Baghdad Journal of Biochemistry
and Applied Biological Sciences
2026, VOL. 7, NO. 1, 8–14, e-ISSN: 2706-9915, p-ISSN: 2706-9907

REVIEW

Comparison of Artificial and Natural Biological Control Mechanisms to Suppress Mycotoxigenic Fungi

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Article Info.

Keywords:
mycotoxins,
phytochemicals,
fungi

Received: 06.30.2025
Accepted: 08.14.2025

Published: 01.09.2026

Abstract

Background: Quality and quantity of plant crops and feeds take a major concern by scientists for sustainable development. Mycotoxins are deleterious secondary metabolites synthesized by diverse species of filamentous fungi, predominantly from the genera *Aspergillus*, *Fusarium*, *Penicillium*, and *Alternaria.* The effects of mycotoxins on crops are complex, encompassing yield reduction, quality degradation, economic losses, food safety and health hazards as well as sustainability and soil health concerns. The World Health Organization, along with the Food and Agriculture Organization, has instituted international safety standards via Codex to govern mycotoxin concentrations in food and feed by establishing maximum permissible limits: aflatoxin B_1: 5–10 µg/kg in food products; total aflatoxins: 15 µg/kg; ochratoxin A: 3–10 µg/kg in cereals and 0.5 µg/kg in infant foods; and fumonisins, with a tolerable daily intake for FB_1: 2 µg/kg body weight per day.

Objective: Multiple modern controlling strategies are developed to limit fungal contaminant growth securing both agricultural yield and quality of productions. Currently this reviews provide comparative study among different biocontrol mechanisms that down-regulate mycotoxins productivity using different traditional biological mechanisms such as microbial degradation, antagonisms, and phytochemicals with recently developed methods focused on manipulation at genomic level for suppressing fungal proliferation and toxins pollution.

Conclusion: Modern biotechnological techniques directly interferes to diminish mycotoxin biosynthesis pathways and triggers innate plants defense systems, which enhance with great specificity, and more efficient and persistent restrictions.

1. Introduction

Globally, agricultural crops and feed are naturally attacked by various plant pathogens, particularly mycotoxigenic fungi [1]. Mycotoxicologist advance investigation confined mycotoxin production to certain fungal genera, such as *Aspergillus*, *Fusarium*, *Penicillium*, and *Alternaria*, regarded as the most important producers of mycotoxins [2,3]. Currently, more than 400 mycotoxins are produced by different types of fungi. However, one type of fungus can make different mycotoxins [4]. Aflatoxin (AFs), fumonisin (FBs), T-2, and HT-2 toxins, ochratoxin (OTA), deoxynivalenol (DON), zearalenone (ZEN), patulin, and ergot alkaloids (EAs) are common and dangerous mycotoins for food safety and public health (Table 1). Mycotoxins are created by fungi during their secondary metabolism in both raw and processed forms of important crops, such as maize, wheat, rice, and nuts. The Food and Agricultural Organization (FAO) has projected global population to be 9.7 billion by 2050; so, agricultural production must increase by at least 60%. Studies in agriculture and crop biotechnology concentrate on enhancing the quality and quantity of crops by developing varieties that are resistant to infections. Therefore, the primary motive

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How to cite this article: Abdulateef, SM. Comparison of Artificial and Natural Biological Control Mechanisms to Suppress Mycotoxigenic Fungi. Baghdad Journal of Biochemistry and Applied Biological Sciences, 2026, VOL. 7, NO. 1, 8–14. https://doi.org/10.47419/bjbabs.v7i1.424

is to devise essential and effective strategies to ensure safety in controlling the growth of fungi and halting the production of mycotoxins [4–6].

The US Food and Drug Administration provides policy guides and updates regulatory limits for different foods and determine maximum limits of mycotoxins. according to united state (US) on 2024 graded as total aflatoxin (0.1–0.2 µg/kg for babies and 20 µg/kg for adults), ochratoxin (3–5 µg/kg), DON (1,000 µg/kg for wheat), fumonicin (2,000–4,000 µg/kg for maize), and patulin (50 µg/kg). The association of these metabolites with health risks is notable, particularly in instances of chronic exposure, which heightens the risk of cancer, immune-toxic effects, and considerable developmental health issues. This includes neural tube birth defects (NTDs) and stunting in children, with additional evidence indicating that certain mycotoxins exhibit teratogenic properties [7].

