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Trichoderma harzianum Suppresses Aflatoxins in *Zea mays*: A Biological Strategy for Pakistan's Agriculture Industry

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Abstract

This study explores the use of endophytic fungi for the biocontrol of harmful aflatoxins (AFTs) in maize (*Zea mays* L.). The main objective of this study was to evaluate the effects of fungal pathogens and biocontrol agents on the corn seed germination and growth of seedlings under controlled conditions. Experiments were conducted under laboratory conditions in a growth chamber and in a greenhouse to assess the influence of environmental factors on seed performance and treatment efficacy. The growth chamber provided uniform conditions for physiological assessment while the greenhouses represented more realistic field conditions. Corn kernels were sown in sterile pots inside the growth chamber at standard conditions or in the greenhouse at controlled conditions and four treatment groups were established: untreated control seeds, seeds treated with non-AFT-producing (non-aflatoxigenic) strains (*Trichoderma harzianum*, *T. asperellum* and *Aspergillus niger*), seeds inoculated with AFT-producing (aflatoxigenic) strains (*A. flavus* and *A. parasiticus*), and seeds co-inoculated with both aflatoxigenic and non-aflatoxigenic strains (*A. flavus* and *A. parasiticus* with *T. harzianum*, *T. asperellum* or *A. niger*). High-performance liquid chromatography was utilized to detect and analyze the presence of AFTs. Co-culturing of *A. flavus* with *T. harzianum* resulted in a significant decrease in AFT levels, achieving a relative reduction of 99.3% compared to aflatoxigenic treatments alone. Among the isolates tested, *T. harzianum* and *T. asperellum* were the most effective at lowering AFT production of the aflatoxigenic strains, reducing the 5120 ± 560 µg/kg AFT level produced by *A. flavus* alone to 50.1 ± 1.10 and 63.1 ± 3.1 µg/kg, respectively. *A. flavus* negatively affected germination and early growth, whereas *T. harzianum* significantly enhanced both parameters. This study demonstrates that non-aflatoxigenic *Trichoderma* isolates can effectively mitigate AFT contamination and improve seedling growth, highlighting their potential as effective. sustainable, and locally adopted biocontrol agents for Pakistan's chronic AFT problem under diverse environmental conditions—an area with minimal prior research and high national relevance.

Keywords: aflatoxin biocontrol; endophytic fungi; *Trichoderma harzianum*; co-inoculation; mycotoxins; maize germination

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1. Introduction

Pakistan is a South Asian nation having a total area of 79.61 million hectares (fiscal year 2023–2024), of which 22.52 million hectares are cultivated for agriculture (fiscal year

2023–2024). Since agriculture accounts for 37.4% of employment and roughly 24% of GDP (2025), the majority of the population depends either directly or indirectly on agriculture, highlighting the influence of the agribusiness industry on Pakistan's economy [1,2]. Maize (*Zea mays* L.) is one of the most widely grown crops in the world for food, feed, and industrial use [3,4]. It is a staple crop in many countries and an essential commodity for global food security [5]. After rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.), maize is the third most important food crop worldwide. Similarly, maize is one of the most common crops grown in Pakistan, where it is cultivated on approximately 1.32 million hectares and yields an average of about 4787 kg per hectare in Punjab Province [6,7].

Maize is highly susceptible to mycotoxin contamination [8–11]. Since contamination frequently occurs in food and feed commodities [12], naturally occurring mycotoxins [13] pose a serious food safety concern in the global food industry. Approximately 25% of the world's food supply is significantly contaminated, among which aflatoxins (AFTs) have been identified as major contaminants [14,15]. First identified in 1960, AFTs are a class of naturally occurring, structurally similar secondary metabolites that are toxic, mutagenic, and carcinogenic, produced by specific *Aspergillus* species [16]. *Aspergillus flavus* and *A. parasiticus* are most frequently associated with AFTs contamination in fields and storage environments; however, many (though not all) *Aspergillus* species produce AFTs [17]. There are several types of AFTs, including aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2) [18–20]. Among these, AFB1 and AFG1 are considered the most significant because they are more prevalent in food [21]. AFB1, which exhibits several detrimental properties such as mutagenicity, carcinogenicity, hepatotoxicity, teratogenicity, nephrotoxicity, and immunosuppressive effects, is the most common and widespread food and feed contaminant in the world [22,23]. It is classified as a Group 1 carcinogen (carcinogenic to humans) by the International Agency for Research on Cancer (IARC) [24] implemented by regulatory agencies [25,26] and by the scientific community [27,28]. Long-term exposure to low amounts of AFTs from contaminated food and feed can lead to serious health effects, including immunosuppression, liver cancer, stunted growth in children, and other systemic diseases [21]. High-dose exposure can result in acute aflatoxicosis, which may cause induction of oxidative stress, neurotoxicity, and promotion of inflammatory gene expression [29], as well as hemorrhage, rapid liver damage, and even death [30]. Because these mycotoxigenic fungi cannot be completely eradicated from agricultural fields due to their extreme stability [31], standard chemical and physical methods have proven ineffective in eliminating them from contaminated food and feed. Consequently, controlling *A. flavus* and *A. parasiticus* and with this the possible production of these AFTs prior to harvest, post-harvest and in derived products remains a significant challenge [32–35].

Chemical techniques involve using agents such as acids, bases, oxidizers, and reducing substances to break down or bind AFTs. However, these approaches can alter the properties of food, pose safety concerns, or leave unwanted residues. Furthermore, chemical methods primarily remove toxins present on the surface of contaminated food, meaning that the breakdown of internal toxins remains a slow process [35]. Physical separation methods, including sorting, washing, milling, microwave heating, irradiation, and pulsed light, focus on physically removing contaminated particles or reducing AFT levels. Although somewhat effective, these techniques may not completely eliminate AFTs and often require significant labor [36]. Biological degradation involves using microorganisms or enzymes to break down AFTs into metabolites that are less harmful or non-toxic.

The range of fungicides registered and remaining applicable against *Aspergillus* spp. is rather limited and further shrinks due to emerging fungicide resistance affecting not only the agriculture sector but also environmental and human health [37–40]. Biocontrol

methods show promise by reducing reliance on chemical fungicides but require precise optimization and management to ensure its effectiveness [41]. Due to its safety, sustainability, and cost-effectiveness, the biocontrol approach is considered a desirable and appropriate alternative for the removal and degradation of AFB1 in agricultural products. Moreover, biocontrol strategies help maintain the acceptability, safety, nutritional value, and sensory quality of agricultural commodities [42]. However, despite these efforts, challenges remain in achieving consistently high levels of AFT detoxification while adhering to food quality and safety standards.

