
Biological Control of Mycotoxigenic Fungi and Their Toxins: An Update for the Pre-Harvest Approach

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Abstract

Over recent decades, laboratory and field trial experiments have generated a considerable amount of data regarding the promising use of beneficial microorganisms to control plant diseases. Special attention has been paid to diseases caused by mycotoxigenic fungi owing to their direct destructive effect on crop yield and the potential production of mycotoxins, which poses a danger to animal and human health. New legislative initiatives to restrict the use of the existing commercial chemical pesticides have been an incentive for developing and registering new bio-pesticides. In this book chapter, we discuss up-to-date pre-harvest biological control agents against mycotoxigenic fungi and their respective toxins. We will focus on the different modes of action of the most frequently studied biological control agents. Furthermore, a comprehensive overview on their ability to suppress mycotoxin biosynthesis will be discussed.

Keywords: biological control, mycotoxigenic fungi, mycotoxins, pre-harvest

1. State of the art

Cereals are a major source of calories consumed by people worldwide on a daily basis. With increasing global population, food production needs to increase by 50 to 70% in the next 30 years to avoid global food insecurity [1]. The danger of food insecurity is particularly serious for the developing countries especially sub-Saharan Africa where more people are suffering from hunger and this situation is expected to deteriorate in the future [2]. The challenge of safely and securely feeding these people, has to be faced in a world with a

shrinking arable land, with less and more expensive fossil fuels, increasingly limited supplies of water, social unrest, economic uncertainty and within a scenario of a rapidly changing climate. Moreover the impact of plant diseases cannot be overestimated. The impact of fungal diseases and new variants of existing pathogens on agriculturally important crops is considered to be one of the main threats to worldwide food availability and safety. It was figured that diseases on our most important agricultural crops resulted in damages that were enough to feed 8.5% of the world's population [3]. The mission of providing food to the growing world population can therefore not be accomplished without a good control of these plant diseases. An important group of plant pathogens are toxigenic plant pathogens which produce mycotoxins, secondary metabolites of unrelated chemical structures and biological properties with a very broad toxic effects to humans and livestock, so in addition to posing a threat for food security, these pathogens also pose a threat to food safety [4–6].

Management of plant diseases can be done by adopting several strategies such as the cultivation of resistant cultivars, the use of sound crop rotation schemes and the use of chemical control. The harmful impact of plant protection products on the environment and human and animal health have prompted the European Union (EU Directive 2009/128/EC) to encourage research on alternative and ecofriendly solutions such as integrated pest management and the use of biological control agents (BCAs). Biological control, henceforth called biocontrol, in plant pathology, aims at utilizing microorganisms to prevent the colonization and/or suppress the spread of harmful plant pathogens [7]. BCAs in this chapter are defined as beneficial microorganisms that are able to antagonize plant pathogens and protect the plant [8–11]. Although the definition includes both pre-harvest and post-harvest strategies, this chapter will focus on pre-harvest biocontrol measures [12, 13].

The most studied mycotoxin producing plant pathogenic genera are *Fusarium*, *Alternaria*, *Claviceps*, *Stachybotrys* and *Aspergillus* spp. [4, 14–16]. These genera infect a wide array of commodities including cereals, nuts, beans, sugarcane, and sugar beet in the field (e.g. *Fusarium*, *Alternaria* and *Claviceps* spp.) and/or during storage (e.g. *Aspergillus* spp.). **Figure 1** illustrates, in term of biological control, the most studied mycotoxigenic fungi in pre-harvest in different crops. *Fusarium graminearum* is a predominant pathogen in wheat and maize, *Fusarium verticillioides* contaminates maize while *Aspergillus flavus* infects groundnuts and maize. Other mycotoxigenic plant pathogens such *Alternaria alternata*, *Claviceps purpurea*, and other members of the genera *Fusarium* (e.g. *F. avenaceum*, *F. acuminatum*, and *F. proliferatum*) and *Aspergillus* (e.g. *A. carbonarius*, *A. niger*, and *A. parasiticus*) received less attention in research to date.

Mycotoxins are ubiquitous in agricultural crops and their production occurs under certain environmental conditions during and/or after plant colonization [4, 17]. Exposure to mycotoxins either in a short and/or long term can lead to diverse toxic effects on a wide range of organisms [5, 6, 14, 17, 18]. Often, these fungal toxins are not only harmful for vertebrates and invertebrates (mycotoxins) but also for plants (phytotoxins). Economically, these natural contaminants hamper the international trade and significantly affect the world economy

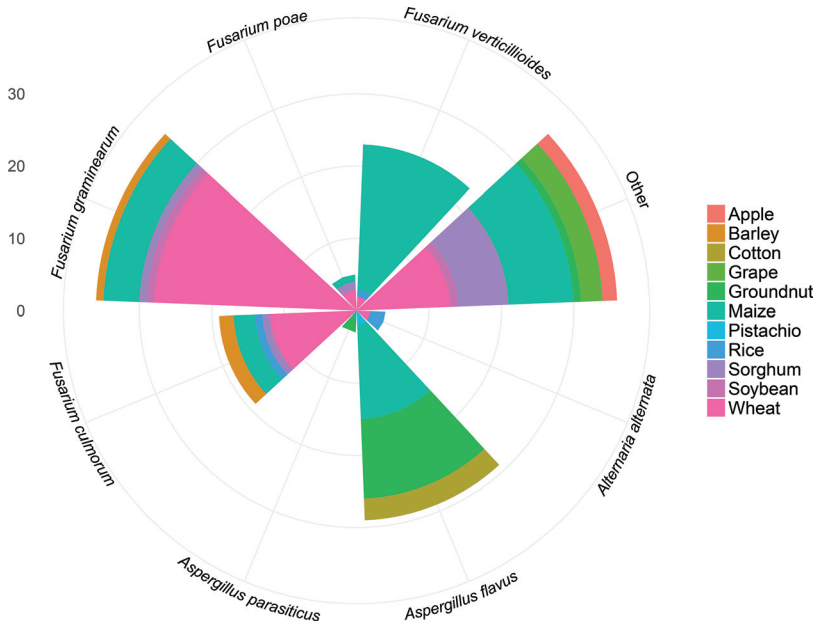


Figure 1. Overview of the number of papers published between 1989 and 2017 which use biological control strategies against, mycotoxigenic plant pathogenic fungi in different crops.

