumarin in Citrus sinensis cultivar Valencia: UV irradiation of fruit; infection of fruit with Penicillium digitatum; Antifungal activity; FB<sub>1</sub> levels: HPLC analyses | In vitro: Induction of 6,7-dimethoxycoumarin in Citrus sinensis cultivar Valencia: concentrations of 6,7-dimethoxycoumarin increased from 0.36 to 15.2 µg/g following UV irradiation; concentrations of 6,7-dimethoxycoumarin increased from 0.36 to 35.1 µg/g following infection of fruit with P. digitatum; Antifungal activity: 6,7-dimethoxycoumarin exhibited antifungal activity against F. verticillioides; FB<sub>1</sub> levels: 6,7-dimethoxycoumarin caused reduction of FB<sub>1</sub> production by F. verticillioides | - | Mohanty and Odhav, 2006 |
TABLE 3 | Continued

| Biocontrol compound | Fusarium spp. studied | Water activity ($a_w$) | Test system with details of experimental model | Reduction criteria | Application | Reference(s) |
|---------------------|-----------------------|-------------------------|-----------------------------------------------|-------------------|-------------|--------------|
| Commercial vanillic acid and caffeic acid | *F. verticillioides* Sheldon 25N; *F. proliferatum* Matsushima Nirenberg 73N | 0.88-0.97 | Can phenolic compounds be used for the protection of corn from fungal invasion and mycotoxin contamination during storage? | In vitro: Effect on fungal growth: maize kernels dispensed as a monolayer in Petri dishes; three $a_w$ values (0.88-0.97) and six phenolic compound concentrations incorporated (0-2500 µg g$^{-1}$ maize); inoculation (mycelial disc) and incubation; measurement of colony diameters; Effect on FB$_1$ production: maize kernels dispensed as a monolayer in Petri dishes; $a_w$ 0.96 and a range of phenolic compound concentrations incorporated (0, 1000 and 2000 µg g$^{-1}$ maize); inoculation and incubation; FB$_1$ levels: HPLC analyses. | Potential application as antifungal compounds to protect stored grains; however, high concentrations of phenolic compounds may reduce its efficacy; high concentrations negatively affected the sensory quality of the maize; commercial application possibly not economically feasible | Samapundo et al., 2007 |
| Commercial preparations of natural plant constituents: trans-2-hexenal; carvacrol; eugenol | *F. verticillioides* strain isolated from maize | N/A | Activity of natural compounds on *F. verticillioides* and fumonisin production in stored maize kernels. | In vitro: Effect on conidial germination: acidified PDA; inoculation; compounds (6.2-147.6 µL/L) added to filter paper and placed inside the dish cover; incubation; determination of percentages of conidial germination; determination of MIC; Effect on mycelial growth: acidified PDA; inoculation; compounds (3.1-49.2 µL/L) added to filter paper and placed inside the dish cover; incubation; determination of percentage mycelial growth compared to the control; determination of MIC. | Trans-2-hexenal effective in controlling *F. verticillioides* growth, also in asymptomatic maize kernels; trans-2-hexenal as fumigant penetrates into the internal part of maize kernels. | Menniti et al., 2010 |
| Biocontrol compound | Fusarium spp. studied | Water activity (a_w) | Test system with details of experimental model | Reduction criteria | Application | Reference(s) |
|---------------------|----------------------|---------------------|-----------------------------------------------|--------------------|-------------|--------------|
| Aqueous and organic extracts of weedy plants | F. verticillioides (MRC 826, 8267, 8559); F. proliferatum (MRC 2301, 6908, 7140) | N/A | Antifungal activity of four weedy plant extracts against selected mycotoxigenic fungi. | In vitro: Inhibition of fungal growth; water extracts of all four plant species exhibited no antifungal activity. | Extracts of V. unguiculata and A. spinosus could potentially be applied in crop disease | Thembo et al., 2010 |

(Continued)
| Biocontrol compound | Fusarium spp. studied | Water activity ($a_w$) | Test system with details of experimental model | Reduction criteria | Application | Reference(s) |
|---------------------|-----------------------|------------------------|-----------------------------------------------|-------------------|-------------|--------------|
| collected in the Gauteng and North West Provinces of South Africa: Tagetes minuta; Lippia javanica; Amaranthus spinosus; and Vigna unguiculata | | | In vitro: Preparation of plant extracts: Drying of aerial parts of plants at room temperature; grinding of dried plants into a powder; sequential extraction with hexane, dichloromethane, methanol, and water; drying of extracts; Determination of the MIC: Serial dilution microplate technique | activity at the highest concentration (2.5 mg mL${}^{-1}$); methanol, hexane and dichloromethane extracts of A. spinosus and V. unguiculata exhibited the broadest spectrum antifungal activity after 48 h; all solvent extracts of A. spinosus and V. unguiculata exhibited the highest inhibitory and stability effects over 120 h against all Fusarium strains. Stability of plant extracts over 120 h: dichloromethane extracts loses its activity more rapidly than methanol and hexane extracts | | |
| THC compounds: F. proliferatum INRA 212 | N/A | In vitro inhibitory effect of tetrahydrocurcuminoids on F. proliferatum growth and FB1 biosynthesis. In vitro: Antifungal activity of THC1: THC1 (2.7, 8.1, and 13.4 µmol mL${}^{-1}$ THC1) solution distributed on surface of PDA plates and air dried; inoculation with F. proliferatum and incubation; determination of fungal growth: measurement of colony diameter; determination of inhibition percentage: radial growth in relation to the control; comparison with results from THC2 and THC3; Effect on FB1 levels: Cultivation in GYEP liquid medium; inoculation; cultures supplemented with THC1 (0.8, 1.3, 1.9, 2.7 µmol mL${}^{-1}$); incubation; FB1 levels: HPLC analyses | THC compounds are promising biocontrol agents due to low inhibitory concentrations; THC1 is a food-grade compound and can be produced on large scale for industrial application | Coma et al., 2011 |
| THC compounds from the roots of Curcuma longa L. (Turmeric) | F. verticillioides N/A | Antimicrobial activity of G. pentaphyllum extracts against fungi producing aflatoxin and fumonisin and bacteria causing diarrheal disease. In vitro: Antifungal activity | G. pentaphyllum is frequently being applied as herbal medicine; extracts could be applied to control F. verticillioides growth | Srichana et al., 2011 |
| Extracts of Gynostemma pentaphyllum (Southern Ginseng) | F. verticillioides N/A | In vitro: Antifungal activity; extracts exhibited antifungal activity against F. verticillioides growth (41-43% reduction) | | |
| 70% Ethanol extracts of Equisetum arvense (Horsetail) and Stevia rebaudiana (Candyleaf) | F. verticillioides (UdL-TA 3.215) 0.93-0.95 | Effect of extracts on growth and mycotoxin production by A. flavus and F. verticillioides in maize seeds as affected by water activity. In vitro: Fungal growth: preparation of maize kernels ($a_w$ levels adjusted to 0.93 and 0.95) | In vitro: Effect of plant extracts on fungal growth: extracts of S. rebaudiana significantly reduced CFU counts of F. verticillioides; (>99% reduction; $a_w$ 0.95); E. arvense reduced CFU counts of F. verticillioides at $a_w$ levels 0.93 and 0.95, but not as effective as S. rebaudiana; | - | Garcia et al., 2012 |
| Biocontrol compound | Fusarium spp. studied | Water activity ($a_w$) | Test system with details of experimental model | Reduction criteria | Application | Reference(s) |
|---------------------|-----------------------|-------------------------|-----------------------------------------------|-------------------|-------------|--------------|
|                    | 0.95, respectively) and supplementation with plant extracts, separately and in 1:1 mixtures, respectively; maize kernels in single layers in Petri dishes; inoculation and incubation; determination of CFU counts after 10, 20 and 30 days of incubation by employing a selective medium for *Fusarium* spp.; FB$_1$ and FB$_2$ levels in maize cultures: HPLC analyses | significant ($P < 0.05$) stimulation of growth observed in a few cases; FB$_1$ and FB$_2$ levels in maize cultures: fumonisin production was not significantly affected | Cinnamon and oregano oils could be effective in controlling growth and FB$_1$ production by *F. proliferatum* in maize pre-harvest | Velluti et al., 2003 |