In addition to non-aflatoxigenic strains of *A. flavus* [43], various antagonistic microbes, including different bacteria and yeasts, have demonstrated significant inhibitory effects on the growth and/or AFT production of aflatoxigenic strains and are considered potential candidates for the biocontrol of AFT contamination during pre-harvest stages. Among these antagonists, fungi belonging to the genus *Trichoderma* are the most widespread biocontrol agents worldwide, available for inoculation, as well as in formulated biopesticides and biostimulants [44,45]. *Trichoderma* fungi are primarily free-living and mostly inhabit soil, consisting of strains that are highly competitive in the rhizosphere and associated with root ecosystems. *Trichoderma* species are predominantly atoxigenic: given strains may produce toxic metabolites, there are only a few *Trichoderma* species with known mycotoxin production. In particular, *T. harzianum* and *T. asperellum* species are considered not to produce common mycotoxins, although certain strains of both species produce secondary metabolites that can be phytotoxic or plant growth promoters, such as trichotecene polyketides, including harzianums A–B, harzianumols A–H, trichoharzin derivatives, and recently identified harzianolides K to Q, as well as terpenoids, lactones, alkaloids, peptides or amphipathic peptaibols [46–51]. In turn, we do not apply the term "atoxigenic" for these *Trichoderma* species as it would suggest the existence of toxigenic strains. The beneficial impacts of *Trichoderma* include their ability to suppress both soil-borne and foliar plant pathogens, such as *Fusarium* species [52], as well as to enhance plant resistance to abiotic stresses and diseases, improve mineral nutrition for plants, promote plant growth, and increase crop yield. The suppression of AFT production by *Aspergillus* species (mainly *A. flavus* and *A. parasiticus*) has been indicated to occur through a multi-faceted process including direct interaction, antibiosis, and environmental modification [53]. *T. harzianum* exerts its inhibitory effects against *A. flavus* species through several coordinated mechanisms, beginning with direct mycoparasitism where its hyphae coil around and penetrate the competitor's cell walls, leading to cytoplasmic leakage and lysis. In addition to physical contact, the fungus emits volatile organic compounds (VOCs) such as 6-pentyl-2H-pyran-2-one that act as biofumigants to inhibit spore germination, while simultaneously secreting non-volatile secondary metabolites that serve as antibiosis agents to suppress radial growth and AFT synthesis. The process is further bolstered by enzymatic degradation, as *T. harzianum* produces extracellular hydrolases—including chitinases and glucanases—that break down *Aspergillus* cell walls and degrade AFB1 into less toxic, polar molecules. Finally, as a fast-growing organism, it outcompetes its rival for nutrients and space, rapidly colonizing the substrate and depleting the resources necessary for *Aspergillus* to thrive or produce harmful toxins [54–59].

T. asperellum is a well-known biocontrol fungus. It has several ways of improving plant health against phytopathogens through mycoparasitism, the secretion of cell wall-degrading enzymes like chitinases and glucanases, and the induction of plant defense responses [60]. Additionally, it promotes seedling strength, root growth, and soil structure, all of which help maintain long-term soil health and stress tolerance [61]. Species of both *Bacillus* and *Trichoderma* also help to solubilize phosphate, which further supports plant growth [62,63]. The recognized biocontrol efficacy of *Trichoderma* and *Bacillus* species is

partly due to their ability to produce a particular glucanase, β-1,3-glucanase, an important enzyme that breaks down β-1,3-glucans in fungal cell walls. This action leads to the destruction and inhibition of pathogens. This enzyme is also crucial in the mycoparasitism and pathogen suppressing features of *T. harzianum*. β-1,3-glucanase often works together with chitinase to boost antifungal effectiveness [64]. *Bacillus* spp., including *B. subtilis* and *B. amyloliquefaciens*, are similarly able to release β-1,3-glucanase that serves as a key factor in their antifungal activity against various plant pathogens [65]. Beyond direct antagonism, both genera can induce systemic acquired resistance. They increase the levels of defense-related enzymes such as phenylalanine ammonia-lyase and peroxidase, along with pathogenesis-related proteins like β-1,3-glucanases. This process combines direct pathogen suppression with improved host defense [66]. Microbial bioproducts (especially *Bacillus* and *T. asperellum*) improved Napier grass growth and physiology in a cultivar-dependent manner with improved chlorophyll and indoleacetic acid contents by *Bacillus* and increased β-1,3-glucanase and peroxidase activities by *Trichoderma* [67].

The remarkable effectiveness of *Trichoderma* spp. as biocontrol agents, classified as safe for human consumption, stems from their ability to employ multiple mechanisms of antagonistic action against plant pathogens. As rapidly growing and metabolically adaptable fungi, they can utilize a wide range of organic materials as a food source, making them highly competitive in soil environments. Furthermore, they produce several secondary metabolites that inhibit plant pathogens, acting as chemical defenses in what is known as interference competition. While *Trichoderma* spp. has been among the most extensively researched agents for the biological control of soil pathogens [68], to date, only a limited number of *Trichoderma* species and strains, particularly *T. harzianum*, have been evaluated for their efficacy in controlling *A. flavus* and reducing AFT production [45,54,69–76].

Despite the growing recognition of AFT contamination as a major threat to Pakistan's maize production, there remains a lack of locally generated evidence on sustainable, biological solutions that can function effectively within the country's distinct agro-ecological environment. Conventional chemical and physical control methods often degrade food quality, raise safety concerns, and fail to completely eliminate internal toxin residues. In contrast, biological management using beneficial fungi offers an environmentally friendly and cost-effective solution that aligns with global goals for sustainable agriculture and food safety. Although *T. harzianum* is widely recognized for its biocontrol potential, it has not been specifically evaluated for their ability to suppress AFT risks in maize systems. This gap limits the development of practical, low-cost, and environmentally compatible mitigation strategies for farmers.

Therefore, the focus of the present study is to investigate the role of biocontrol of AFT levels produced by aflatoxigenic strains (*A. flavus* and *A. parasiticus*) in *Zea mays* by three fungal strains not producing mycotoxins (*T. harzianum*, *T. asperellum* and *A. niger*). The effects of biocontrol agents were systematically evaluated in controlled experiments, with artificial co-inoculation, on germination and seedlings under laboratory conditions and in greenhouses as a context-appropriate biological approach for reducing AFT levels in *Zea mays*.