due to borders rejection when mycotoxin concentrations exceed the maximum permissible levels. Although the production of mycotoxins by these toxigenic plant pathogens is of economic importance, many research groups do not take them into account when studying biological control strategies. These studies are then limited to the fungicidal or fungistatic effects of the BCAs while the effect of the BCAs on mycotoxin production is often overlooked. **Figure 2A** subscribes this issue and shows the number of papers on mycotoxigenic fungi with and without considering mycotoxins under *in vitro*, greenhouse and field conditions over the last 30 years. The figures presented in **Figure 2A** are even an underestimation, as they comprise research on *A. flavus* (**Figure 2B**). Many of these papers deal with “Aflasafe” and all include aflatoxin measurements. Omitting these *A. flavus* data provides a more correct view on the lack of studies investigating the effects of BCAs on mycotoxin production (**Figure 2C**).

In view of the importance of mycotoxins for animal and human health, this review will focus on the effect of BCAs on the mycotoxin production by toxigenic plant pathogenic fungi. In a first part, we will provide an overview on the diverse modes of action BCAs can have. Secondly, a more in depth insight into the effect of BCAs on production of the major mycotoxins is provided. Finally, we end by providing some perspectives for future research and hurdles that might have to be taken.

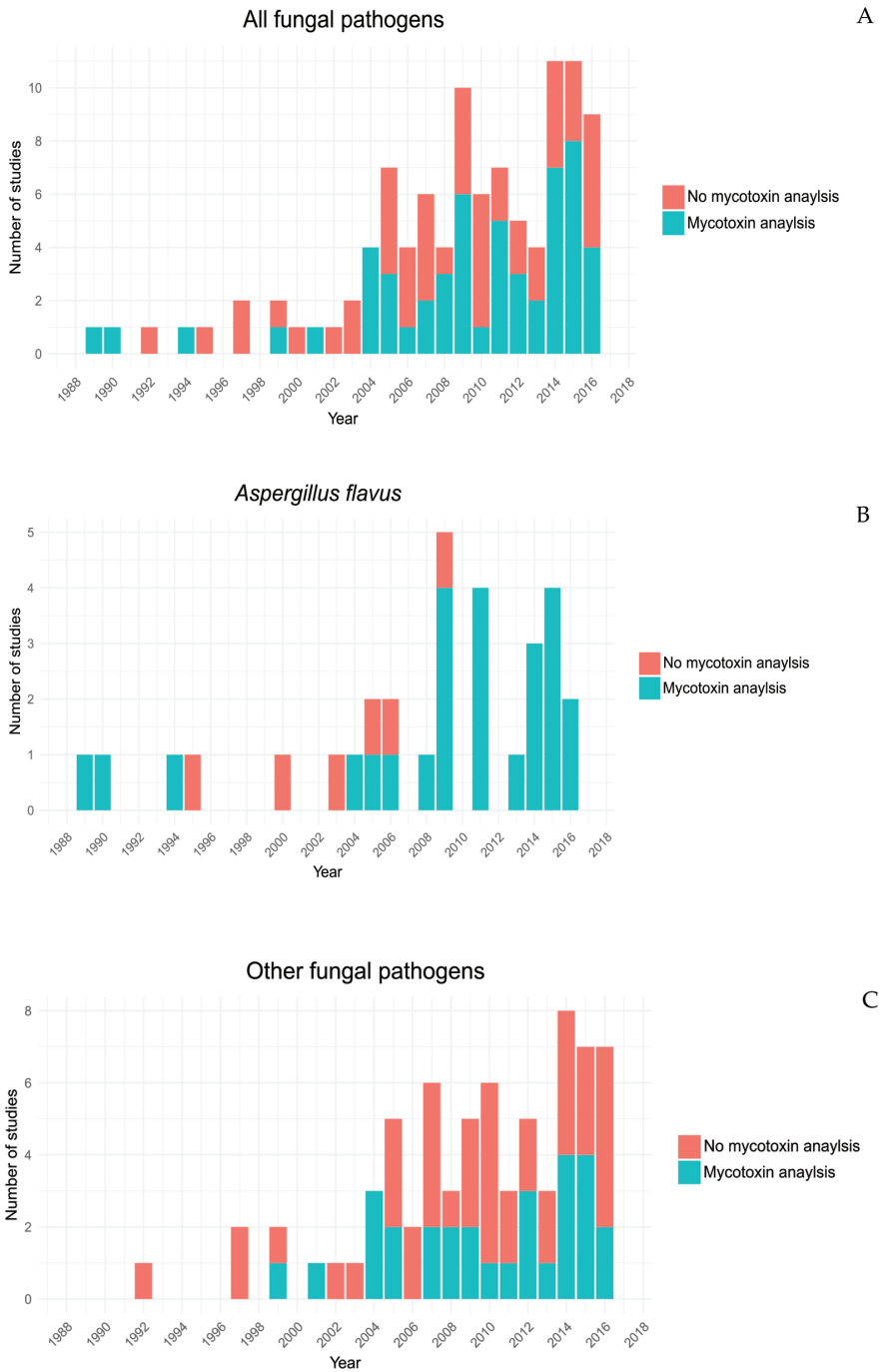


Figure 2. Number of published papers between the period of 1988–2017 addressing biocontrol of mycotoxigenic fungi with and without considering the effect on mycotoxins.

2. Modes of action of BACs

The main modes of action of BCAs are **antibiosis**, **competition**, **mycoparasitism**, and **stimulation or enhancement of plant defense** [7]. BCAs usually rely on more than one mode of action to antagonize the pathogen i.e. presence of one dominant mode of action does not exclude the others. **Table 1** summarizes the reported modes of action used against mycotoxigenic fungi in each crop.