**ESSENTIAL OILS**

| Essential oils extracted from cinnamon, clove, oregano, palmarose and lemongrass | *F. proliferatum* (three different isolates) | 0.95 and 0.995 | Inhibitory effect of cinnamon, clove, lemongrass, oregano and palmarose essential oils on growth and FB$_1$ production by *F. proliferatum* in maize grain. | In vitro: Effect of essentials oils on growth rate of *F. proliferatum*: All five essential oils had a significant ($P < 0.05$) inhibitory effect on growth of *F. proliferatum* at $a_w$ 0.995 at both temperatures; At $a_w$ 0.95, the effect of essential oils on growth rates was dependent on the temperature; incubation at 20$^\circ$C: Oil of cinnamon, clove and oregano (1000$\mu$g essential oil.g$^{-1}$ of maize) had a significant ($P < 0.05$) inhibitory effect on *F. proliferatum* growth; at concentrations of 500 $\mu$g essential oil.g$^{-1}$ of maize only cinnamon and oregano were effective; incubation at 30$^\circ$C: none of the essential oils analyzed had an inhibitory effect on any of the fungal growth rates; FB$_1$ levels in maize cultures: At $a_w$ 0.995 and both temperatures, cinnamon, oregano and palmarose oils had a significant ($P < 0.05$) inhibitory effect on FB$_1$ production by all three fungal strains; clove and lemongrass oils only exhibited a significant inhibitory effect at 30$^\circ$C; At $a_w$ 0.950 none of the essential oils had a significant effect on FB$_1$ production; essential oil concentration did not affect FB$_1$ production | Velluti et al., 2003 |

Culture conditions: single layer of maize kernels in Petri dishes; inoculation (agar disk method); variables: essential oil concentration; water activity; temperature (20 and 30$^\circ$C), fungal isolates; Fungal growth; measurement of colony diameter; FB$_1$ levels in maize cultures: HPLC analyses
| Biocontrol compound | *Fusarium* spp. studied | Water activity (a<sub>w</sub>) | Test system with details of experimental model | Reduction criteria | Application | Reference(s) |
|---------------------|-------------------------|-------------------------------|-----------------------------------------------|-------------------|-------------|--------------|
| Essential oils and oleoresins extracted from *Zingiber officinale* (Ginger); Synthetic antioxidants BHA, BHT and PG | *F. verticillioides* (= *F. moniliforme*) | N/A | Chemistry, antioxidant and antimicrobial investigations on essential oil and oleoresins of *Z. officinale*. In vitro: Extraction from *Z. officinale* rhizomes: essential oils were extracted by hydrodistillation; oleoresins were extracted with ethanol, methanol, carbon tetrachloride and isooctane, respectively; Phytochemistry and identification of extracted components: GC-MS; Antioxidant activity of components compared with BHA, BHT and PG; peroxide-, anisidine- and thiobarbituric acid values; DPPH radical scavenging and total antioxidant activity by ferric thiocyanate methods; Antifungal activity: “Poisoned food” technique: ginger oil and oleoresins (2, 4, and 6 µl) mixed with CDA culture medium and poured into Petri plates; inoculation (mycelial discs) and incubation; measurement of radial growth; average colony diameters; calculation of the percentage mycelial zone inhibition; In vitro: Extraction from *Z. officinale* rhizomes: a large number of components extracted; major components: geranial (essential oil), eugenol (ethanol oleoresin extract) and s-furane (methanol, carbon tetrachloride and iso-octane oleoresin extracts); Antioxidant activity of components compared with BHA, BHT and PG: the presence of the essential oils, oleoresins and antioxidants resulted in reduced peroxide- and anisidine values and DPPH radical concentration; antioxidant activity of essential oils and oleoresins is comparable to BHA and BHT, but less than PG; the essential oils and ethanol oleoresin extracts exhibited better antioxidant activity than other oleoresins and the synthetic antioxidants; Antifungal activity of components: essential oils and oleoresins moderate to good inhibition *F. verticillioides* growth; ginger oil and the CCl<sub>4</sub> oleoresin extract (6 µl dose of each) highly effective against *F. verticillioides* growth (100% inhibition); Essential oils generally more effective than the oleoresins | Preservation of edible oils and other foodstuffs against autoxidation and microbial spoilage | Singh et al., 2008 |
Antioxidants

The food-grade antioxidants butylated hydroxyanisole (BHA) and propylparaben (PP) have shown potential for controlling F. verticillioides and F. proliferatum growth and fumonisin production at a variety of water activities and incubation temperatures in vitro (Etcheverry et al., 2002; Table 3). Both fungal species were more sensitive to BHA and PP than the other antioxidants evaluated, i.e., trihydroxybutyrophenone (THBP) and butylated hydroxytoluene (BHT). In another study, combination treatments of BHA and PP resulted in further reduction of fumonisin production (Reynoso et al., 2002). BHA, PP, and BHT alone or in combination also resulted in a significant ($P < 0.001$) reduction in hydrolytic enzyme activity, which is required for early fungal growth. Similar results were reported by Torres et al. (2003). BHA is produced naturally by Botryococcus braunii, Cylindropermopsis raciborskii, Microcystis aeruginosa, and Oscillatoria sp., while PP is a natural compound extracted from plants. Both antioxidants are also produced synthetically, are considered GRAS by the US FDA and frequently employed as preservatives in the food and cosmetic industries (Reynoso et al., 2002; Rawal et al., 2010; US FDA, GRAS substances evaluated by US FDA, GRAS substances evaluated by the US FDA, 2003; Samapundo et al., 2007 and antifungal activities differ between the respective THC (Ginger) rhizomes exhibit clear antimicrobial activity against F. moniliforme in vitro (Singh et al., 2008; Table 3). Ginger oil and carbon tetrachloride oleoresin extracts have shown highly effective inhibition of F. verticillioides growth. The antioxidative potential of the essential oil and oleoresins, in terms of peroxide content, anisidine and thiobarbituric acid values, 1,1-diphenyl-2-picrylhydrazyl free radical scavenging activity and total antioxidant activity was in general comparable to the antioxidants BHA and BHT, but not as effective as propyl gallate. The phenolic compound geranial is dominant in the oleoresin biosynthetic pathway; effects on colony morphology; granulation of the cytoplasm; and rupture of the cytoplasmic membrane (Garcia et al., 2012).

Phenolic Compounds

Investigations into the effects of the natural phenolic compounds vanillic and caffeic acid on F. verticillioides and F. proliferatum growth and FB₁ production at different water activities in maize in vitro indicated that an increase in phenolic compound concentration results in an increase in the lag phase of growth, and a decrease in fungal growth rate and FB₁ production (Samapundo et al., 2007; Table 3). In general, complete inhibition of Fusarium growth was observed at relatively high phenolic concentrations and low water activities. F. proliferatum was more sensitive, exhibiting complete inhibition of growth in the presence of the compounds. Both compounds significantly reduced FB₁ production by F. verticillioides and F. proliferatum, with vanillic acid being more effective. No FB₁ was produced by F. verticillioides in the presence of vanillic acid at the lowest concentration tested.