According to severity and ubiquitously, this review has focused on highly poisonous secondary metabolites: aflatoxins (AFB1 and AFB2), fumonisin (FB1 and FB2), and their controlling mechanisms, which are applied to increase producers' growth. AFB1 is generated by *Aspergillus flavus* and *Aspergillus parasiticus*. AFB1 is regarded as a carcinogenic, teratogenic, and mutagenic agent. The International Agency for Research on Cancer (IARC) has graded it as level 1 for carcinogenicity, demonstrating a substantial link with liver cancer, immunological suppression, and growth impairment because of chronic exposure [8,9]. Fumonisins are significant pollutants of maize and its by-products, generated by *Fusarium verticillioides* and *Fusarium proliferatum*. Fumonisins are harmful both individually and in conjunction with other mycotoxins, and are associated with immunological suppression, hepatotoxicity, nephrotoxicity, and chronic liver disorders [10]. They are the causal agents of numerous diseases affecting plants, animals, and humans. The IARC has determined fumonisin as a level 2 carcinogen [11].

Mycotoxins biological down-regulation

A variety of methods, such as chemical (enzymes) and physical (radiation, ozone, etc.) methods, are utilized to control fungal growth in order to reduce mycotoxins' creation, including biological degradation.

This review focused on biological methods, an attempt to implement sustainable development. Mycotoxin biocontrol is classified in two main categories: Natural biological control and artificial biological control. Natural methods include applying plant extracts and phytochemicals [19], microbial competition or antagonistic nontoxic fungi—those able to reduce mycotoxins action by antifungal activity, mycotoxins biosynthesis inhibition, antioxidants action, and mycotoxin degradation [20]. On the other hand, artificial biological control techniques involve genomic manipulation at both crops and pathogens levels.

Application of plant extract and phytochemicals

Recently, evidence-based uses of plant-derived phytochemicals are observed, especially essential oils (EOs) and phenolics for inhibiting mycotoxin-producing fungus and lowering toxin levels in crops and stored foods as presented on Table 2.

According to Vaičiulienė *et al*. [21], extracts from oregano and thyme can reduce mycotoxin levels and enhance the sanitary quality of corn silage. Maize silage

Table (1): Crop mycotoxins and their pathogenic effects.

Mykes genera + species	Mycotoxins	Target crops	Plant diseases	Human health effects	References
Aspergillus flavus	Aflatoxins (B1, B2, G1, G2)	Rice, seeds (cotton, maize) and nuts	Accumulative on seeds	Carcinogenic, teratogenic, and mutagenic	[12]
Aspergillus parasiticus	Aflatoxins (B1, B2, G1, G2)	Spices, ground nuts, zea mays	Accumulative on seeds	Carcinogenic, teratogenic, and mutagenic	[13]
Fusarium verticillioides	Fumisins (B1, B2)	Maize	Root and stem rot	Neurotoxic, carcinogenic	[14]
Fusarium graminearum	Deoxynivalenol (DON) zearalenone	Wheat, maize, and barley	Head blight (scab)	Vomiting Immunosuppression, reproductive disorders	[15]
Fusarium sporotrichioides	T-2 and HT-2 toxins	Oats, wheat, barley	Accumulative on seeds	Cytotoxic Immunosuppressive	[16]
Penicillium verrucosum	Ochratoxin A	Barley, wheat, rye	Accumulative on seeds	Neurotoxic Genotoxic Carcinogenic	[17]
Penicillium expansum	Patulin	Apples, pears	Blue mold rot in apples	Mutagenicity Neurotoxicity Genotoxicity	[17]
Alternaria alternata	Alternariol (AOH), tenuazonic acid	Tomatoes, cereals, oilseeds, fruits		Cytotoxic Genotoxic Endocrine disruption	[18]

Table (2): Survey about diverse plant extract and phytochemicals.

Sources/plants	Active components	Mechanism of action	Targets	References
Thymus vulgaris (thyme)	Thymol, carvacrol	Antifungal	*A. flavus* *Fusarium*	[21]
Cinnamomum	Cinnamaldehyde	Antifungal	Aflatoxin Ochratoxin	[23]
Zingiber officinale (genger)	Gingerol, shogaol	Antifungal Antioxidant	Aflatoxins Fumonisins	[25]
Curcuma longa (turmeric)	Curcumin	Antioxidant Gene expression modulator	Aflatoxins Fumonisins	[26]
Allium sativum (garlic)	Allicin	Antifungal ROS modulation	Aflatoxin Ochratoxin	[27]
Cymbopogon citratus (lemongrass)	Citral	Antifungal	*Aspergillus*	[28]

samples were subjected to fermentation under laboratory conditions utilizing MIX of thyme [TE] + oregano [OE]. Silos were opened after 90 fermenting period and silage samples were collected to evaluate levels of mycotoxins and sanitation standard. The levels of mycotoxins on silo decreased variably; ochratoxin (TE + MIX] post-treatment came down below the detection threshold; minimal level of DON was recorded with [OE] post-treatment. On other hand, [T-2] toxin levels were markedly reduced following [MIX] application [21].