2. Results

2.1. Effects of Single and Co-Inoculation of Aspergillus flavus and Trichoderma harzianum on Germination Seedling Growth Parameters

The influence of fungal treatments on maize seed germination and early seedling growth is presented in Table 1. The results revealed significant variation among treatments. Seeds inoculated with the aflatoxigenic strain of *Aspergillus flavus* exhibited the

lowest germination percentage (50.0 ± 2.8%) and delayed germination (germination time 5.3 ± 0.3 days) indicating a significant negative effect of the pathogen on seed viability and early development ($p < 0.01$). In contrast, seeds treated with the non-toxin-producing strain *T. harzianum* showed the highest germination percentage (90.0 ± 2.5%) and the shortest mean germination time (3.8 ± 0.2 days), suggesting that this bioagent significantly promotes seed vigor and rapid emergence ($p < 0.05$). Growth parameters followed a similar trend, where the longest shoot length, root length, and total seedling length were recorded in the *Trichoderma*-treated group. The co-inoculation treatment showed improved germination and seedling growth compared with the pathogen-only treatment, indicating that the atoxigenic strain can mitigate the inhibitory effects of *A. flavus*. Overall, the results suggest that the application of *T. harzianum* enhances seed germination and supports early seedling development in maize.

Table 1. Effects of fungal treatments on maize seed germination and development. Values are represented as mean ± standard deviation of three independent replicates. Germination and development parameter values in single and co-inoculation treatments are compared statistically to the control group by *t*-test at significance level of 0.05. Significant differences compared to the control group for each parameter are indicated in parentheses after the values, *: $p < 0.05$, **: $p < 0.01$. The distribution of all datasets was found normal by the Shapiro–Wilk test ($p = 0.86$–0.91), and homogeneity of the variances did not differ from each other significantly based on Bartlett's test ($p = 0.75$–0.82).

Fungal Species	Germination (%)	Germination Time (Days)	Shoot Length (cm)	Root Length (cm)
Control	80.0 ± 3.2	4.2 ± 0.2	10.6 ± 0.5	7.8 ± 0.4
Single inoculation				
A. flavus	50.0 ± 2.8 (**)	5.3 ± 0.3 (**)	7.4 ± 0.4 (**)	5.2 ± 0.3 (**)
T. harzianum	90.0 ± 2.5 (*)	3.8 ± 0.2 (*)	12.3 ± 0.6 (*)	9.1 ± 0.5 (*)
Co-inoculation				
A. flavus/*T. harzianum*	85.0 ± 3.0	4.0 ± 0.2	11.8 ± 0.5 (*)	8.7 ± 0.4 (*)

2.2. Detection of Aflatoxins by Instrumental Analysis

The high-performance liquid chromatography (HPLC) system equipped with a UV detection confirmed the presence of AFB1, AFB2, AFG1 and/or AFG2 in the samples. AFTs detection in HPLC analysis is displayed by the corresponding chromatograms (Figure 1). The identity of AFB1, AFB2, AFG1 and AFG2 was verified by comparing the detected peaks in standard AFT solutions and processed maize samples (see Section 4.4). Identification of AFB1, AFB2, AFG1, and AFG2 was carried out using standard reference materials. The retention times were 4.15, 4.77, 5.38 and 6.27 min for AFG2, AFG1, AFB2 and AFB1, respectively, closely matching the national standard procedure [77], with negligible variations between the two instrumental analytical systems. Calibration curves were prepared with standard solutions at 0.01, 0.05, 0.125, 0.25, 0.5, 1.25, 2.5, 5.0, 10 and 20 µg/mL concentrations for all AFT types investigated, and peak areas for each AFT showed a linear correlation with the AFT concentration in this concentration range. The limit of detection (LOD) in the extract was found to be 0.01 µg/mL using the HPLC protocol applying UV detection. This LOD in the extract corresponds to AFT levels of 0.67–1.43 µg/kg in the maize kernels (based on 15–25 g maize samples subjected to the sample preparation procedure). A consistent pattern of similar peak areas was observed in the majority maize samples.

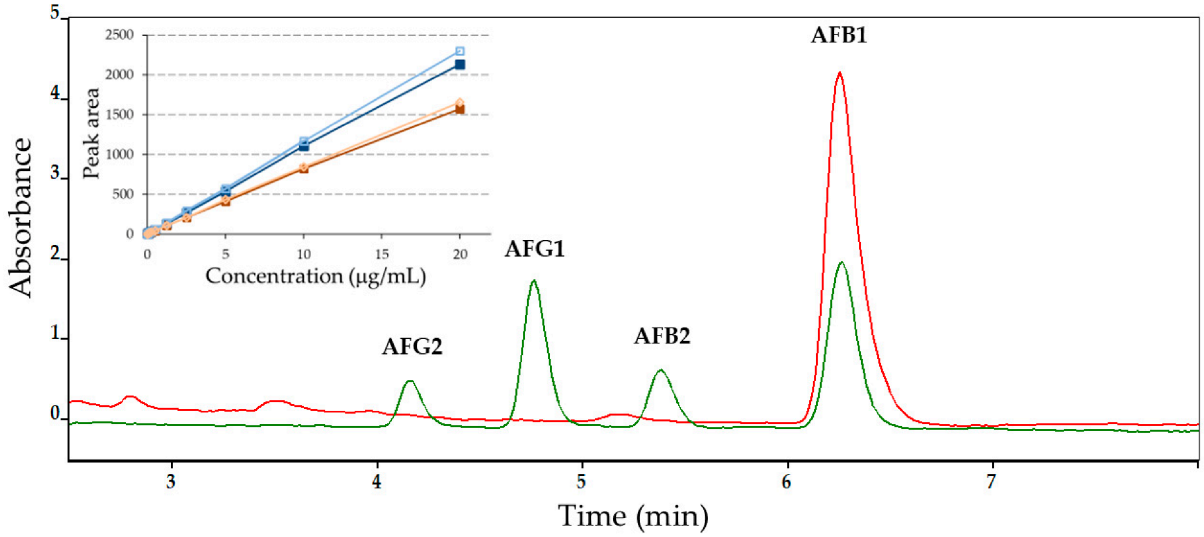


Figure 1. High-performance liquid chromatography chromatograms showing 0.15 µg/mL of aflatoxin G2 (AFG2), 0.5 µg/mL of aflatoxin G1 (AFG1), 0.15 µg/mL of aflatoxin B2 (AFB2), and 0.5 µg/mL of aflatoxin B1 (AFB1) standard (green line), and 0.90 µg/mL of AFB1 produced by *Aspergillus flavus* co-inoculated with *T. harzianum* (red line). The standard calibration curves for each mycotoxin are depicted in the inserted diagram in the concentration range of 0.01 to 20 µg/mL of AFB1 (dark blue line), AFB2 (light blue line), AFG1 (dark brown line), and AFG2 (light brown line).