F. verticillioides growth and FB₁ production are inhibited by several other plant phenolic compounds in vitro (Table 3). Chlorophorin, iroko, maakianin, vanillic acid, and caffeic acid inhibits F. verticillioides growth, while FB₁ production is inhibited by chlorophorin, iroko, vanillic acid, caffeic acid, and ferulic acid (Beekrum et al., 2003; Table 3). Flavonoids, phenolic acid, and terpine rich 70% ethanol extracts of the non-toxic food-grade plants Equisetum arvense (Horsetail) and Stevia rebaudiana (Candyleaf), effectively inhibited F. verticillioides growth, with S. rebaudiana being more effective (Garcia et al., 2012). However, fumonisin production was not affected. Essential oils of the herbaceous climbing vine of the family Cucurbitaceae, Gynostemma pentaphyllum (Southern Ginseng), inhibited growth of F. verticillioides (Srichana et al., 2011). G. pentaphyllum is frequently applied as herbal medicine and exhibits high antioxidant activity. Fumigation by trans-2-hexanal (extracted from fruits and vegetables), carvacrol (extracted from oregano and thyme), and eugenol (extracted from cinnamon and clove) effectively inhibits F. verticillioides conidial germination and mycelial growth in maize kernels, with trans-2-hexanal the most effective (Menniti et al., 2010). Trans-2-hexanal fumigation was also effective in controlling the fungus in asymptomatic kernels. However, the treatment does not reduce fumonisin levels post-harvest, but reduces the germ-ability of maize kernels. The compound 6,7-dimethoxycoumarin, occurring in Penicillium digitatum infected Citrus sinensis cultivar Valencia fruit (Valencia orange), reduces F. verticillioides growth and FB₁ production (Mohanlall and Odhav, 2006). Possible mechanisms of inhibition by phenolic plant extracts include disruption of the fumonisin biosynthetic pathway; effects on colony morphology; granulation of the cytoplasm; and rupture of the cytoplasmic membrane (Garcia et al., 2012).
by *F. verticillioides* and *F. proliferatum* in vitro (Velluti et al., 2003; Table 3). The inhibitory effect of the essential oils was overall more pronounced at higher water activities, probably due to more effective penetration of oils into kernels in the presence of water. The antimicrobial activity of these oils could be attributed to the presence of aliphatic alcohols and phenols in their chemical composition. Oils of cinnamon and oregano were most promising for control of fungal growth and FB1 production by *F. proliferatum*, and cinnamon, oregano and lemongrass oils for *F. verticillioides*. These oils could be effective in controlling fungal growth and FB1 production in maize under pre-harvest conditions.

### Developing Resistant Crops through Breeding and Genetic Engineering

Studies in breeding and genetic engineering for resistance in crops are mainly aimed at preventing invasion by insects, contamination by mycotoxinogenic fungi and detoxification of mycotoxins in planta through various molecular strategies (Duvick, 2001; Cleveland et al., 2003). Selection of resistant genotypes is complex, it requires sufficient genotypic variation within the breeding material; is affected by climatic conditions; and should be tested across several locations and years (Löffler et al., 2010). Lower mycotoxin levels measured in United States and Canadian maize, where no fungicide was introduced, was attributed to successes with breeding resistant maize varieties.

Extensive genomic resources are essential for investigations into the biochemical and regulatory pathways of mycotoxin biosynthesis, pathogenesis of fungal–plant interactions, and the development of targeted and innovative approaches for breeding and engineering crops for resistance (Cleveland et al., 2003; Brown et al., 2006; Desjardins and Proctor, 2007). Whole genome sequences and expression sequence tags (ESTs) are important tools for understanding disease caused by fungi, fungal lifecycles and secondary metabolism. Available genomic resources include genetic maps, genome sequences, an EST library, and an integrated gene index. Next-generation RNA sequencing was used to study transcriptional changes associated with *F. verticillioides* inoculation in resistant and susceptible maize genotypes by including an extensive range of maize inbred lines (Lanubile et al., 2014). The technique generated extremely useful data on genetic markers involved in recognition, signaling, and controlling host resistance mechanisms. It also provided quantification of expression, thus enabling interpretation of defense responses. The data provides an important genomic resource for the development of disease resistant maize genotypes. Genetic markers identified through this technique could be added to existing information on single nucleotide polymorphism markers.

### Natural Resistance in Crops

Comprehensive knowledge on the biochemical and molecular mechanisms involved in natural resistance of crops is imperative for the further development of resistance to *Fusarium* infection and insect infestation in crops (Cleveland et al., 2003). The whole genome sequence of maize is available (Schnable et al., 2009), permitting genome-wide expression analysis of the maize–*Fusarium* interaction. Studying maize varieties with varying degrees of resistance enables researchers to associate resistant crops with specific genetic, biochemical and anatomical traits. Regions on chromosomes associated with natural resistance to insect invasion, fungal contamination, or mycotoxin production are identified, resistant traits mapped and resistant lines crossed with commercially acceptable lines. Chromosomal regions could be associated with resistance to fungal growth; with mycotoxin production; or with both traits, indicating the possibility of separate genetic control (Cleveland et al., 2003). Comparison of kernel protein profiles between susceptible and resistant genotypes through proteomic analyses contributes to identifying resistance associated proteins. Resistant inbred lines are distinguished from susceptible lines and serve as sources of resistant germplasm.

Expression profiles for maize genes during infection with *F. verticillioides* indicated up-regulation of genes encoding a range of proteins related to cell rescue, defense, and virulence in both resistant and susceptible maize lines, including pathogenesis related (PR) proteins (e.g., chitinase (reducing chitin in fungal membrane); permatin (fungal hyphae leak and rupture)); proteins involved in detoxification response (e.g., cytochrome P450 monooxygenase, peroxidases, and glutathione-S-transferases); heat-shock proteins (regulating folding of resistance proteins); and proteinase inhibitors (Lanubile et al., 2010). Resistance in maize lines could be due to constitutive defense mechanisms that resist fungal infection (Lanubile et al., 2010; Campos-Bermudez et al., 2013). In resistant maize lines defense-related genes, encoding constitutively expressed PR, detoxification enzymes, and β-glucosidases, were transcribed at high levels before infection, and provided defense against the fungus. In susceptible maize lines, defense genes are induced as a response to pathogen infection, though not sufficiently enough to prevent progress of the disease.

Host–pathogen recognition and interaction processes underlie resistance and susceptibility (Campos-Bermudez et al., 2013). Sucrose is one of the compounds that play an important role in host-pathogen recognition and in the outcome of interactions. During fungal infection plant carbohydrate metabolism is manipulated by induced invertase and sucrose synthase enzymes and the formation of hexoses required for fungal growth. Maize lipoygenase (*ZmLOX*) derived oxylipins (e.g., jasmionic acid) are known for regulating plant defense against pathogens, and also play an important role in recognition during host-pathogen interactions, as indicated by up-regulation of LOX genes *ZmLOX5* and *ZmLOX12* in a response to *F. verticillioides* infection (Maschietto et al., 2015).

Mapping of chromosomal regions encoding *Fusarium* ear mold resistance as quantitative trait loci (QTL) and the employment of marker-assisted QTL in selection for *Fusarium* ear mold resistance are valuable tools being developed for maize hybrid development (Duvick, 2001). Ear mold resistance can be mapped as QTL using large segregating plant populations. Molecular markers linked to these QTL could be valuable during inbred development. Other factors that enhance the susceptibility of maize genotypes include: late-maturing cultivars...
where grain moisture content decreases slowly; upright cobs and thin grain pericarp which increase susceptibility to fungal infection; tightness of husks; and the competitive advantage of *F. verticillioides* by having a broader optimum temperature range than *F. graminearum* (Butrón et al., 2006).