Romoli *et al*. showed that the evaluated EOs reduced half ZEA generation by *F. graminearum* and DON generation. For DON and ZEA respectively, the EO and GEO were the EOs with most strong anti-mycotoxigenic effect. These EOs may eventually replace synthetic fungicides, since they have shown encouraging *in vitro* suppression of mycotoxin generation [22].

According to the findings of Gwad *et al*., cinnamon oil, followed by peppermint oil, exhibited the highest fungicidal activity against the growth and aflatoxin production of *A. flavus* on wheat grains. This activity was enhanced when the *nor-1*, *afl*R, *pKs*A, and *aflLJ* genes were suppressed by 94–96% because of the application of cinnamon oil at a concentration of 0.062%, in comparison to the control [23].

Wang *et al.* investigated the effectiveness of cinnamaldehyde as anti-aflatoxigenic agent when applied on *A. flavus*. The results revealed high percentage of studied genes (about 85%) out of the genome cluster exhibiting suppression due to extract reaction [24].

Competitive exclusion by antagonistic microorganisms

A broad spectrum of microorganisms, such as non-toxic and friendly fungi, yeast, and bacteria are employed for naturally controlling mycotoxin synthesis strategy.

Akbar *et al*. employed environment-friendly microbes such as *Lactobacillus casei*, *Bacillus licheniformis*, *Rhodococcus erythropolis*, *Aspergillus niger*, and *Saccharomyces crevice* in their investigation, which have potential reduction effect against aflatoxins to tolerable consumption levels. The utilization of these bacteria for biodegradation positively influences biocontrol efforts for mycotoxins. Their findings indicated that *Rhodococcus erythropolis* and *Bacillus licheniformis* might degrade aflatoxins to undetectable levels, resulting in reductions of 0.57 ppb and 0.95 ppb for *Rhodococcus erythropolis* and *Bacillus licheniformis*, respectively [29].

Mahmoud *et al*. showed considerable potential for prevention of toxigenic fungus growth against *A. flavus* and *F. proliferatum* for *L. macroides* [30]. *L. macroides,* the antagonistic bacterium, displayed chitinase activity and production that suggests its use as a biocontrol agent. The bacterial supernatant showing antifungal properties revealed an excess of Pyrrolo [1,2-a] pyrazine-1,4-dione, Hexahydro by GC-MS analysis. *A. flavus* produced *L. macroides* to decease substantially AFB1 and AFG2. It also displayed significant 90% suppression of fumonisin B1 resulting from *F. proliferatum* [30].

Different *Lactiplantibacillus plantarum* strains isolated compounds have demonstrated antifungal activity against various types of fungi. *L. plantarum* primarily demonstrates inhibitory effects on the genera *Aspergillus*, *Fusarium, Penicillium*, *Rhizopus*, and *Mucor*. The primary recognized components consist of volatile organic compounds (VOCs), organic acids, and proteinaceous substances [31].

Dobrzyński and Naziębło gave statistics in their review; they assumed that additional strains from the *Paenibacillus* genus besides *P. polymyxa* could be employed for plant protection in future. Enhancing comprehension in this domain could augment the efficacy of biocontrol agents, such as *Paenibacillus* spp., thus ensuring the safe and sustainable utilization of biological fungicides [32].

Application of genomic manipulation strategy

Recent insights have shown that the application of genome manipulation at transcriptional regulator factor levels within microbes or hosts has potential indications against toxin production. Figure 1 briefly explains the transformation process, which involves formation of RISC complex