- (i) **Antibiosis** encompasses the production of secondary metabolites such as antibiotics [19–21], lytic enzymes [22] and other proteins [23] that are able to suppress the growth, weaken the virulence or kill the pathogenic fungi.
- (ii) **Competition** occurs when two or more fungi compete for the same essential nutrients required for their growth and development [24, 25]. Another type of competition is exclusion by occupying the same niche [26, 27].
- (iii) **Mycoparasitism** or **hyperparasitism** is a direct parasitic attack of one fungus by another one which eventually causes death of the host pathogen [28–30].
- (iv) **Colonization of the plant**, by beneficial micro-organisms can trigger local or systemic defense responses, thus enhancing resistance against plant pathogens [31, 32].

2.1. Antibiosis

Production of a wide range of antibiotics, enzymes and other antifungal compounds which contribute to adverse impacts on plant pathogen are characteristic features of different fungal BCAs such as *Trichoderma* spp. and *Clonostachys* spp. [8, 11, 24, 33]; bacterial BCAs such as *Bacillus* spp., *Pseudomonas* spp., *Streptomyces* spp. and *Lactobacillus* spp. [19, 20, 34, 35]; and yeast BCAs such as *Cryptococcus* spp., *Kluyveromyces* spp. and *Saccharomyces* spp. [10, 36]. All these BCAs have an arsenal of metabolites targeting different structures of the pathogen which thereafter curtails the growth or kills the pathogen.

A. Enzymes hydrolyzing fungal cell wall

The fungal cell wall is a complex structure containing mainly glucan polymers and chitin. For several BCAs, molecules which interfere with this cell wall have been described. Peptaibols, linear oligopeptides produced by *Trichoderma* spp., inhibit beta-glucan synthase which prevents the pathogen from reconstructing its cell wall [37]. Culture filtrates of a *T. harzianum* isolate changed the colony color of *A. flavus* and had a clear effect on the growth. A microscope study showed alterations in the morphology of *A. flavus* represented by abnormal vesicle formation and various aberrant conidial heads reflecting cell wall deformity [38]. Production of some extracellular enzymes (amylolytic, cellulolytic, pectinolytic, lipolytic and proteolytic) were also demonstrated, however the inhibition was directly associated with source of carbon (glucose or sucrose) or nitrogen (L-alanine or other) available in the medium [38].

B. Production of metabolites that affect fungal membrane

Production of antifungal metabolites interfering with membrane structures have been described in several BCAs. The most important class is the lipopeptides which interfere with the membrane and the sterols in the membrane [39]. These lipopeptides have been proven to be effective against several genera of toxigenic fungi such as *Aspergillus* and *Fusarium* spp.

The presence of two antibiotic lipopeptides, iturin and surfactin, revealed the potent antifungal activity [20] of two *Bacillus* spp. (P1 and P11) against *A. flavus* [40]. Similarly, *B. subtilis* BS119m was able to completely inhibit *A. flavus* growth which was associated to its ability to produce a high amount of surfactin [41]. Crane et al. monitored iturins produced by *B. amyloliquefaciens* in wheat under greenhouse and the field conditions and found an inverse relationship between iturins levels and *Fusarium* disease incidence [42]. Fengycin, another lipopeptide purified from *Bacillus subtilis* IB culture showed an inhibitory effect against *F. graminearum* [19].

C. Production of antifungal compounds having antibiotic effects not related to membrane and cell wall effects

Where antibiotics have been described as powerful allies in the battle against bacterial contaminants, several molecules have been described which are fungicidal. The polyketide compound 2,4-diacetylphloroglucinol (DAPG) produced by *P. fluorescens* has received a particular consideration due to the broad spectrum activity against various fungal pathogens [43–46]. The molecule was isolated from *Pseudomonas* spp. strain F113 present in the rhizosphere of sugar beets [46] and has later been isolated from the rhizosphere of different crops [47]. DAPG has been shown to have antifungal effects against *Fusarium* and *Alternaria* spp. [48].

Although antibiosis has been proven to be a major weapon against plant pathogenic, fungal resistance might arise. One example is known for *F. verticillioides* in which a Lactamase encoding gene (FVEG_08291) has been identified which enables the pathogen to resist benzoxazinoid phytoanticipins produced in plant but also possibly microbial xenobiotic lactam compounds [49]. This information therefore raises an important question about the ability of mycotoxigenic plant pathogens to cope with the antifungal compounds produced by BCAs. In case that reported fungal resistance may be present against BCAs, this may necessitate the continuous exploration of new antibiotics.

2.2. Competition for niche and nutrition

Competition for niche or competitive exclusion is a restriction of access to the habitat of a pathogen on the plant or seeds by another microorganism while competition for nutrients happens when two or more microorganisms compete for the same source of macro- and micro-nutrients required for growth and secondary metabolites production [7].

One of the most famous and promising examples on competition for ecological niche and nutrition is found in *A. flavus* control [26]. However, competition of other mycotoxigenic pathogens such as *F. pseudograminearum* through nutrient competition [50] and *F. culmorum* and *F. graminearum* [51] were also reported. It has been demonstrated that atoxigenic *A. flavus*

strains are powerful BCAs to control the toxigenic strains of *A. flavus* in cottonseed [52–54], maize [27, 55–57] and various types of nuts [58–61]. Currently, different strains of atoxigenic *A. flavus* are being used depending on the endemic area and sometimes a mixture of strains is used in the field. This competitive exclusion theory has been recently confirmed in situ by co-inoculating corn kernels with GFP-labeled AF70 and wild-type AF36. The study showed that there is a population difference (up to 82% reduction) between the co-inoculated kernels with both fungi and the control one inoculated only with GFP-labeled AF70 after visualizing under UV. Furthermore, aflatoxins (AFs) analysis showed a 73% reduction compared to the control [62].

However, AFs are not the only toxic compounds produced by *A. flavus*. Cyclopiazonic acid (CPA) is another mycotoxin produced by certain strains of *A. flavus*, including the atoxigenic strains, affecting mainly the liver and muscles of livestock [63, 64]. As an example, the commercially registered BCAs AF36, while it is effective against toxigenic *A. flavus*, it has been confirmed for its CPA production in cottonseeds. Therefore, researchers screened and tested new strains lacking the production of both toxins for the same previously mentioned crops [65–67]. Testing atoxigenic strains of *A. flavus* against other AFs producing fungi such as *A. parasiticus* was less common because *A. parasiticus* is less virulent and not predominantly occurs in the soil as *A. flavus* [59].