**Genetic Engineering for Resistance to Insect Infestation and *Fusarium* Infection in Crops**

Natural fungal and insect resistance mechanisms could be further enhanced in commercially acceptable crops through genetic engineering (Cleveland et al., 2003). The role of hemicellulose, cysteine protease, peroxidase, *α*-amylase inhibitors, as well as maize ribosomal inactivating protein in insect resistance mechanisms are important focus areas. Genetically modified *Bt* maize expressing *cry* proteins from the bacterium *Bacillus thuringiensis*, has the potential to reduce insect damage and fumonisin levels compared to non-*Bt* hybrids. Furthermore, chitinase enzymes for digestion of chitin, an integral part of the exoskeleton of insects, have been applied for control of *Sesamia inferens* (corn borer; Osman et al., 2015). A chitinase gene from the cotton leaf worm, *Spodoptera littoralis*, was expressed in transgenic maize, and resulted in enhanced resistance against *S. cretica*. The development of transgene resistance to fungal disease appears to be more challenging than insect resistance (Duvick, 2001). Although, moderate resistance was demonstrated in model systems, no transgenic crops with effective resistance to fungal disease are commercially available. However, genetics of *Fusarium* infection of maize kernels, development of disease symptoms and biosynthesis of fumonisins is a rapid developing field and could provide more insights for developing transgenic resistance to *Fusarium* infection in the near future.

Genetic engineering approaches include the cloning and expression of genes encoding maize secondary metabolites with antifungal properties and the overexpression of pathway-limiting enzymes (Duvick, 2001). However, it should be kept in mind that diversion of metabolic pathways could compromise other vital biosynthetic routes. Expression of antifungal protein in tissue critical for fungal infection could be a strategy, while different types of resistance could be employed by pyramiding different types of resistance genes into commercial germplasm. Host plant–pathogen interactions are complex, involving multiple proteins and metabolites as well as competition for biomass and nutrients. Signaling pathway genes control a variety of cellular defense pathways involving protein–protein interactions. Engineering of the main signals controlling defense gene expression could result in more effective defense response including constitutive response or a chemically induced response and the development of enhanced disease resistance phenotypes.

Another approach involves the expression of catabolic enzymes to detoxify mycotoxins *in situ* before it accumulates in the plants (Duvick, 2001). Success depends on several factors: the extent to which the plant-produced enzyme reaches its target substrate and the stability of the detoxification step; enzyme localization in the seed in relation to mycotoxin accessibility; kinetic parameters of the enzyme in the context of its localization in the plant; stability and activity of the enzyme pre- and post-harvest; and the identity and toxicity of breakdown products.

**Bt Maize**

Genetic modification of maize plants to express insecticidal *Cry* proteins of *Bacillus thuringiensis* (called *Bt* maize) provides a safe and highly effective method for insect control and accompanying *Fusarium* infection and fumonisin production (Betz et al., 2000). Corn borers cause considerable damage to maize stalk and ear tissue, which in turn stimulates germination of *F. verticillioides* spores, leading to progressive ear and kernel rot and eventually production of increased levels of fumonisins. A significant correlation was reported between the extent of insect damage and total fumonisin levels in maize (Dowd, 2001). *Cry1Ab* protein in *Bt* protected maize reduces corn-borer damage in maize dramatically, resulting in considerable less *Fusarium* infection and reduced fumonisin levels (Betz et al., 2000). *Cry* proteins are selectively active against a specific range of insects including lepidopteron and coleopteran insect pests. Extensive field trials across the USA and Europe confirmed frequently lower fumonisin concentrations detected in maize using *Bt* maize hybrids (Hammond et al., 2004), thereby increasing the percentage maize grain suitable for human consumption. In South Africa, there has been a decrease over the last 20 years in the amount of chemical insecticides used, due to the cultivation of *Bt* crops (Kunert, 2011). In the US States the annual benefits that *Bt* maize provides in terms of lower fumonisin and aflatoxin contamination are estimated at about $23 million (Wu, 2006). *Bt* maize could especially be a useful tool in developing countries.

The insecticidal nature of the *Cry* proteins has led to the development of a variety of commercial *Bt* microbial pesticide products since 1961 (Betz et al., 2000). Extensive toxicological studies by the US Environmental Protection Agency (EPA) and the World Health Organisation (WHO) have proven the safety of *Bt* protected crops and products to humans, animals and the environment [US EPA, 1998a,b; International Programme on Chemical Safety (IPCS), 1999]. Food derived from *Bt* crops has also been fully approved by numerous regulatory agencies throughout the world. Safety considerations were further supported by the more than 50 years history of safe use of these products (McCintock et al., 1995). The potential for human and non-target exposure is extremely low, as *Cry* proteins exhibit a high degree of specificity toward the target insect species, should be ingested to activate in the target species and should have no contact activity (Betz et al., 2000). *Bt* products are considered to reduce the risks posed by insecticides, thereby impacting less on the environment. It also functions as a supplementary pest control by enhancing the presence of beneficial natural occurring non-target insects (Gianessi and Carpenter, 1999). The cultivation of *Bt* protected maize by growers increased rapidly throughout the world since its commercial introduction in 1996 (Betz et al., 2000). Grower approval could be ascribed to increased crop yields, reduced crop damage and input costs as a result of reduction in the use of chemical pesticides; and highly effective pest control. *Cry* proteins in the plant tissue are not affected by application timing, accuracy of application, concentration, rain or sunlight. *Bt* crops are entirely equivalent to non-recombinant
plants, except for the presence of cry genes and proteins. Bt protected crops and products meet important standards for biological control agents regarding technical viability, need, safety and efficacy. 

Recently, increasing insect resistance and accompanied occurrence of resistance alleles in insects against first generation Bt crops have been reported (Kunert, 2011; Abbas et al., 2013). Efforts to reduce the development of target insect resistance to Bt crops include introduction of a refuge strategy, which involves the cultivation of non-Bt crops nearby Bt crops to prevent domination of resistant insect species. The effectiveness of Bt crops is also influenced by fluctuation of the Bt protein concentrations produced in plants, which in turn is determined by factors such as plant maturation and photosynthesis. Possible structural changes of Bt proteins, including changes in microRNA and protein profiles were also reported. Bt maize genotype plays a determining role in the efficacy of insect damage control (Clements et al., 2003). Bt (Cry1Ab) protein protected plants could reduce fumonisin concentration in maize during seasons when the European corn borer (O. nubilalis Hübner) dominates, but not in seasons when the corn earworm (H. zea Boddie) dominates. Tende et al. (2010) evaluated sensitivity of the stalk borer species Chileus partellus (Lepidoptera, Crambidae) and Busseola fusca (Lepidoptera, Noctuidae) toward endotoxins constitutively produced by two Bt maize inbred lines frequently cultivated in Kenya. The Bt maize inbred lines (Event 223 cry1AB::Ubiquitin and Event 10 cry1Ba::Ubiquitin) reduced C. partellus survival significantly and sensitivity remained constant through eight generations. However, B. fusca invasion could not be sufficiently controlled by these inbred lines and remained unchanged through five generations. More efficient transgenic Bt crops could be produced through gene pyramiding (Kunert, 2011).

**POST-HARVEST BIOLOGICALLY BASED CONTROL METHODS FOR REDUCTION OF THE FUMONISINS IN FOOD AND FEED**

**Natural Clay Adsorbents**

Introduction of natural clay adsorbents during food processing leads to detoxification of contaminated food through adsorption of mycotoxins (Aly et al., 2004; Robinson et al., 2012). The bioavailability of mycotoxins in animal feed is also reduced in this manner, thereby preventing toxic interactions and absorption across the gastrointestinal tract.