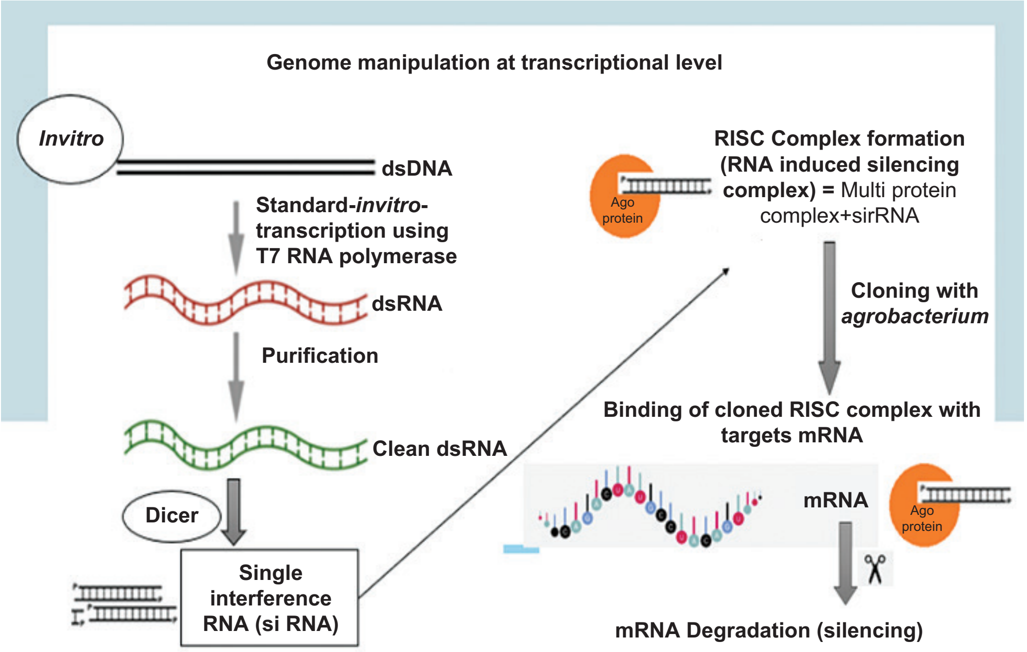


Figure (1): General plant genome manipulation strategy.

that plays center role in RNA interference (RNAi) and then either degrade it or block its translation [33,34].

Yulfo-Soto *et al.* studied the wheat strain (FgTRI101), which was cloned and incorporated into the wheat cultivar Bobwhite to investigate whether wheat could convert and excrete 3-ADON and lower *Fusarium graminearum* trichothecene 3-O-acetyltransferase, to determining whether wheat could reduce *Fusarium* head blight (FHB) and trichothecene contamination [35]. Following infection with *F. graminearum*, FgTri101-transgenic wheat plants exhibited significantly enhanced resistance to FHB and decreased levels of DON; 3-ADON was not detected [35].

Yang *et al*. showed inhibition capacity of both non-aflatoxigenic *Aspergillus* strains and *Aspergillus oryzae* that cultured on cell-free cultivation through chemicals that produced. Inhibitory component efficiently suppress growth and reproduction, leading to reduced toxicity. Additionally, it was demonstrated that genes linked to the metabolic AFs pathway were markedly down-expressed, diminishing certainly *Far*B and *Afl*S [36]. Wang *et al*. demonstrated that disruption of *afl*R substantially impaired aflatoxin biosynthesis pathway, leading to a marked reduction in aflatoxin production [37]. The *afl*R deletion markedly modified the expression of genes associated with growth and development, along with carbohydrate-related metabolic pathways, corroborating the findings of Kong *et al.* [37,38].