Competition for nutrient and niche can also be seen in *Trichoderma* and *Clonostachys* spp. when they are applied before pathogen occurrence [11, 68]. *Trichoderma* spp., especially *T. harzianum*, produce siderophores, low-molecular-mass ferric-iron-specific chelators, when the available iron in the environment is low [23]. Siderophores chelate the oxidized ferric ions ($\text{Fe} + 3$) making it available as an iron source [24, 37, 69] and this enables *Trichoderma* spp. to compete for iron which is an essential element for the development of many plant pathogens [24, 68].

2.3. Mycoparasitism

Mycoparasitism is a direct parasitic relationship between one fungus and another fungal host [24]. The mycoparasitic interaction is mediated through certain gene involved in synthesis of some metabolites (mainly chitinases, glucanases, and proteases) allowing the parasitic fungi to degrade and invade the host cells [24, 29, 70]. A wide array of BCAs employ this strategy to compete against several mycotoxigenic pathogens especially against *Fusarium* spp. Among these, *Trichoderma* spp., are a widespread mycoparasitic BCA naturally present in the soil and the plant [11, 70, 71]. The fungi are mainly biotrophic, perform mycoparasitic interaction with living fungi, although the species also compete for niche and nutrients, enhance the plant systemic and localized resistance and secrete secondary antifungal metabolites [29, 68]. Upregulation of some chitinase-encoding genes occurred upon mycoparasitic contact of *Trichoderma* spp. with *Fusarium* [71, 72]. *T. viride* showed a potent antagonisms of *F. verticillioides* in an *in vitro* assay which was proven by the suppression of radial extension of the fungus by 46% after 6 days and by 90% after 14 days [73].

On rice, *T. harzianum* performed very well against *F. verticillioides* through mycoparasitism and showed a mutual antagonism by contact [74]. Some metabolites such as cell wall-degrading enzymes, chitinases and β -1,3 glucanases were suggested by the author to be involved in the mechanism as the evidence of mycoparasitism in this study was supported by cryo scanning

electron microscopic observations. The same experimental setup was previously done using the same BCA on rice but against *Alternaria alternata* and similar results and conclusions were reported [75]. Upon fungal cell wall degradation by chitinases produced by *Trichoderma* spp., another type of enzymes called exochitinases are secreted and the attack starts to kill the pathogen [24].

Trichoderma spp. have mostly been tested as a BCA against *F. graminearum* in wheat [38, 51, 76–78]. In a field trial, T-22 strain, reduced formation of perithecia of *F. graminearum* by 70% [77].

Clonostachys is another genus famous for mycoparasitism and demonstrates a promising BCA against a wide range of plant pathogens including *F. graminearum*, *F. verticillioides*, *F. poae*, and *F. culmorum*. However, compared to *Trichoderma*, *Clonostachys* spp. are poorly studied. Within *Clonostachys* spp., *C. rosea* is the most researched and has been associated with multiple modes of action such as antibiosis [33], induction of plant resistance, [79], and niche and nutrient competition [80]. The fungus *C. rosea* secretes a number of antibiotics such as peptaibols, gliotoxin, trichoth as well as cell wall degrading enzymes such as chitinases, glucanases. *C. Rosa* ACM941 was reported to produce chitin-hydrolysing enzymes capable of degrading cell wall of *F. culmorum* [81].

Recently, *Sphaerodes* spp. have been discovered as a potential biocontrol agent against *Fusarium* spp. relying on mycoparasitism tactics with promising results. Among these species *Sphaerodes mycoparasitica* was isolated in association with *Fusarium* spp. from wheat and asparagus fields [82] and has shown its ability to limit *Fusarium* infection in both 3-ADON and 15-ADON chemotypes and limit DON synthesis both in vivo and in planta [82, 83]. For bacterial BCAs, Palumbo et al. [84] reported the production of antifungal metabolites and chitinase by *P. fluorescens* (strains JP2034 and JP2175) which had negative effects on the growth of *A. flavus* and *F. verticillioides*.

2.4. Indirect through the plant

Enhancement of systemic plant resistance using plant growth-promoting rhizobacteria, which results an effective protection against a broad spectrum of pathogens, has been well described [85–87]. *P. fluorescens* is known to produce various plant growth regulators such as indole acetic acid, gibberellins and cytokinins which interfere with plant signaling [88]. In addition, it also produces antibiotics, volatile compounds, enzymes [21, 89]. The production of indole-3-acetic acid by *P. fluorescens* MPP4 is triggered by the presence of some pathogens such as *F. verticillioides* M1 which in turn contributes into its antagonistic activity [90]. *P. fluorescens* CHA0 prevented the carbon diversion and plant biomass reduction due to *F. graminearum* infection in barley [91]. The antagonistic activity of *P. fluorescens* MKB158 against *F. culmorum* was documented by Khan et al., however, the author mentioned that an indirect effect through enhancement of the plant systemic resistant is involved in the antagonistic activity [92]. *Lysobacter enzymogenes* strain C3 exerts also its biocontrol effect through induction of resistance in wheat against *F. graminearum* beside the production of lytic enzymes [93]. Effective reduction of the pathogen after heat treatment of C3 broth cultures to inactivate the bacterial cells and lytic enzymes was a confirmation for the presence of some fungal elicitors.

Besides rhizobacteria, the fungus *T. harzianum*, while, has also been shown to promote plant growth, increase nutrient availability and enhance the resistance against fungal diseases through

colonization of plant roots [24, 37, 70]. Extensive research has been done to use *Trichoderma* spp., against *F. verticillioides* [94], *F. graminearum* [78] and *A. flavus* [95]. *T. harzianum* was reported to limit *F. verticillioides* in maize through the induction of systemic resistance by inducing ethylene and jasmonate signaling pathways [96]. Recently, novel species of *Trichoderma* (*T. stromaticum*, *T. amazonicum*, *T. evansii*, *T. martiale*, *T. taxi* and *T. theobromicola*) are classified as true endophytes as they have been reported to invade the plant tissue away from the root and induce transcriptomic changes in plants and protect the plants from diseases and abiotic stresses [97].