Montmorillonites are a group of phyllosilicate clay minerals that have the ability to adsorb organic compounds through cation-exchange (Aly et al., 2004). The adsorption abilities of montmorillonite clays are higher than other clay minerals due to their large molecular structure and surface area that increases considerably when wet. Their chemical structures are characterized by alternating layers of tetrahedral silicon and octahedral aluminum coordinated with oxygen atoms. Montmorillonite clay minerals effectively reduce FB1 in aqueous solutions in vitro, and in human- and animal models in vivo through adsorption (Table 4). The adsorption is saturable and occurs largely within the interlaminar regions of the clay (Mitchell et al., 2013). Certain clay minerals, particularly naturally occurring aluminum oxides have structure-selective affinities for different mycotoxins and the degree of adsorption depends on the polarity of the molecules, while the particle size of clays could also influence binding affinity (He and Zhou, 2010). A correlation exists between the binding capacity of the clays and the ratio of their surface acidity to pore volume. In this regard, the slightly higher adsorption of AFB1 than FB1 to hydrated sodium calcium magnesium silicate hydroxide (Egyptian montmorillonite, EM) and hydrated sodium calcium aluminum silicate (HSCAS) in spiked malt extracts, could be ascribed to the difference in polarity between the molecules (Aly et al., 2004). The adsorption capacity of montmorillonite clays can be enhanced by addition of phosphate and polyphosphate salts, bentonite, or calcined attapulgite (He and Zhou, 2010). A combination of clay minerals (1–10%) and modified yeast cell wall extracts (90–99%) could be beneficial for adsorption of multiple mycotoxins, including the fumonisins (Howes and Newman, 2000).

Because natural clay mineral adsorbents are considered GRAS by the US FDA (2015), they could be applied effectively and economically in the food and feed industries and several clay minerals have been proven to be acceptable for commercial uses [US FDA, GRAS substances evaluated by the Select Committee on GRAS substances (SCOGS); He and Zhou, 2010]. However, application of clay minerals often requires high levels to be included into animal feed; interaction of natural clays with food- and gut-based nutrients remains unclear; and the possibility of accumulation of dioxin (a toxic trace component in montmorillonite) in animals remains a concern.

**Microbial Transformation of the Fumonisins**

Development of control methods to detoxify the fumonisins through transformation should be directed toward deamination of the free amino group at C-2 and hydrolysis of the ester bonds at C-14 and C-15 (Gelderblom et al., 1993). Microorganisms capable of transforming FB1 to less toxic end products include Exophiala spinifera ATCC 74269, Rhinocladiella atrovirens ATCC 74270, Bacterium ATCC 55552, and Sphingopyxis macrogoltabida MTA144 (Duvick et al., 1998a,b; Blackwell et al., 1999; Heinl et al., 2010). Transformation of FB1 by the black-yeast E. spinifera was mainly achieved through decarboxylation by inducible extracellular esterase enzymes and amino oxidases converting hydrolysed fumonisin (HFB1) to unknown end products. Degradation by Bacterium ATCC 55552 and S. macrogoltabida MTA144 is achieved through de-esterification by carboxylesterases and subsequent deamination of HFB1 by aminotransferases, with the formation of 2-keto HFB1 (Heinl et al., 2010; Hartinger et al., 2011). The microbial gene sequences coding for these enzymes were determined by employing degenerate polymerase chain reaction (PCR) primers, inverse PCR and gene walking techniques. Carboxylesterase (FumD) and aminotransferase enzymes (FumI) of S. macrogoltabida MTA144 and Bacterium ATCC 55552 were expressed in Pichia pastoris.
### Current information on reduction of fumonisin B₁ in aqueous solutions (in vitro), and human and animal models (in vivo) through adsorption to clay minerals.

| Clay mineral | Test system with details of experimental model | Reduction criteria | Application | Reference(s) |
|--------------|-----------------------------------------------|--------------------|-------------|---------------|
| HSCAS; EM    | Application of adsorbent agent technology in the removal of AFB₁ and FB₁ from malt extract.  
In vitro: Adsorption ability of HSCAS and EM for FB₁ in aqueous solutions: Adsorbents (0.5; 1; 2; 4% w/v) weighed out in glass tubes; FB₁ added (5, 10 and 50 ppm in aqueous solution); reaction: 1 h at 25°C; centrifugation; determination of FB₁ levels in the supernatant; Adsorption ability of HSCAS and EM for FB₁ in aqueous malt extract: preparation of FB₁ contaminated malt (50, 100 and 200 ppm FB₁); preparation of malt extract: steeping of spiked malt extract; collection of steep; addition of HSCAS and EM (0.5% w/v); shaking for 30 min; centrifugation; filtration; determination of FB₁ levels in filtrate; FB₁ levels: HPLC analyses. | In vitro: Adsorption ability of HSCAS and EM for FB₁ in aqueous solutions: Both sorbents (0.5% w/v) exhibited high affinity to adsorb to FB₁ in aqueous solutions at different contamination levels: Adsorption ability of HSCAS and EM for FB₁ in aqueous solutions: adsorption ability of HSCAS 85.1-92.4%; adsorption ability of EM 78.2–92.2%; lower levels of adsorbents (0.5%) resulted in more effective adsorption; Adsorption ability of HSCAS and EM (both 0.5% w/v) for FB₁ in aqueous malt extract: adsorption ability of HSCAS 85.25–91.97%; adsorption ability of EM 88.4–92.47% | Food and beverage industries: removal of FB₁ from aqueous solutions, i.e. during the extraction of malt | Aly et al., 2004 |
| NS (Novasil)  | Calcium montmorillonite clay reduces urinary biomarkers of FB₁ exposure in rats and humans.  
In vivo: Rodent model: Male Fisher 344 rats; FB₁ and NS added to feed; treatment groups: absolute control, FB₁ control, and FB₁ plus NS (2% w/w); acclimation period; FB₁ dosage (25 mg/kg bw) based on an average of 150 g bw; supplemented feed administered to rats by single aqueous gavage; urine samples collected daily; Human study: participants recruited from six communities within the Ejura-Sekyedumase district of Ghana; three study groups: High dose (NS 3 g/day; low dose (NS 1.5 g/day) and placebo control; study period: 3 months; collection of urine at multiple time points; UFB₁ biomarker levels: HPLC analyses; Creatinine levels in urine samples: MALDI-TOF MS. | In vivo: Effect of dietary NS on UFB₁ levels in rats and humans: NS significantly reduced the excretion UFB₁ in urine: Rodent model: NS treatment significantly reduced UFB₁ by 20% in 24 h and 50% after 48 h; Human study: week 8 and 10 high and low dose NS treatments resulted in decreased percentage of participants with detectable UFB₁; median levels of the high dose group at week 8 were significantly (P < 0.05) lower than the placebo group; week 10 median UFB₁ levels for both high and low dose groups were significantly (P < 0.05) reduced. | Reduction of fumonisin exposure in communities at risk in Ghana: NS could be a suitable enterosorbent for reduction of the bioavailability of fumonsins in the gastrointestinal tract of animals and humans; intervention methods in the form of capsules or other dose forms; further studies: to determine whether a time-related effect exists, to confirm the efficacy and safety of NS clay as a multifunctional intervention and to determine the nutritional implications of NS supplementation of diets | Robinson et al., 2012 |