In inoculation experiments performed under laboratory growth chamber and greenhouse conditions, AFT determination for single and co-inoculated treatments showed variation in the results (Table 2). In co-inoculation compared to single inoculation, total AFT concentrations were significantly reduced in kernels when aflatoxigenic isolates were paired with non-aflatoxigenic ones. The comprehensive statistical analysis demonstrated that both the biocontrol agent (*T. harzianum*, *T. asperellum* or *A. niger*) and the experimental conditions (growth chamber or greenhouse), as well as the toxicogenic pathogen (*A. flavus* or *A: parasiticus*), had a significant effect on AFT production. ($p = 0.001$).

2.3. Total Aflatoxin Concentration in Grains Under Laboratory and Greenhouse Conditions

In inoculation experiments conducted both under laboratory (growth chamber) and greenhouse conditions, AFT levels varied significantly between single and co-inoculated treatments. Overall, co-inoculation with non-aflatoxigenic isolates consistently reduced AFT concentrations compared to single inoculations with toxigenic strains in both experimental systems.

In single inoculation, *Aspergillus flavus* produced significantly higher AFT levels than *A. parasiticus* under both growth chamber ($p = 0.002$) and greenhouse conditions ($p = 0.001$). Under growth chamber conditions, total AFT concentrations were 6720 ± 880 µg/kg in kernels treated with *A. flavus* (AFB1 and AFB2 producer) and 3504 ± 71.8 µg/kg in kernels treated with *A. parasiticus* (AFB1, AFB2, AFG1, and AFG2 producer). Under greenhouse conditions, corresponding values were 5120 ± 560 µg/kg and 1479 ± 20.8 µg/kg, respectively. For both aflatoxigenic *Aspergillus* strains (*A. flavus* and *A. parasiticus)*, significantly lower toxin levels were observed in the greenhouse compared to the growth chamber ($p = 0.03$ for *A. flavus* and $p = 0.001$ for *A. parasiticus*).

Across all co-inoculation treatments, AFT concentrations were significantly lower in the greenhouse than in the growth chamber ($p \leq 0.03$). In both experimental systems, co-inoculation of toxigenic strains with biocontrol agents (*Trichoderma harzianum*, *T. asperellum*, and *Aspergillus niger*) significantly reduced AFT levels compared to single inoculations

($p < 0.001$). Under growth chamber conditions, the greatest reduction for *A. flavus* was observed with *T. harzianum* (77.4 ± 17.8 µg/kg), followed by *T. asperellum* (146 ± 2.9 µg/kg) and *A. niger* (295 ± 20.5 µg/kg). *T. harzianum* not only exceedingly reduced AFB1 levels, but decreased AFB2 levels to below the LOD. Similar substantial reductions were observed for *A. parasiticus*, where co-inoculation with *T. harzianum* resulted in the lowest AFT level (346 ± 31.6 µg/kg), significantly lower than other combinations ($p < 0.001$).

Table 2. Total aflatoxin (B1, B2, G1, G2) concentration in maize kernels and aflatoxin level reduction rates (where applicable) when aflatoxigenic *Aspergillus* species (*A. flavus* and *A. parasiticus*) or non-aflatoxigenic *Aspergillus* (*A. niger*) or *Trichoderma* species (*T. harzianum* and *T. asperellum*) were administered in single inoculations or combined inoculations of aflatoxin producers and non-aflatoxigenic isolates under laboratory growth chamber or greenhouse conditions. Values are represented as mean ± standard deviation. Homogenous groups determined at a significance level of 0.05 by one-way analysis of variance and Tukey's honest significant difference are presented for growth chamber (small letters in parenthesis) and greenhouse (capital letters in parentheses) after the total aflatoxin concentration values for single and co-inoculation on grains. The distribution of all datasets was found normal by the Shapiro–Wilk test (p = 0.63–0.96), and homogeneity of the variances did not differ from each other significantly based on Bartlett's test (p = 0.55–0.97). * Total combined concentration of aflatoxin B1, aflatoxin B2, aflatoxin G1, and aflatoxin G2; ** Aflatoxin reduction% = [1 − (total aflatoxin in co-inoculation/total aflatoxin in aflatoxin producers)] × 100; *** *A. flavus* and *A. parasiticus* are aflatoxin producers, and their corresponding aflatoxin content is based on kernel inoculation using these isolates.

Fungal Species	Total Aflatoxin Concentration * (µg/kg)	Aflatoxin Reduction Rate ** (%)	Total Aflatoxin Concentration * (µg/kg)	Aflatoxin Reduction Rate ** (%)
	in growth chamber		in greenhouse	
Single inoculation on grains				
A. flavus ***	6720 ± 880 (g)	–	5120 ± 560 (G)	–
A. parasiticus ***	3504 ± 71.8 (f)	–	1479 ± 20.8 (F)	–
Co-inoculation on grains				
A. flavus/*T. harzianum*	77.4 ± 17.8 (a)	98.8	50.1 ± 1.1 (A)	99.0
A. flavus/*T. asperellum*	146 ± 2.9 (b)	97.8	63.1 ± 3.1 (AB)	98.8
A. flavus/*A. niger*	295 ± 20.5 (c)	95.6	154 ± 1.5 (D)	97.0
A. parasiticus/*T. harzianum*	346 ± 31.6 (d)	90.1	70.6 ± 16.5 (BC)	95.2
A. parasiticus/*T. asperellum*	827 ± 95.5 (e)	76.4	99.7 ± 14.7 (C)	93.2
A. parasiticus/*A. niger*	850 ± 99.7 (e)	73.7	394 ± 64.4 (E)	73.4

In the greenhouse experiment, a comparable pattern was observed, although overall toxin levels were lower than those recorded under growth chamber conditions ($p \leq 0.03$). Co-inoculation treatments further reduced AFT concentrations to 50.1 ± 1.1–154 ± 1.5 µg/kg for *A. flavus* and 70.6 ± 16.5–394 ± 64.4 µg/kg for *A. parasiticus*. Among biocontrol agents, *T. harzianum* consistently showed the strongest reduction in AFT production, followed by *T. asperellum*, while *A. niger* showed comparatively weaker effects ($p \leq 0.001$).

Overall, *T. harzianum* was the most effective biocontrol agent in reducing aflatoxin accumulation in both growth chamber (AFT reduction rates were 98.8 and 90.1% for *A. flavus* and *A. parasiticus*, respectively) and greenhouse conditions (AFT reduction rates were 99.0 and 95.2% for *A. flavus* and *A. parasiticus*, respectively) generally enhanced the reduction effect compared to growth chamber conditions (Figure 2).