Another approach to enhance the plant resistance is through colonization. Extensive research is being done to discover endophytic microorganisms which colonize plant (tissue) without harming the plant [98] to reduce the plant diseases and mycotoxins in crops [99–103]. Endophytes can enhance plant growth and fitness, and offer protection against biotic and abiotic stresses by inducing plant defense responses. However, it should be noted that some of them are pathogenic to the plant in some phases of their lifecycle or under certain environmental conditions [98]. Some endophytes exert its role to enhance the host immune system against several fungal pathogens through the improvement of the nutrient uptake from the soil such as *Piriformospora indica*, a cultivable root fungal endophyte belonging to the order Sebaciales in Basidiomycota [104, 105]. The ability of *Piriformospora indica* to protect barley from root rot caused by *F. graminearum* was confirmed [103]. This was supported by a positive correlation between the relative amount of fungal DNA and disease symptoms and the absence of an inhibition on the growth of *F. graminearum* when co-inoculated with *Piriformospora indica* in an *in vitro* assay. Another endophyte such as *Epicoccum nigrum* has also proven its biocontrol activity against several plant pathogens [106], however it is ability to control diseases caused by mycotoxin producing fungi were scarcely studied [107, 108].

Pathogen	Host	Mode of action of BCAs				References
		Mycoparasitism	Antibiosis	Competition for niche / nutrients	Indirect through the plant	
<i>Alternaria alternata</i>	Wheat	✓	✓	✓		[48, 107]
	Rice	✓	✓	✓		[75, 109]
<i>Aspergillus terreus</i> HAP1	Apple	✓	✓	✓		[110]
	carbonarius		✓	✓		[111, 112]
	flavus			✓		[52–54]
	Pistachio nuts		✓	✓		[113, 114]
	Peanuts	✓	✓	✓		[58–61, 66, 115–119]
	Maize	✓	✓	✓	✓	[27, 55, 56, 65, 67, 84, 95, 118, 120–124]
<i>niger</i>	Peanuts		✓			[125]
	Grape		✓	✓		[111]
<i>parasiticus</i>	Peanuts			✓		[59, 60]
<i>Fusarium</i>	Maize			✓		[121]
	acuminatum	Maize			✓	[126]
	Sorghum				✓	[126]

Pathogen	Host	Mode of action of BCAs				References
		Mycoparasitism	Antibiosis	Competition for niche / nutrients	Indirect through the plant	
	Wheat				✓	[126]
<i>avenaceum</i>	Maize				✓	[126]
	Sorghum				✓	[126]
<i>culmorum</i>	Wheat	✓	✓		✓	[126, 127]
	Barley		✓		✓	[92, 102]
	Maize	✓	✓			[72, 127–129]
<i>equiseti</i>	Wheat	✓	✓	✓	✓	[48, 51, 130–132]
	Rice	✓	✓	✓		[133]
	Maize				✓	[126]
<i>graminearum</i>	Sorghum				✓	[126]
	Wheat				✓	[126]
	Barley				✓	[103]
	Maize	✓	✓		✓	[99, 128, 129, 134]
	Sorghum				✓	[126]
<i>langsethiae</i>	Wheat	✓	✓	✓	✓	[35, 48, 51, 72, 76–78, 89, 93, 100, 101, 107, 108, 126, 127, 129–131, 135–144]
	Soybean	✓	✓	✓		[145]
	Wheat	✓	✓			[127]
<i>nivale</i>	Maize				✓	[126]
	Sorghum				✓	[126]
	Wheat				✓	[126]
<i>poae</i>	Maize					[126]
	Sorghum				✓	[126]
<i>proliferatum</i>	Wheat	✓	✓	✓	✓	[107, 126, 127]
	Maize	✓	✓			[129, 146]
	Wheat	✓	✓			[129]
<i>sambucinum</i>	Maize				✓	[126]
	Sorghum				✓	[126]
	Wheat				✓	[126]
<i>sporotrichioides</i>	Maize				✓	[126]
	Sorghum				✓	[126]
	Wheat	✓	✓		✓	[126, 127]
<i>verticillioides</i>	Rice	✓		✓		[74]
	Maize	✓	✓	✓	✓	[73, 84, 90, 94–96, 146–158]
	Wheat	✓	✓			[127]
<i>crookwellense</i>	Maize				✓	[126]
	Sorghum				✓	[126]
	Wheat	✓	✓		✓	[78, 126]

Table 1. Different modes of action used by BCAs against mycotoxigenic fungi.

3. Biocontrol and mycotoxins

3.1. Trichothecenes toxins

Fusarium head blight (FHB) and *Fusarium* ear rot (FER) are two of the most serious diseases affecting wheat and maize respectively throughout the world [130, 131, 139]. Over the last few years, FHB was predominantly caused by three species of *Fusarium*: *F. graminearum*, *F. avenaceum* and *F. culmorum* [108, 159] while FER is mainly caused by *F. verticillioides*, *F. proliferatum*, *F. subglutinans*, and *F. graminearum* [154, 156]. However FHB mostly occurs as a complex of several species [14, 160]. Each disease has multi-destructive effects on the crop through reducing the yield and grain quality. Over 180 types of trichothecenes are produced by *Fusarium* spp. contaminating mainly agricultural staples such as maize, wheat, and barley [14, 15]. The most prominent members are deoxynivalenol (DON), nivalenol (NIV) and T-2 Toxin. The biochemical importance of DON for fungal growth and development is not fully clear yet; however, it may have an important role during fungal infection and colonization and act as a virulence factor [160]. In animals, DON interferes with the cellular protein synthesis and clinically causing animal feed refusal and vomiting while NIV may induce genotoxic effect and leucopenia on long term exposure [4, 5, 17]. T-2 toxin triggers apoptosis to immune cells [161]. Due to the complexity of the life cycle of *Fusarium* spp., researchers mostly tried two application strategies to biologically control the disease, treatment of the crop residue with the antagonist or treatment of wheat ears at anthesis [162]. Most of the performed experiments used bacterial BCAs rely on antibiosis mainly to control the diseases and DON level. Less research discussed the effect of BCAs on NIV [51] and T-2 toxin [107].