(Continued)
| Clay mineral | Test system with details of experimental model | Reduction criteria | Application | Reference(s) |
|--------------|---------------------------------------------|-------------------|-------------|--------------|
| Refined UPSN, particle size 45-100μm | Calcium montmorillonite clay reduces AFB$_1$ and FB$_1$ biomarkers in rats exposed to single and co-exposures of aflatoxin and fumonisin. In vivo: Rodent model: Male Fisher 344 rats; treatment groups: absolute control, AFB$_1$ (25 mg/kg bw) treatment, AFB$_1$ (25 mg/kg bw) + FB$_1$ (0.125 mg/kg bw) treatment; FB$_1$ treatment groups were supplemented with UPSN (0%, 0.25%, and 2%); acclimation period; supplemented feed administered to rats by single aqueous gavage; collection of urine at multiple time points over 72 h; UFB$_1$ levels: HPLC analyses | In vivo: FB$_1$; FB$_1$ treatment: UPSN (2% w/w) significantly ($P < 0.0001$) decreased UFB$_1$ levels at 12, 24 and 36 h; 2% UPSN treatment more effective than the 0.25% UPSN treatment: 2% UPSN treatment 85 and 98% reduction at 12 and 24 h, respectively; 0.25% UPSN treatment 45 and 55% reduction at 12 and 24 h, respectively; AFB$_1$/FB$_1$ co-treatment: Lower efficacy than with separate UPSN treatments; a dose-dependent reduction in UFB$_1$ for the UPSN treated AFB$_1$/FB$_1$ groups: 2% UPSN more effective than the 0.25% UPSN treatment: 2% UPSN treatment 51 and 59% reduction at 12 and 24 h, respectively; 0.25% UPSN treatment 28 and 39% reduction at 12 and 24 h, respectively; 2% UPSN treatment significant reduction at 12 h ($P < 0.0177$), 24 h ($P < 0.0234$ and 72 h ($P < 0.0001$); 0.25% UPSN treatment: reduction only statistically significant at 72h ($P < 0.0036$); AFB$_1$; UPSN treatment reduced AFM$_1$ biomarkers in a dose-dependent manner with the largest reduction in the 2% treatment group (97 and 99% reduction after 12 and 24 h, respectively); AFB$_1$/FB$_1$ co-treatment: Lower efficacy than with separate UPSN treatments; UPSN treatment dose-dependently reduced AFM$_1$ excretion; 0.25% UPSN treatment more effective than the 2% treatment group | Economical and sustainable intervention to reduce exposure to FB$_1$ and AFB$_1$; utilization of the clay as a binder for both FB$_1$ and AFB$_1$; application could selectively reduce levels below carcinogenic thresholds | Mitchell et al., 2013 |

HSCAS, Hydrated sodium calcium aluminum silicate; EM, Egyptian montmorillonite (Hydrated sodium calcium aluminum magnesium silicate hydroxide); NS, Calcium montmorillonite; UPSN, calcium montmorillonite Uniform particle size Novasil; FB$_1$, Fumonisin B$_1$; UFB$_1$, Urinary FB$_1$, AFB$_1$, Aflatoxin B$_1$, AFM$_1$, Aflatoxin M$_1$; Bw, Body weight.
| Method of mycotoxin reduction | Test system with details of experimental model | Reduction criteria | Application | Reference(s) |
|-------------------------------|-----------------------------------------------|--------------------|-------------|--------------|
| Hand-sorting of maize         | Occurrence of Fusarium spp. and mycotoxins in Nepalese maize and wheat and the effect of traditional processing methods on mycotoxin levels. Field study; Study area: Kathmandu, Nepal; Maize samples: purchased at a market in Kathmandu; the samples contained large amounts of visibly diseased kernels; Hand-sorting: participants: four trained plant pathologists, three untrained urban women, five women from smallholder farms in the Lamjung district of Nepal; removal of visibly diseased kernels; maximizing the recovery of the starting sample; Fumonisin and DON levels: immunoassay or HPLC | Field study; Hand-sorting: all participants were able to produce a product with acceptable fumonisin and DON levels; large differences between participants with regards to maximizing the recovery of the starting sample; plant pathologists and two rural women (86% recovery), three untrained urban women and three rural women (49% recovery); Fumonisin and DON levels: maize samples prior to hand-sorting: > 1000 ng toxin/g maize; maize samples after hand-sorting: < 1000 ng toxin/g maize | Hand-sorting is economically viable for populations with limited food resources; most of the starting material should be recovered in the cleaned product; educational campaigns to raise awareness among Nepalese consumers on the occurrence of mycotoxins in maize and the efficacy of hand-sorting methods | Desjardins et al., 2000 |
| Hand-sorting, winnowing, washing, crushing, and dehulling of maize | Fate of aflatoxins and fumonisins during the processing of maize into food products in Benin. In vitro: Impact of sorting, winnowing, washing, and crushing of maize on fumonisin levels in maize intended for the preparation of traditional maize-based food: Sorting: removal of visibly moldy, insect damaged and broken kernels; Winnowing (complementary to sorting): removal of impurities from sorted maize by collecting maize in a metallic tray, throwing contents into the air and allowing impurities and broken kernels to be blown away; Maize washing (complementary to sorting and winnowing): maize to water ratio 1:2 (w/v); hand rubbing of kernels (15 min); removal of floating grains and impurities; Crushing and dehulling (complementary to sorting, winnowing and washing) (removal of pericarp and embryo): crushing with plate disc mill; sieving to obtain separately grits, hulls and fine fractions; hand washing of grits (10-15 min); soaking in water (2 l) [grits to water ratio 1:3 (w/v)]; Total fumonisin levels in fractions: ELIZA (VICAM) | In vitro: Sorting and winnowing: 68.75% reduction in total fumonisin content of maize; total fumonisin levels were high in the moldy and damaged kernels; Maize washing (complementary to sorting and winnowing): additional 15.34% reduction in total fumonisin content of maize; total fumonisin levels were high in the upper floating grain fractions; significant amount of fumonisins detected in washing water; Crushing and dehulling (complementary to sorting, winnowing and washing): significant (P < 0.05) reduction of total fumonisin levels; no fumonisins detected in washed grits | Reduction of fumonisins in maize intended for traditional food preparation in rural subsistence farming households: systematic cleaning of maize, involving sorting and washing, performed prior to preparation of maize-based food | Fandohan et al., 2005 |
| Mechanical shelling and dehulling of maize | Impact of mechanical shelling and dehulling on Fusarium infection and fumonisin contamination in maize. In vitro: Impact of shelling methods on Fusarium and fumonisin contamination: all mechanical shelling methods caused damage to maize kernels; Fusarium colony count highest (P < 0.05) in maize shelled with mechanical sheller; Fusarium colony count positively and significantly correlated with percentage of kernel damage (r = 0.6; P < 0.01); total fumonisin levels the highest (P < 0.01) in maize shelled with mechanical sheller; fumonisin levels | In vitro: Impact of shelling methods on Fusarium and fumonisin contamination: all mechanical shelling methods caused damage to maize kernels; Fusarium colony count highest (P < 0.05) in maize shelled with mechanical sheller; Fusarium colony count positively and significantly correlated with percentage of kernel damage (r = 0.6; P < 0.01); total fumonisin levels the highest (P < 0.01) in maize shelled with mechanical sheller; fumonisin levels | Promotion of dehulling for reduction of mycotoxins in maize; introduction of dehulling methods in African countries where it is still uncommon; selection of appropriate shelling methods to limit kernel damage and reduce mycotoxin contamination | Fandohan et al., 2006 |
### Table 5 | Continued