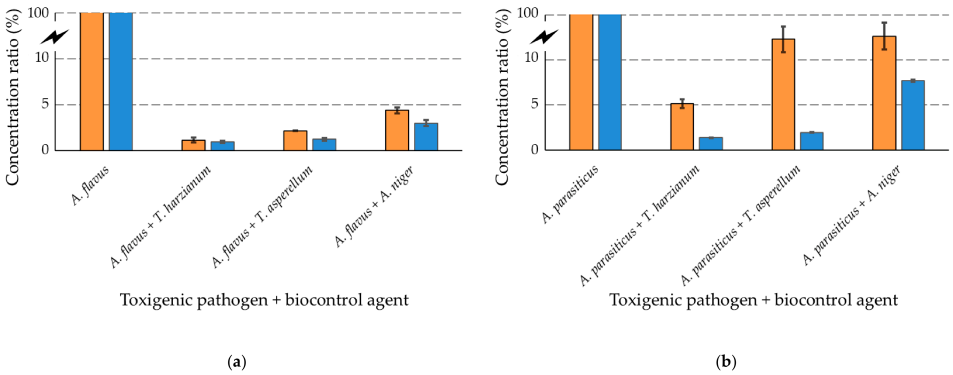


Figure 2. Aflatoxin production by *Aspergillus* species *A. flavus* (**a**) and *A. parasiticus* (**b**) suppressed by co-inoculation with biocontrol agents *Trichoderma harzianum*, *T. asperellum*, or non-aflatoxigenic *A. niger*, expressed as percentage ratios relative to the maximal aflatoxin production capacity of the given *Aspergillus* species in single inoculations, under growth chamber (orange columns) and greenhouse (blue columns) conditions. The ↯ signs on the concentration axes indicate axis breaks.

2.4. The Effect of Biocontrol Agents on Grain Quality

Based on evaluations of grain quality from harvested ears, the effectiveness of biocontrol agents against mycotoxigenic fungi was evident. The highest-quality kernels in terms of color were observed in ears treated with *T. harzianum* in the co-inoculation treatment. The kernels treated with *A. flavus* alone were affected by the discoloration (Figure 3).

2.5. Assessment of the Antifungal Potential of Trichoderma harzianum and T. asperellum Against Aspergillus flavus and A. parasiticus

The endophytic *T. harzianum* and *T. asperellum* were evaluated for their antagonistic properties against *A. flavus* in an in vitro setting (Figure 4a–c). Results from the dual culture test indicated that *T. harzianum* exhibited a faster growth rate than both *A. flavus* and *T. asperellum*. The findings revealed that both *T. harzianum* and *T. asperellum* inhibited the growth of *A. flavus*, with *T. harzianum* being more effective. After five days of incubation, a distinct clear zone appeared between the two colonies, restricting the mycelial expansions of *A. flavus*. On the seventh day, the 77% inhibition percentage of *T. harzianum* was significantly higher ($p = 0.040$) against *A. flavus*, compared to 60% for *T. asperellum* relative to the control (Figure 4d). After three additional days, *A. flavus* was completely overgrown by both *T. harzianum* and *T. asperellum*. The findings demonstrate that these *Trichoderma* species, particularly *T. harzianum*, possess antagonistic activity against *A. flavus*.



Figure 3. Discoloration effect caused by toxic fungus. (**a**) Treated with *Aspergillus flavus* only, (**b**) co-inoculation of *A. flavus* with *Trichoderma harzianum*.

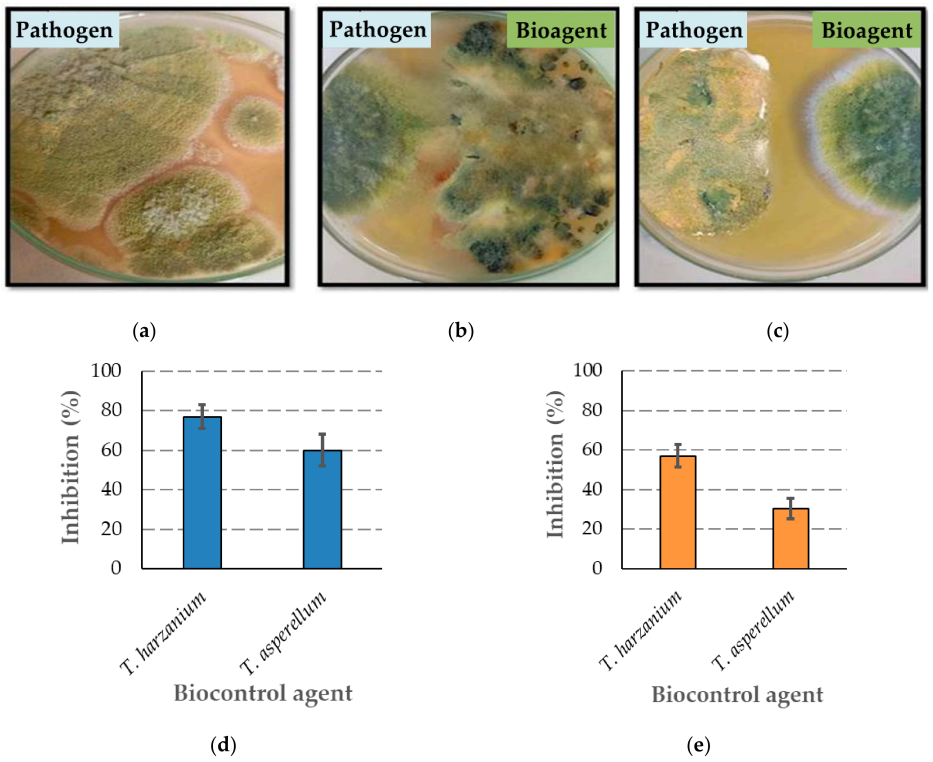


Figure 4. Effects of bioagents on *Aspergillus flavus* and *A. parasiticus* linear growth reduction percentage using dual culturing technique. (**a**) Control; *A. flavus* only, (**b**) interaction between *Trichoderma harzianum* and *A. flavus*, (**c**) interaction between *T. asperellum* and *A. flavus*, (**d**) percentage growth inhibition of *A. flavus* by each bioagent, (**e**) percentage growth inhibition of *A. parasiticus* by each bioagent.