An isolate of *Trichoderma*, *T. gamsii* 6085, was selected as a potential antagonist against *F. culmorum* and *F. graminearum*. The strain exhibited the capacity to negatively affect DON production by both pathogens up to 92% [72]. A field experiment on winter wheat for two seasons was conducted to evaluate the efficacy of different BCAs against ear blight and associated DON presence. Two strains of *F. equiseti* were the best performing strains and decreased the mycotoxins level produced by *F. culmorum* and *F. graminearum* by 70 and 94%, respectively. However, low levels of NIV in the cereals treated with *F. equiseti* were detected [51]. Recently, *Piriformospora indica* has proven its promising ability to reduce the severity the disease caused by *F. graminearum* and mycotoxin DON contamination in wheat by 70–80% and increase the total grain weight of *F. graminearum*-inoculated samples by 54% [100]. Novel bacterial endophytes predicted to be *Paenibacillus polymyxa* and *Citrobacter* were able to detoxify DON *in vitro*, but the performance of some of these isolated strains under field condition or in green house has not been reported yet [99].

Three stains of the yeast *Cryptococcus* spp. (*Cryptococcus nodaensis* OH182.9, *Cryptococcus* spp. OH 71.4, and *Cryptococcus* spp. OH 181.1) were tested in several field experiments and they could control the disease by 50–60% on susceptible winter wheat. However DON content was the same as control [137]. Later, the same group cultivated another strain, *Cryptococcus flavescens* OH 182.9, and applied it at early anthesis but found no effects on DON level [142].

Besides fungal and yeast BCAs, bacteria have also been used to control DON produced by *F. graminearum* in wheat [35, 139, 144, 163] and in maize [99]. A complete reduction in DON

content was achieved when *B. subtilis* RC 218 and *Brevibacillus* spp. RC 263 were applied at anthesis for two seasons [144] which was consistent with previous findings under greenhouse conditions by the same authors [163], although there was no constant reduction in the disease incidence. Opposite to that, Khan and Doohan tested three strains of *Pseudomonas* spp., two strains of *fluorescens* and one strain of *frederiksbergensis*, against *F. culmorum* and DON production in wheat and barley in a small scale field experiment. The results showed that DON was reduced in wheat and barley by 12 and 21%, respectively [164].

Other types of trichothecenes were not well researched as the previously mentioned toxins due to their low incidence in crops. Variable results for T-2 toxin after spraying the ears of susceptible and resistant wheat cultivars with *Trichoderma* spp. under greenhouse conditions were documented. The author used four fungi, *Epicoccum* spp., *Trichoderma* spp., *Penicillium* spp. and *Alternaria* spp. however the last one is known for production of *Alternaria* toxins [107].

3.2. Zearalenone

Although zearalenone (ZEN) is an important mycotoxin in many cereals, less attention has been paid to control this toxin. ZEN is a potent mycoestrogen which competitively binds to estrogen receptors causing reproductive disorders in farm animals and human [5]. Other forms of ZEN include α and β zearalenol, zearalanone and, α and β -zearalanol which are often detected at variable concentration usually lower than ZEN.

Trichoderma isolates have recently been reported to detoxify ZEN by transforming ZEN into reduced and sulfated forms [165]. This was in accordance with previous results by Gromadzka et al. who tested two isolates of *Trichoderma* and several isolates of *Clonostachys in vitro* against two isolates of *F. graminearum* and two isolates of *F. culmorum*. Despite the high rate of ZEN reduction (over 96%), the performance of these isolates under greenhouse or field experiments was not confirmed [128].

C. rosea converts ZEN into less toxic compounds through an enzymatic alkaline hydrolysis by lactonohydrolase *in vitro* [23, 166]. This has been proved after cloning the coding region of the responsible gene, *zhd 101*, and expressing in *Schizosaccharomyces pombe* [167] and *Escherichia coli*, but not with *Saccharomyces cerevisiae* which exhibited weak detoxification activity against ZEN [168]. Through this approach which involves the direct interaction between BCAs and pathogen toxin, resistance of BCAs to mycotoxin itself is an important feature to ensure the efficacy and durability. Also, it was proven that *C. rosea* is tolerant to ZEN exposure due to the presence of high numbers of ATP-binding cassette transporters [169].

3.3. Fumonisin

Fumonisin B1 (FB1), the main member of fumonisins, is produced by *F. verticillioides* and *F. proliferatum* which usually infect maize [14]. The mycotoxin suppresses ceramide synthase and causes neurological toxicities in horses, pulmonary edema in pigs, and may pose hepatotoxicity and esophageal cancer in human [18]. Therefore, several trials have been conducted to effectively control the mycotoxin in maize using different strategies. Most of the field studies were done using bacterial BCAs [147, 148, 150, 158] while other types of BCAs, and fungi, were

restricted to *in vitro* testing [73, 154–156]. Maize rhizobacterial isolates belonging to *Pseudomonas* and *Bacillus* genera significantly reduced the mycotoxin production by 70 to 100% [157]. However, in another study, a mixture of *P. Solanacearum* and *B. subtilis* was not able to affect FB1 concentration [151]. Seed treatment with *B. amyloliquefaciens* Ba-S13 was sufficient to reduce fumonisins B1 concentration in maize field tests [148]. That has been confirmed in a 2-year field study with the same bacteria, *B. amyloliquefaciens*, after application of two different treatments: inoculating seeds during pre-sowing and maize ears at flowering [150].

P. fluorescens isolated from maize rhizosphere by Nayaka et al. had a clear reduction of FB1 content and the disease incidence after challenge with *F. verticillioides* during a 3-years study [147]. Seed treatment followed by spray treatment with a pure culture of *P. fluorescens* reduced the incidence of fumonisins by 88% [147]. Bacon et al. suggested the use of the endophytic bacterium, *B. subtilis* to control FB1 production as a convenient approach to prevent the vertical transmission of the fungi. Under greenhouse conditions, FB1 was reduced by 50% [154].