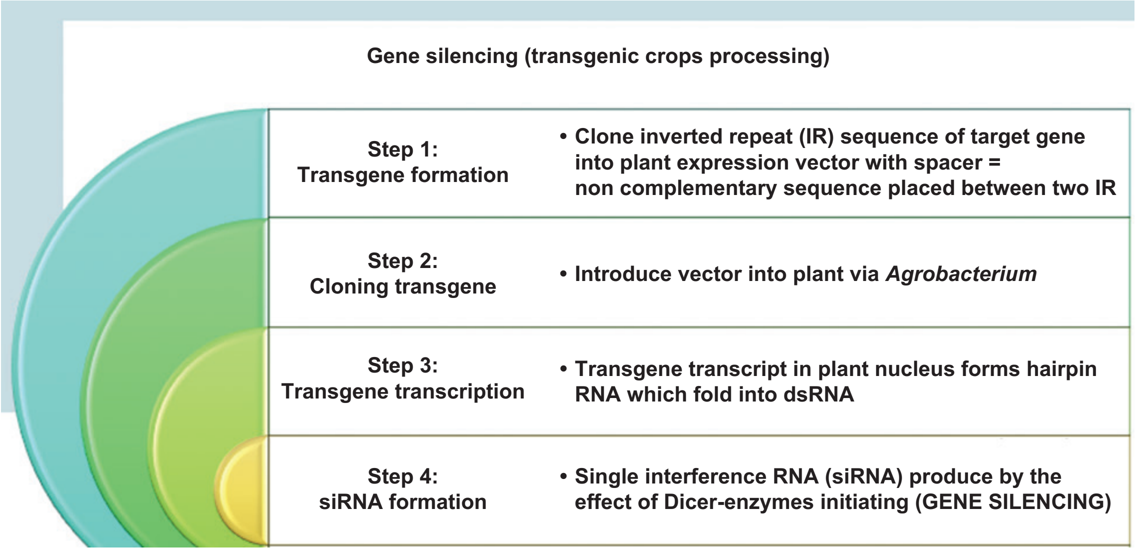
Masanga *et al.* developed an approach known as host-induced gene silencing, which has significant potential for creating aflatoxin-resistant plant germplasm suitable for the African context, where farmers cannot make additional investments beyond accessing the germplasm [39]. They modify maize using a hairpin design that targets the transcription factor *afl*R involved in aflatoxin production. The engineered transgenic maize was exposed to an aflatoxigenic strain of *A. flavus* from Kenya, the area prone to AFs epidemics. Masanga *et al.*'s findings indicated that *afl*R was down-regulated in *A. flavus* colonizing transgenic maize. Moreover, maize kernels from transgenic plants exhibited a dramatically reduced accumulation of aflatoxins by about 14-fold, compared to wild-type plants [39]. Figure 2 shows how genetically modified crops become transgenic using hairpin or spacer.

Peng *et al.* focused on transcription factor of oxidative stress *Atf*A and approved that it is crucial for the pathogenicity of *A. flavus* [40]. Indications revealed that knockout strains from *atf*A reduced sporulation of *A. flavus*, decreased AFB1 synthesis, and impaired invasive capacities under oxidative stress.

In agricultural crop protection, RNAi has been employed to combat pests and pathogens; however, challenges and limitations remain, including the environmental instability of double-stranded RNA (dsRNA) caused by UV exposure, precipitation, and microbial degradation, suboptimal uptake in specific insect species, elevated production costs, and regulatory obstacles concerning genetically modified organisms (GMOs) or RNA applications.

Conclusions

This review highlights how targeted strategies, including conventional biological control methods and newly created genetically engineered solutions for mycotoxin mitigation, act actively. Our findings complemented both strengths and unique differences in efficacy, sustainability, and spectrum of application. Conventional methods, such as biological control with antagonistic bacteria, and the application of natural plant extracts, have historically been utilized to mitigate fungal infections and mycotoxin accumulation.



Gene silencing (transgenic crops processing)

Step	Description
Step 1: Transgene formation	• Clone inverted repeat (IR) sequence of target gene into plant expression vector with spacer = non complementary sequence placed between two IR
Step 2: Cloning transgene	• Introduce vector into plant via *Agrobacterium*
Step 3: Transgene transcription	• Transgene transcript in plant nucleus forms hairpin RNA which fold into dsRNA
Step 4: siRNA formation	• Single interference RNA (siRNA) produce by the effect of Dicer-enzymes initiating (GENE SILENCING)

Figure (2): Gene silencing (transgenic crops processing).

Researchers' solutions are often environmentally sustainable, economically viable, and suitable for various agronomic circumstances. Nevertheless, they frequently encounter constraints, such as variable outcomes, susceptibility to environmental factors, and limited effectiveness, particularly under intense fungal stress. Conversely, genetically engineered methods, such as the creation of transgenic crops with improved resistance to fungal infections and targeted gene editing to inhibit mycotoxin producing genes in fungi, present potential and more precise solutions. Contemporary biotechnology interventions can directly disrupt mycotoxin production pathways or augment the plant's intrinsic defense mechanisms. Although they exhibit significant specificity and potential for prolonged protection, their implementation remains hindered by legislative, ethical, and acceptance challenges, in addition to technical constraints in large-scale deployment.

Acknowledgments

Author's appreciation to all the researchers whose contributions supported this evaluation. Their contributions were vital to the advancement of this paper.

Conflicts of Interest

The author confirmed that he was the sole author of this review paper. All information sources and previously published materials were appropriately credited and acknowledged. This manuscript has not been submitted for publication elsewhere.

Author contributions (CRediT Taxonomy)

SMA: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Writing-original draft; Writing-review and editing.

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