3. Discussion

In the present study, the efficacy of non-aflatoxigenic fungal isolates (*T. harzianum*, *T. asperellum* and *A. niger*) in reducing AFT contamination caused by aflatoxigenic strains of *A. flavus* and *A. parasiticus* in maize was examined under controlled laboratory and greenhouse conditions. The findings clearly indicate that co-inoculation with non-aflatoxigenic isolates resulted in a significant reduction in AFT production, supporting the potential of improved inoculation strategies as a viable approach for biological control.

In the growth chamber experiments, the AFT concentration produced by *A. flavus* co-inoculated with *T. harzianum* was the lowest (77.4 ± 17.8 µg/kg) compared to the aflatoxigenic control (6720 ± 880 µg/kg). This drastic decrease can be explained by previous studies that attribute the antagonistic mechanisms of *Trichoderma* spp. to competition for space and nutrients, mycoparasitism, and secretion of antifungal metabolites such as peptaibols and gliotoxin [70–80]. The superior effectiveness of *T. harzianum* compared to other isolates suggests that it achieves stronger competitive exclusion against *A. flavus*, likely due to its rapid colonization ability and production of cell wall-degrading enzymes [53,81]. The relatively higher AFT concentrations observed in co-inoculations of *A. niger* and *A. parasiticus* (850 ± 99.7 µg/kg) may be attributed to the less aggressive antagonistic nature of *A. niger* against aflatoxigenic fungi. Similar findings were reported by Ehrlich [82], who noted variable efficacy depending on the aflatoxigenic strain and environmental conditions. Nevertheless, even in these cases, AFT levels were significantly reduced compared to the aflatoxigenic control, indicating the partial biocontrol potential of *A. niger*. However, this utility is limited in practical applications by the known potential of the genus to produce ochratoxin. Laboratory data were corroborated by greenhouse experiments, confirming the efficacy of *T. harzianum* under semi-field conditions. Kernels

inoculated with combined *A. flavus*/*T. harzianum* showed the lowest measurable AFT levels (50.1 ± 1.1 µg/kg) still well above the LOD, whereas kernels inoculated with *A. flavus* alone accumulated high AFT content (5120 ± 560 µg/kg). This result aligns with previous findings by Atehnkeng et al. [83,84], who demonstrated that non-aflatoxigenic isolates can significantly reduce AFT biosynthesis in maize under field conditions. Additionally, co-inoculation with *T. asperellum* also resulted in a markedly low AFT level when paired with *A. flavus*, reinforcing the need for broader use of biocontrol agents.

Although the particularly high AFT reduction efficacy values reported in this study exceed the average reduction rate published in the scientific literature, under certain conditions these high reduction rates can be achieved. In a field study conducted during the growing season of 2022 in Novi Sad, Serbia, maize samples from *T. harzianum* K179-treated plots contained AFTB1 under the LOD (<0.4 µg/kg) compared to the untreated control (3.05 ± 0.21 µg/kg) and synthetic fungicide-treated plots (0.70 ± 0.10 µg/kg) [76]. Also, in a field experiment, groundnut seeds were treated by *T. harzianum* and *T. viridae* each at 5 g/kg in Ethiopia. In this study, concentrations of 5704.4 µg/kg AFTB1 and 2219.0 µg/kg AFTB2 were measured in untreated control plots, while plots treated with *T. harzianum* and *T. harzianum* + farm yard manure were free from AFT (concentrations were under the LOD value) [85]. Under in vitro circumstances performed on Czapek Dox-Agar supplemented with 2% peanut flour, AFTB1 production by *A. flavus* was reduced by *Trichoderma* spp. at a maximum level of 84.9% [52]. A storage study of *Sorghum bicolor* proved the effective biocontrol importance of genus *Trichoderma*, with the AFTB1 production reduction rate by 90.5% and 83.5% by *T. harzianum* and *T. afroharzianum*, respectively, compared to the inoculated positive control (682.6 µg/kg) [86].

The observed decrease in AFT levels can be explained by several mechanisms. A possible mode of action is that *Trichoderma* spp. outcompetes aflatoxigenic fungi by colonizing maize kernels and rhizospheres, occupying ecological niches, and producing volatile organic compounds (VOCs), while also inducing host plant defense responses [79,87,88]. Another mechanism involves *A. niger*, which secretes organic acids and enzymes that alter the microenvironment, making it less favorable for aflatoxigenic fungi [82]. Furthermore, suppression of AFT production may occur not only through fungal inhibition but also via disruption of AFT biosynthetic pathways through molecular signaling [89,90].

Although the efficacy of non-aflatoxigenic isolates was observed in both experimental setups, greenhouse findings revealed a lower amount of residual AFT compared to the flask experiment, particularly when *A. niger* was co-inoculated. This distinction highlights the influence of environmental factors—such as humidity, temperature, and microbial community dynamics—under semi-field conditions in altering the interactions between aflatoxigenic and non-aflatoxigenic fungi [84].

Considering the high AFT production levels observed in the current laboratory and greenhouse study due to the intensive inoculation procedure applied, it is important to note that the objective of this study was not to reproduce typical field contamination levels but rather to evaluate the efficacy of the biocontrol treatment under highly challenging conditions. That is why the experimental setup employed artificial inoculation with aflatoxigenic fungi and conditions specifically designed to maximize fungal growth and AFT production. Such a stringent experimental setup represents a worst-case scenario that places substantial pressure on the biocontrol agent. Consequently, although the residual AFT concentrations remained above the regulatory limits established by the European Union, the observed reduction is noteworthy because it was achieved under conditions that strongly favored AFT biosynthesis. The high efficacy observed in this challenge model suggests that the biocontrol strategy has considerable potential for reducing aflatoxin contamination under practical agricultural conditions, where infection pressure and environmental conditions

may be less extreme than those imposed in the laboratory. At the same time, field environments are inherently more complex than laboratory systems, and therefore direct extrapolation should be made with caution. The practical application of biocontrol agents in the field offers additional opportunities for reduction in AFT production when integrated with good agricultural practices, proper drying, optimized storage conditions, and the use of competitive non-toxigenic strains. Future studies should focus on field-scale evaluations, optimization of application methods and timing, and the integration of biocontrol with other management strategies to achieve aflatoxin levels that comply with international regulatory standards.