When *T. viride* was co-inoculated in corn kernels with *F. verticillioides*, a reduction of FB1 by 72–85% was obtained depending on the time of inoculation [73]. The fungus was also proposed as a postharvest agent to prevent the accumulation of the toxins during storage [73, 154]. It was proven that *C. rosea* can inhibit the synthesis of fumonisins by *F. verticillioides* but does not degrade it [170]. Constant reduction of FB1 by 60–70% depending on the temperature when a 50:50 mixture of the pathogen and *C. rosea* 016 applied at different ripening stage of maize cobs. These investigations were done as *F. verticillioides* may attack maize at ripening under suitable environmental conditions [156]. Previously, similar results at the same concentration (50:50/ pathogen: *C. rosea* 016) in milled maize agar were also reported [155]. It could be concluded that using bacterial BCAs rely on antibiosis was more effective to control FB1 *in vitro* and in field trials.

3.4. Aflatoxins

AFs are the most natural carcinogenic substance in the history targeting mainly liver and are classified as Group 1 according to the International Agency for Research on Cancer [4, 6, 16, 171]. *A. flavus* and *A. parasiticus* infect mostly groundnuts, maize, cottonseed, soybean and tree nuts in the field and/or during storage producing a wide range of secondary toxic metabolites including AFs [60, 172]. Researchers have mostly been focusing on *A. flavus* as the fungus is highly invasive and more widespread in nature compared to *A. parasiticus*. Regarding their ability to synthesize mycotoxins, toxigenic *A. flavus* strains produce aflatoxin B1 (AFB1) and B2 (AFB2) while *A. parasiticus* produces four types of AFs (AFB1, AFB2, AFG1 and AFG2). CPA is only produced by *A. flavus* including strains which lack the potential to produce AFs [173].

In general, reduction of AFs in different crops has mostly been performed with non-toxicogenic *A. flavus* strains [27, 52, 54, 60, 65, 114, 120, 123]. Some of these strains (AF36 as an example) are commercially available in the market [53, 65]. Two theories are suggested on the mode of action for the reduction of AFs by non-toxicogenic *A. flavus* BCAs; (i) reduction due to competitive exclusion on toxigenic wild *A. flavus* population and (ii) inhibition of biosynthetic pathways involved in aflatoxin production, however the exact mechanism is still obscure [62].

Doster et al. used *A. flavus* strain AF36 as a BCA to control AFs in pistachio orchards for four consecutive seasons (2008–2011) and he could diminish AFs level by 20–45% [114]. In groundnuts, more trials *in vitro* [61, 66] and in the field [58–60] have been done. Zhou et al. 2015 found a positive correlation between AFs reduction rate and inoculum dose while Hulikunte Mallikarjunaiah et al. 2017 measured total AFs in rhizospheric and geocarpospheric soil and groundnut seeds after he treated them with two strains isolated from India. A significant reduction of mycotoxin concentration below the maximum permissible levels for ground nuts was obtained [61]. Field trials in Argentina were designed to control AFs in groundnut. However, the author reported a high level of AFs reduction, and the results were inconsistent between the two seasons [58, 59].

High levels of AFs and CPA control in maize field were achieved after challenging two strains of *A. flavus* with atoxigenic strains K49 and NRRL 21882 [65]. Mauro et al. could obtain similar results *in vitro* after screening for local atoxigenic strains from Italy [67]. In Nigeria, a successful maize field trial exhibited the promising use of two locally isolated strains, La3279 and La3303, in controlling AFB1 and AFB2 up to 99.9% [120]. When these two strains mixed with other two strains to make a mixture applied to the soil before flowering, a similar conclusion was obtained [55] with the advantage of persistence of the biocontrol effect during storage.

Researchers have also tested different species of *Trichoderma* such as *T. viride*, *T. harzianum* and *T. asperellum* [38, 95, 115, 116]; bacteria [84, 121, 124]; yeast [36, 174]; and algae [118] as a potential alternative BCAs to control *Aspergillus* spp., although not all have looked into mycotoxins (**Figure 2B**). Production of two volatile compounds, dimethyl trisulfide and 2,4-bis(1,1-dimethylethyl)-phenol, by *Shewanella algae* strain YM8 showed a 100% inhibition on aflatoxin synthesis in maize and peanuts stored at different water activities [118]. Previously, *B. subtilis* RCB 90 *in vitro* was also reported to completely inhibit AFB1 [121]. The yeast, *Candida parapsilosis* IP1698 was also able to inhibit aflatoxin production (90–99%) at different pH and temperatures [174]. This was also in line with the same reduction percentage obtained but with *Bacillus* spp. P1 and *Bacillus* spp. P11 [40]. Aiyaz et al. tested in the field, four BCAs and all the formulations, by maize seeds treatment application, had a significant reduction in AFs level [95].

4. From lab bench to field trials

Hundreds of BCAs have been tested against different types and strains of mycotoxigenic fungi *in vitro*. However, not all of them were effective against mycotoxigenic fungi under field conditions. For instance, Johansson et al. selected 164 bacterial isolates out of 600 for a field experiment to control *F. culmorum* infection in wheat and three strains of *Fluorescent pseudomonads* and a species of *Pantoea* gave a high level of control and consistent results [159].

In general, the difference in BCAs performance from *in vivo* condition to field conditions might be related to the influence of other factors present in the field such as meteorological parameters, soil characteristics, nutrient availability, microbial community which may affect the efficacy of the screened BCAs. Other important parameters which are not present in *in vivo* studies include the way of delivery of the BCAs to the host (spray or direct inoculation), form of

delivery (conidial or spore suspension/with or without carrier), application time (during seeding or flowering) and application route (to the soil or directly to the seed) to ensure the interaction of BCAs against the pathogen. Examples for the available BCAs in the market include AF36 and Afla-Guard® which are commercial BCAs for pre-harvest application to control aflatoxin contamination in the United States [62], Polyversum®, a recent authorized commercial product in France (Pythium oligandrum strain ATCC 38472) to be used against *Alternaria* spp., *Fusarium* spp., and other plant pathogens, and Plant Shield™ which is the registered product for *T. harzianum* 22.