| Method of mycotoxin reduction | Test system with details of experimental model | Reduction criteria | Application | Reference(s) |
|------------------------------|-----------------------------------------------|--------------------|-------------|--------------|
| **Hand-sorting of maize**    | Effect of sorting on incidence and occurrence of fumonisins and *Fusarium verticillioides* on maize from Nigeria. Analyses of field samples: Collection of maize samples in the Kaduna state of Nigeria; hand-sorted “good” and “poor” quality maize were collected from farmers’ stores; Incidence of *F. verticillioides* in maize: mycological analyses: isolation, identification and quantification of *Fusarium* spp.; maize kernels plated out on semi-selective *Fusarium* medium peptone-pentachloronitrobenzene agar; single-sporers transferred to carnation leaf agar for identification; identification with standard morphological criteria; Confirmation of the identity of selected *F. verticillioides* strains: amplified fragment length polymorphisms; Fumonisin levels in maize: ELIZA | Cause by the method, mean *Fusarium* population (cfu g\(^{-1}\)) and total fumonisin levels: ELIZA (VICAM) | An appropriate method for reducing fumonisin exposure in rural subsistence farming communities of West Africa; only effective if “good” quality maize is consumed alone and “poor” maize discarded; educational and awareness campaigns should be performed in rural Africa: information on hand-sorting as reduction method and the health risks of using sorted moldy maize as animal feed | Afolabi et al., 2006 |
| **Hand-sorting of maize**    | Co-occurrence of fumonisins with aflatoxins in home-stored maize for human consumption in rural villages of Tanzania. Analysis of field samples: Study area: rural subsistence farming communities in high maize production regions of Tanzania; Sampling of maize: shelled and unshelled maize for human consumption from households and stores; 5-6 months after harvest; FB1 and FB2 levels in maize samples: HPLC; Determination of the percentage of defective kernels; Collection of information from the community: questionnaires on practices with regards to the type of staple food and the handling, storage, sorting and discarding of maize; involving heads of households | Cause by the method, mean *Fusarium* population (cfu g\(^{-1}\)) and total fumonisin levels: ELIZA (VICAM) | Reduction of fumonisin exposure in rural subsistence maize farming communities at risk; sorting of maize prior to storage; implementation of sorting methods by farmers and households in affected rural areas; educational and awareness campaigns on the health risks of using sorted moldy maize as animal feed or as raw material for beer making | Kimanya et al., 2008 |
| **Hand-sorting and washing of maize** | Simple intervention method to reduce fumonisin exposure in a subsistence maize-farming community in South Africa. Field study: Study area: rural subsistence farming communities in the Centane magisterial district of the Eastern Cape Province of South Africa; | Cause by the method, mean *Fusarium* population (cfu g\(^{-1}\)) and total fumonisin levels: ELIZA (VICAM) | An effectively implemented simple, practical and culturally acceptable intervention method for reduction of fumonisin exposure in rural subsistence maize farming communities | Van der Westhuizen et al., 2010 |
| Method of mycotoxin reduction | Test system with details of experimental model | Reduction criteria | Application | Reference(s) |
|-------------------------------|-----------------------------------------------|-------------------|-------------|--------------|
| **Participants:** females who prepare traditional maize-based food from home-grown maize; Baseline phase of study: Preparation of maize-based stiff porridge by participants according to their customary practices; consumption of porridge (2x 0.5 kg portions for two consecutive days); Assessment of porridge intake: 24 h dietary recall questionnaire by utilizing full-scale photographs of portions; | **Intervention phase of study:** Hand-sorting: training of participants by field workers demonstrating the removal of infected and damaged kernels with the aid of photographs; sorting of a 4 kg maize kernel batch by participants under the supervision of the field workers; Washing of maize: demonstration of a 10 min maize water washing procedure; 5 min hand agitation and 1 min agitation prior to the 10 min end point; washing of good sorted kernels by participants under supervision of the field workers; Drying of subsamples of sorted and washed kernels; Preparation of traditional stiff porridge by field workers; consumption of a weighed portion (0.5 kg) by each participant; extra portion of stiff porridge supplied to participants; Assessment of porridge intake: 24 h dietary recall questionnaire; Determination of total fumonisin levels in sorted and washed maize, and in maize porridge from the baseline and intervention phases: HPLC; Determination of fumonisin exposure: total fumonisin levels in the stiff porridge consumed by each participant during the baseline and intervention phases of the study | **Intervention study:** Hand-sorting and washing of maize: significant ($P < 0.05$) reduction in FB$_1$ levels (84% reduction); PDI assessment: Mean PDI of FB$_1$ at baseline significantly ($P < 0.05$) reduced with 62% following the intervention: before the intervention PDI levels of 71% participants exceeded JECFA recommended PMTDI level for FB$_1$; following the intervention only 53% of participants exceeded the recommended PMTDI level; Urine: UFB$_1$ in urine was reduced with 52% ($P = 0.02$) following the intervention; normalization with UF$_1$C indicated a 41% reduction ($P = 0.06$); | Simple, practical and culturally acceptable intervention for reduction of fumonisin exposure in rural subsistence farming communities exposed to high levels of fumonisins in their staple diet | Van der Westhuizen et al., 2011a |
| Method of mycotoxin reduction | Test system with details of experimental model | Reduction criteria | Application | Reference(s) |
|-------------------------------|-----------------------------------------------|-------------------|-------------|--------------|
| **Laboratory-optimized hand-sorting and washing of maize** | Optimising sorting and washing of home-grown maize to reduce fumonisin contamination under laboratory-controlled conditions. | In vitro: Questionnaires on customary sorting and washing of maize: focus groups; females who traditionally prepare maize meals; interviews with field workers; Maize: obtained from rural subsistence farming households; Hand-sorting and washing procedures: as described above Van der Westhuizen et al., 2010; Effect of water temperature (5 min wash): 25 and 40°C; Effect of wash duration (25°C): 5, 10, 30 and 60 min; Mycological analyses: determination of percentage kernels infected; determination of the frequencies of Fusarium and Stenocarpella species Total fumonisin levels in maize samples: HPLC | to larger number of both male and female participants including children | Van der Westhuizen et al., 2011b |
| **Hand-sorting, flotation/washing, dehulling of maize and combinations thereof** | Effectiveness of hand-sorting, flotation/washing, dehulling and combinations thereof on the decontamination of mycotoxin-contaminated white maize. Analysis of field samples: Maize: Visually moldy white maize kernels purchased from a local market in the Chikwawa district of Malawi; winnowing and mixing; Three factorial design experiment with variables sorting, flotation/washing and dehulling in 8 independent experiments (including the control); Hand-sorting: removal of visibly moldy kernels; Removal of moldy kernels by flotation and washing of non-floating kernels: maize to water ration 1:2 (w/v); stirred by hand and allowed to stand for 5-10 s; removal of top floating fraction; repetition of procedure until all floating kernels and particles were removed; Washing of non-floating kernels: maize to water ratio 1:2 (w/v); 2x 1 min wash; Dehulling: untreated maize and maize without the fractions removed through hand-sorting and flotation (4.5 kg); addition of water (200 ml); dehulling with a mortar and pestle; manual winnowing; FB₁, FB₂ and FB₃ levels in maize samples: LC-MS/MS | Analysis of field samples: Fumonisins are concentrated in moldy, broken and discolored maize kernels; Hand-sorting had the largest effect among the single methods, followed by dehulling and flotation (in this order); Percentage reduction of fumonisin levels in maize: Hand-sorting: 91.6–96.7%; Dehulling: 85.2–90.3%; Rotation: 67–77.8%; Percentage reduction of fumonisin levels in maize after combined treatments: Rotation*Hand-sorting: 63.8–76.5%; Rotation*Dehulling: 60.7–70.4%; Hand-sorting*Dehulling: 79.2–87.3%; Rotation*Hand-sorting*Dehulling: 58.6–61.9%; Hand-sorting of maize resulted in much lower mass loss than dehulling | Reduction of fumonisin exposure in rural subsistence farming communities at risk: hand-sorting of maize kernels proved very effective and is recommended as last line of defense; dehulling might not be necessary if hand-sorting is thoroughly applied; integration of hand-sorting into the maize production and utilization chain; campaigns by governments and relevant developing partners to raise public awareness and promote the hand-sorting method | Matumba et al., 2015 |
and E. coli, respectively, by employing episomal pET-3a vectors. Production of the recombinant enzymes were induced in liquid cultures by isopropyl-beta-D-thiogalactopyranoside, where after degradation of FB1 and HBOB was demonstrated with the recombinant culture supernatant as well as with purified enzyme preparations. HBOB prepared through enzymatic transformation by FumD carboxylesterases exhibited considerably less toxicity than FB1 when evaluated in a pig intestine model as indicated by the modified sphingamine/sphingosine ratios in the liver and plasma, modified intestinal immune response, and absence of hepatotoxicity and impaired intestinal morphology (Oswald et al., 2012). Although, certain of these technologies are considered safe for humans, animals and the environment by the European Food Safety Authority (EFSA), applications of microbial enzymes are presently mainly directed toward the animal feed industry (Duvick et al., 1998b, 2003; Moll et al., 2011). Recombinant enzymes are mass produced in a bioreactor and are applied during storage and food-processing to incorporate into animal feed and act in the intestinal tract of animals, or for treatment of grains in the form of a wash, additive or spray. Other post-harvest methods involving microbial transformation include the engineering of ruminal organisms and supplementation to feed in the form of a probiotic inoculant.