4. Materials and Methods

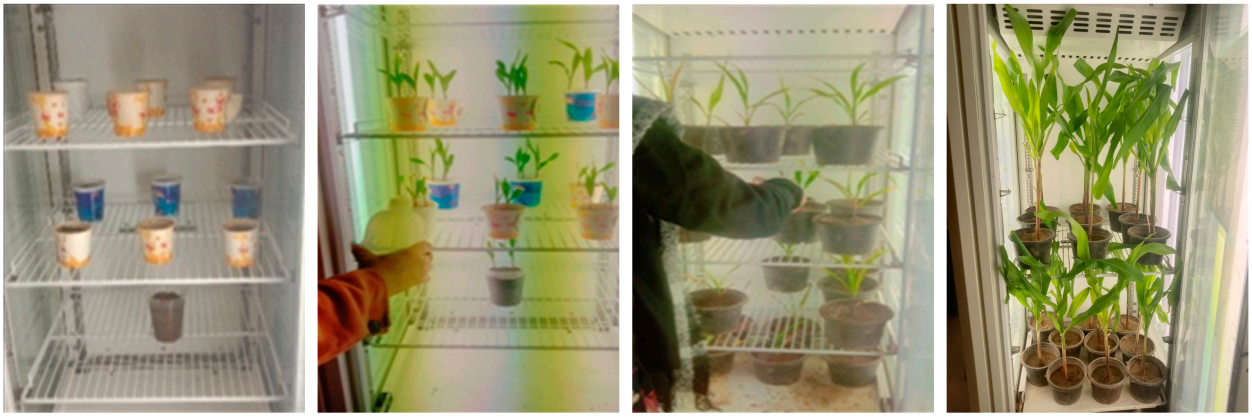
4.1. Isolation of Fungal Strains

Fungal isolates used in this study, comprising aflatoxigenic *A. flavus* and *A. parasiticus* and non-aflatoxigenic *T. harzianum*, *T. asperellum*, and *A. niger*, were obtained from maize kernels collected from farmer's fields at the National Agricultural Research Centre, Punjab, Pakistan. The absence of AFTs in those samples was verified using analytical thin layer chromatography [83,91]. The maize agar plate technique was utilized to isolate fungal strains. Maize samples were first sanitized three times with sodium hypochlorite and then with distilled water before being placed on a potato dextrose agar (PDA) plate. From each sample randomly twenty grains were picked and about three to five grains were aseptically placed on each PDA plate [19]. For complete fungus development, the plate was cultured for 5–7 days at 28 °C and number of kernels showing fungus in each Petri dish was counted [92]. The fungi that emerged were purified and stored. Fungal isolates were maintained on PDA slants and were kept in laboratory conditions for further analysis. The cultures of fungal species were purified by single spore isolation method and were identified based on colony morphology—including the color, texture, and pattern—and microscopic examination of spore characteristics. In addition, the amplified and purified 5.8S region of samples was sequenced using the services provided by Macrogen Co. Ltd. (Seoul, Republic of Korea). The resulting sequences were analyzed using BLASTn (version 2.17.0) (National Center for Biotechnology Information (NCBI), Bethesda, MD, USA) against the NCBI GenBank database, and species identity was confirmed based on $\geq$99% sequence similarity with reference strains. The purified strains have been deposited in the fungal strain collection of the Department of Environmental Sciences, Fatima Jinnah Women University (Rawalpindi, Pakistan). During the experiments, they were stored at 4 °C along with continuous subculturing after every 15–16 days in order to avoid contamination.

4.2. Growth Chamber and Greenhouse Inoculation

For maize seed germination and development experiments in the growth chamber, corn seeds were initially sterilized to eliminate surface fungi and bacteria. Corn kernels were surface sterilized with 1% (*v*/*v*) sodium hypochlorite for 3 min, followed by 70% ethanol for 1 min. They were then rinsed five times with sterile distilled water. Seeds were subsequently sown (4–5 kernels per cup) in 6–7 cm diameter paper cups containing 200 g autoclaved soil and maintained at 21–26 °C in a growth chamber with 14 h light: 10 h dark cycle for 120 days (4 months). Plants were watered every 2 days to maintain soil moisture at approximately 70–80% of field capacity throughout the experiment (Figure 5). Temperature and relative humidity were maintained at 21–26 °C and 55–65%, respectively. Upon germination and subsequent growth, plants were transplanted to larger pots (4–5 gallon) in a greenhouse, arranged in a randomized block design, and grown under natural light with regular irrigation and fertilization to ensure optimal growth. The day and night cycles were modified according to the condition of corn seedlings. Illumination

was maintained under a constant 14:10 h (light:dark) photoperiod, and light intensity was regulated by adjusting the number of fluorescent light tubes turned on out of the 15 tubes (40 W each) installed in the lighting cassette of the PHCBI MLR-352 plant growth chamber (PHC Holdings Corp., Tokyo, Japan). Illumination at plant canopy height with one to four tubes on is 1350 to 5400 Lux, respectively, corresponding to photosynthetic photon flux densities ranging from 10 to 40 µmol photons/m^2/s. The growth chamber cycle was started at 10:00 AM with maximum 4 lights for the entire day. At 6:00 PM, the number of lights shifted to 3. After this time, dark conditions were given to the seeds till 4:00 AM, when only 1 light was turned on to growing seeds. As the day progressed, the number of lights again turned to maximum 4 lights. Temperature fluctuations were also started at 10:00 AM with maximum temperature 26 °C which continued till 3:00 PM. At 6:00 PM the temperature was lowered to 24 °C. Further temperature was decreased to 22 °C at 9:00 PM and kept dropping to 20 °C at 12:00 PM. Finally, temperature fell to its lowest point of 18 °C at 4:00 AM. In a similar manner, temperature began rising again from daybreak to nightfall and went down as night began to fall and increased as the daybreak approached. Relative humidity in the growth chamber was maintained within a range of minimum 55% at 10:00 AM, increased to 60% at 3:00 PM and remained constant at 6:00 PM. However, humidity level rose at 9:00 PM to its maximum range that is 65% and again this value stayed the same to 12:00 PM and persisted unchanged until 4:00 AM. Afterward relative humidity started to decrease to 60% and continued to fall further to 55% as morning arrived. This signified that relative humidity fluctuated between these two values of min. 55% to max. 65% which provided optimal conditions for growth of corn seedlings.



(**a**) (**b**) (**c**) (**d**)

Figure 5. Cultivation of corn plants from seeds inoculated with aflatoxigenic and non-aflatoxigenic isolates of fungal spore suspension maintained under regulated conditions and watered at regular intervals to attain optimal growth. (**a**) Corn seeds sown in sterilized soil and grown in a controlled chamber, (**b**) corn seedlings emerged, (**c**,**d**) plants transferred into larger pots upon growth.