It is crucial to test all the application related parameters in the field as these parameters may give significantly variable results which are not usually followed in many of the performed field trials against mycotoxigenic caused diseases. For example, point inoculation of *Streptomyces* sp. BN1 was not effective to control FHB in wheat while spraying of bacterial spores during wheat flowering gives better results [175]. Successful formulation of *C. rosea* ACM941 guaranteed its efficacy to control FHB in corn, soybean and wheat under filed conditions [176], while most of the field trials used a conidial or spore suspension of the BCAs which may give variable and inconsistent results. Ear inoculation with *B. amyloliquefaciens* and *Enterobacter hormaechei* exhibit highly changeable results while treatment of seeds showed more stable results for managing *F. verticillioides* infection and toxin content in maize [150]. On the other hand, *B. subtilis* strains SB01, SB04, SB23, and SB24 were performing better to control root rot disease when they were applied to soil than treatment of soybean seeds [145]. Omitting one or more of the above parameters may lead to misevaluation of the selected BCAs.

In some cases, a mixture of two more BCAs maybe advisable in the field for a better disease control in case they have a synergistic effect. For example, mixture of *L. plantarum* SLG17 and *B. amyloliquefaciens* FLN13 showed more efficacy in controlling FHB in wheat durum [131].

Although the field trials are exhausting and time consuming, it should consider the application way, application time, effective dose and the best formula in order to precisely evaluate the performance of the selected BCAs and thereafter ensure an effective control of the mycotoxigenic fungal infection and their mycotoxins.

An important obstacle in the commercialization of BCAs is legislation. Current legislations in Europe classify BCAs as Plant Protection Products/Pesticides and hence they must follow the according regulations of the pesticides. This entails that for each BCA the mode of action must be documented and their use should be rational [177].

5. Conclusions and future perspectives

Despite the considerable amount of research that have been done to screen and select effective BCAs to control mycotoxigenic pathogens and their mycotoxins, still there are several pitfalls for using BCAs. For instance, the broad spectrum antagonistic activity of some BCAs such as *Trichoderma* spp., against several pathogenic fungi may also affect other beneficial organisms present in rhizosphere [178] and this may require more research for target specific BCAs. Even though implementation of a biological control strategy is strongly recommended to replace the

use of synthetic pesticides, there are several concerns regarding the biological and environmental stability of BCAs. For example, the population of *A. flavus* including atoxigenic strains is highly diverse. This entails that there is a risk under certain environmental conditions that atoxigenic strains outcross with toxigenic *A. flavus* and thereafter produce mycotoxins [26, 62]. In addition, it is not guaranteed whether the atoxigenic strains can survive for a long time and what is the short term and long term effect on the soil microenvironment.

Care should be taken that besides successful control of plant pathogens, and BCAs themselves do not produce toxic substances. For instance, *C. rosea* secretes gliotoxin which is toxic metabolite to human. Also, it was reported that some *Trichoderma* strains harbor trichothecenes (*Tri*) genes that translate into proteins similar to *Fusarium Tri* proteins [179, 180]. This entails that *Trichoderma* spp. share the production of trichothecenes toxins (such as T-2 toxin) with *Fusarium* spp. In addition, gliotoxin and viridian produced by *T. harzianum*, *T. viride* and *T. virens* showed their phytotoxic effect by reducing seed germination rate in wheat and human toxicity [28]. Therefore, spreading such a microorganism into the environment may impose an extra burden to food safety and public health. Additionally, from the economical point of view, it is necessary to estimate the total cost of application and the need for seasonal reapplication of the BCAs, so it does not exceed costs of current practices.

Controlling mycotoxins is an important aspect in the management of mycotoxigenic pathogens, which adds an extra challenge to find an effective biocontrol agent to control the fungal growth and toxin production simultaneously. It is very well known that one fungal pathogen can produce simultaneously several unrelated mycotoxins, as an example *F. graminearum* produces DON and ZEN which both have two different biosynthetic pathways. The scientific research has mostly been focusing to control one type of mycotoxin. Consequently, it will be more valuable to select a single biocontrol agent able to simultaneously suppress the production of both toxins. It is crucial that the selected BCAs are tolerant to mycotoxins [169] which will guarantee the long term efficiency in the field.

Some mycotoxins can be modified by the plant through alteration of their chemical structure "i.e. conjugation to a glucose moiety and hence called plant metabolites of mycotoxins or modified or masked mycotoxins" [181]. For example, DON is transformed to deoxynivalenol-3-glucoside (DON3G) in the plant as a part of the plant defense mechanism. These masked forms of mycotoxins can be hydrolyzed back into their parent forms "DON" inside human and animal body. Therefore, it is of paramount importance to take into account the effect of biocontrol agents on the production of (masked) mycotoxins and to deeply investigate whether the efficacy of the selected BCAs is due to an actual reduction of mycotoxin content based on a direct inhibition of their production by the pathogen or due to enhancing the plant immunity which may increase the plant ability to form more DON3G as in this case the total mycotoxin content in the plant will remain unchanged. Furthermore, the underlying mechanism between the parent mycotoxin, host and BCAs remains obscure and should be further investigated. In addition, other categories of mycotoxins, however they pose health risks, are underexplored such as enniatins, emerging mycotoxins produced by *Fusarium* spp., [14, 182] have not been tested with BCAs and this necessitates the need for further investigation.

Different BCAs with different modes of action, formulation, treatments, application time were tested showing that it may be difficult to have a single BCA able to diminish all the regulated

mycotoxins “one fits for all may not be the case here” [183, 184]. To tackle this problem, maybe a combination of multiple BCAs or with fungicides could be considered. Application dose should be deeply investigated to achieve the desirable control. As in previous research, it has been shown that a suboptimal or sublethal treatment with fungicides [185] may lead to induction of mycotoxins production by the pathogen as a stress response. Searching for new BCAs with novel modes of action can assist to effectively control mycotoxigenic plant pathogens. Recently, *Enterobacter* spp., a root-inhabiting bacterial endophyte, was reported to have a different mode of action than those previously described through formation of physicochemical barrier that blocks the invasion of *F. graminearum*. However it is unclear whether this mode of action can be applied to maize and wheat [186]. Finally, the sound implantation of pre-harvest strategies can help in saving crop loss but does not fully ensure the safety of food as the fungal attack can also happen during storage or during processing which necessitate a post-harvest control.

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Conflict of interest

The authors declare no conflict of interest.

Other declarations

The authors have mentioned some trade names of certain BCAs for the scientific purpose only and this does not reflect any recommendation for use.

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