### Commercialization of Biological Methods of Control

The lack of effective and environmentally safe chemical control methods against fungal growth and mycotoxin production in food crops has led to investigations into biologically safe alternatives to prevent these contaminants from entering the food chain (Beekrum et al., 2003). Biological pesticides and methods involving natural resources such as plants, microorganisms, genetic factors thereof, and clay minerals are popular alternatives being evaluated for control of mycotoxigenic fungi in grains (Alabouvette et al., 2009). *Fusarium* growth and fumonisins production pre-harvest and post-harvest are effectively reduced by several natural and biological methods involving plant material, microorganisms and minerals, as evident by the extensive research done on this subject in recent years.

Several commercial products for biological control of *Fusarium* diseases and the fumonisins have been developed for application alone, in combination or as part of an integrated control strategy. Products containing biocontrol microorganisms are mainly aimed at application as seed and soil treatments as outlined by Fravel et al. (1998) and Kahn (2013):

- “Fusaclean” and “Biofox C” (non-pathogenic *F. oxysporum* for control of *F. oxysporum* and *F. verticillioides* in a variety of vegetables).
- “Epic” and “Kodiak” (*B. subtilis* for control of *Fusarium* in cotton and legumes).
- “Intercept” (*Pseudomonas cepacia* for control of *Fusarium* in maize, vegetables and cotton).
- “Mycostop” (*Streptomyces griseoviridis* for control of *Fusarium* in ornamental and vegetables crops).
- T-22G and T-22HB (*Trichoderma harzianum* for control of *Fusarium* in grains, soya, cotton and vegetables).
- “Biofungus” (*Trichoderma spp.* for control of *Fusarium* in citrus and pome fruit).
- “Blue circle” (*Burkholderia cepacia* for control of *Fusarium* in vegetables).
- “Deny” (*B. cepacia* for control of *Fusarium* in a variety of grain crops).
- “Cedomon” and “Cerall” (*Pseudomonas chlororaphis* for control of *Fusarium* in wheat, rye and triticale).
- Commercial GRAS products developed from clay minerals include Novasil® and Nevalite® (calcium montmorillonite) (Robinson et al., 2012).
- Fumzyme® (Biomin, Austria) was developed from the carboxylesterase enzyme of *S. macrogoltabida* (Heinl et al., 2010).

Although, there is an increased interest in biological control methods, much effort is put into details of natural compounds capable of controlling fungal growth and mycotoxins in vitro. However, the growing knowledge base on this subject should be further developed for application in planta and in the field pre-harvest, post-harvest, and during storage and food-processing. In order to develop the available information into appropriate methods for application in planta and in the field, there are many economic and technological hurdles to overcome. The effectiveness of antioxidants, essential oils, phenolic compounds and combinations for example, has been demonstrated at laboratory scale, and bioactivity in the vapor phase makes it promising as fumigant for protection of grains on the field immediately after harvest or during storage (Chulze, 2010). However, evaluation studies in grains are limited due to cost implications and the inhibitory effect in maize generally achieved with higher concentrations than in synthetic media, because of possible matrix interference and reduced bioavailability relating to distribution on kernel surfaces and penetration into the pericarp (Torres et al., 2003; Samapundo et al., 2007). In certain cases, high concentrations of phenolic compounds could also affect the sensory quality of the maize. Certain antioxidants such as BHA and PP, clay minerals, and plant extracts are considered GRAS, making it very promising for biocontrol purposes. Mixtures of antioxidants or combinations with other food preservatives (i.e., benzoic and sorbic acids) could further enhance the antifungal efficacy (Reynoso et al., 2002).

Even though biologically based treatments most likely will have a reduced effect than chemical methods on the desired nutritional value, quality, safety, or sensory attributes of foods and feed and impact on the environment, compliance to food safety assessment guidelines, such as those prescribed by the European Network on Safety Assessment of Genetically Modified Food Crops (ENTRANSFOOD) and the FAO/WHO, have to be met (He and Zhou, 2010). Assessments could include compositional analyses of key components of treated food including nutrients, micronutrients, and predictable secondary metabolites; assessment of possible toxicity, allergens; potential environmental impact; long-term nutritional impact; influence of food/feed processing; potential dietary intake and change
in dietary pattern. While there are several opportunities for further exploring and developing biological control methods for *Fusarium* growth and fumonisins, each method has its own challenges. However, an integrated approach, involving good agricultural management practices, HACCP models and storage management, together with appropriately selected biologically based microbial treatments, mild chemical and physical treatments could reduce *Fusarium* diseases and fumonisins effectively pre- and post-harvest (da Cruz Cabral et al., 2013).

**Practical and Culturally Acceptable Methods for Mycotoxin Reduction—Approaches in Sub-Saharan Countries**

Methods for prevention of chronic exposure to the fumonisins, particularly in low socio-economic rural subsistence farming communities, remain critically important. In developed countries high standards of the major food suppliers and retailers are upheld and the regulatory controls deter the importation and marketing of seriously contaminated products. In developing countries only a limited number of countries have legislative maximum levels for fumonisins, and implementation thereof is often poor. In rural subsistence farming communities, legislation is not applicable and with continued pressure on food security, an increased *mycotoxin exposure* on a daily basis is the norm. In addition, due to the stringent mycotoxin standards in developed countries, the best-quality food products are normally exported resulting in highly contaminated foods being utilized domestically which increases the risk of mycotoxin exposure and the associated adverse health effects (Pitt et al., 2012).

High risk population groups include rural communities and/or subsistence farmers heavily reliant on maize as their staple diet. Although, commercial maize is contaminated with lower levels, daily exposure could be a risk factor for disease development in impoverished communities.

In developing countries, where resources are limited and sophisticated technologies are lacking, the importance of cost-effective and simple intervention methods, predominantly at population level, has been emphasized. In this regard, culturally acceptable simple, practical and biologically based methods of reduction are relevant, as a last line of defense in rural subsistence farming communities exposed to high levels of the fumonisins in their staple diet. Effective reduction has been demonstrated with hand sorting, flotation, washing, dehulling of maize kernels and combinations thereof in vitro and in field studies (Table 5). Dehulling and shelling of maize are common practices in West-Africa (Fandohan et al., 2006), with the removal of the pericarp an effective way to reduce mycotoxin contamination (Sydenham et al., 1994; Bullerman and Bianchini, 2007; Burger et al., 2013). The effectiveness of hand-sorting of maize by removing visibly infected and damaged kernels, resulting in a significant reduction of fumonisins has been demonstrated in several African countries, including Benin (Fandohan et al., 2005), Nigeria (Afolabi et al., 2006), Tanzania (Kimanya et al., 2008), South Africa (Van der Westhuizen et al., 2010), and Malawi (Matumba et al., 2015). In South Africa a simple, practical and culturally acceptable hand-sorting and washing intervention method was developed and implemented for reduction of fumonisin exposure in a subsistence maize-farming community (Van der Westhuizen et al., 2010, 2011b). The efficacy of the maize kernel wash method could possibly be further enhanced by incorporating clay minerals or fumonisin detoxifying enzymes. Advantages of interventions involving practical methods usually take the form of improved health outcomes rather than market outcomes (Wu and Khlangwiset, 2010a,b).

Public health interventions should be culturally acceptable; be implemented through educational campaigns; and must have financial and infrastructural support to be feasible in remote rural areas where they are most needed. Sustainability of these reduction strategies is, however, dependent on the available maize supply (food security), as well as the socio-economic status and education of a community.

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Dr. JA, Wrote article; Prof. WG, Coordinated and assisted in writing article; Prof. WV, Assisted in writing article.

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