The two-stage potting system (in growth chamber and greenhouse) was adopted to optimize early seedling establishment and subsequent experimental conditions. Seeds were initially sown in smaller pots in a controlled growth chamber to ensure rapid and uniform germination under consistent temperature, light, and moisture conditions. Seedlings reached the V10–V12 stage, prior to tasseling. Tasseling and silking occurred approximately 7 days after transfer to the greenhouse. The initial 120-day growth period in the growth chamber ensured uniform seedling establishment under controlled conditions. They were transferred to larger pots in the greenhouse to provide greater root volume and environmental conditions suitable for the later experimental treatments. This approach minimized

early-stage variability and allowed better control during inoculation, while preventing root restriction in the later growth phase.

Inoculations with aflatoxigenic and non-aflatoxigenic isolates were carried out for both growth chamber and greenhouse experiments. For preparation of the inoculation, spore suspensions of 10^6 spores/mL were prepared from 5-day-old cultures grown on PDA at 28 °C, using sterile distilled water containing 0.02% Tween 80, and adjusted with a hemocytometer [83]. The efficacy of non-aflatoxigenic isolates was tested on maize grains under laboratory conditions (growth chamber study design). Grains were sterilized, inoculated with aflatoxigenic and non-aflatoxigenic isolates in 2 single (*A. flavus* or *A. parasiticus*) and in 6 co-inoculation treatments (*A. flavus* + *T. harzianum*, *A. flavus* + *T. asperellum*, *A. flavus* + *A. niger*, *A. parasiticus* + *T. harzianum*, *A. parasiticus* + *T. asperellum*, *A. parasiticus* + *A. niger*). There were 23 individual treatments (including growth chamber and greenhouse) consisting of water control with 0.02% Tween 80, individually 11 treatments for each isolate (200 mL, 1×10^6 spores/mL) and non-aflatoxigenic isolate seeded with the same proportion as the aflatoxigenc isolate (100 mL each, 2×10^6 spores/mL) with 12 treatments. The aflatoxigenic isolate in co-inoculated treatments was inoculated one hour before the non-aflatoxigenic isolate. In order to make sure that the surfaces of kernels were entirely coated with inoculums the vials were shaken for thirty seconds in a vortex mixer. For exchange of gas, caps of vials were slightly closed and incubated for seven days at 31 °C then oven dried for one day at 45 °C to stop further activity of fungus, and were finally ready for aflatoxin analysis.

The germination experiments were set up in a randomized design with three replicates per treatment. For each replicate, 20 treated seeds were placed evenly on two layers of sterile Whatman No. 1 filter paper (Merck KGaA, Darmstadt, Germany) in 9 cm Petri dishes moistened with 5 mL of sterile distilled water. The plates were sealed with Parafilm and incubated in a growth chamber at 25 ± 2 °C with a 12 h light and 12 h dark photoperiod and 70% relative humidity. Germination percentage was recorded after 7 days. Seeds were considered germinated when the radicle length reached ≥2 mm. Germination time was calculated as the average number of days needed for germination. After 21 days, the shoot and root lengths of 10 randomly selected seedlings per replicate were measured in centimeters using a digital caliper. All seedling length measurements were expressed as mean ± standard deviation of three replicates (60 seeds per treatment for germination; 30 seedlings per treatment for growth).

Greenhouse inoculation experiments using artificial wounding and inoculation were conducted similarly as reported in the scientific literature in similar Petri dish or other format experiments to model natural infection processes by bypassing physical barriers for the entry of toxigenic *Aspergillus* [93] and *Fusarium* spp. [94]. Inoculations were carried out using two aflatoxigenic and three non-aflatoxigenic fungal strains. The non-aflatoxigenic strains included *T. harzianum*, *T. asperellum*, and *A. niger*, while the aflatoxigenic strains were *A. flavus* and *A. parasiticus*. *A. flavus* was selected for its strong AFT-producing ability in both growth chamber and greenhouse conditions. Inoculation was performed in single (*A. flavus* or *A. parasiticus*) and in co-inoculation treatments (*A. flavus* + *T. harzianum*, *A. flavus* + *T. asperellum*, *A. flavus* + *A. niger*, *A. parasiticus* + *T. harzianum*, *A. parasiticus* + *T. asperellum*, *A parasiticus* + *A. niger*). Maize ears were inoculated with *A. flavus* and *A. parasiticus* at the early dough stage. A 3 mm diameter cork borer was used to create a wound through the cob husk, and 10 µL of spore suspension (1×10^6 conidia/mL) was injected directly into the wound using a syringe needle (Figure 6). Maize samples were harvested eight weeks after inoculation in all greenhouse sampling events, and dried to below 15% moisture to halt fungal growth. At maturity, five cobs per plot were harvested and sun-dried in their husks for 14 days. The grains combined from these cobs were then assessed to evaluate the

effectiveness of non-aflatoxigenic isolates in competitively excluding AFT-producing fungi, thereby preventing AFT contamination at both the wound site and in unwounded kernels. Kernels were carefully removed using a spatula, grouped by treatment plot, and stored in separate envelopes at 4 °C until further analysis.

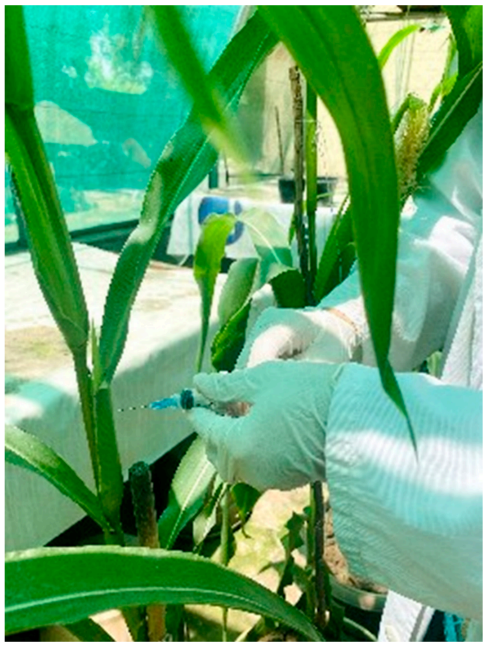


Figure 6. Inoculation of corn seedling under greenhouse conditions.

4.3. Aflatoxin Analysis by High Performance Liquid Chromatography

Prior to AFT analysis, grains were washed with sterile distilled water under a laminar flow hood to maintain sterile conditions and eliminate surface spores. The uncontaminated grains were then dried in an oven at 45 °C for 24 h. AFT analysis was performed by HPLC according to our previous studies [95,96].

Parallel samples of 15–25 g kernel mass were separately prepared for extraction by suspending them in 70% aqueous ethanol and subsequently being ground in a blender for 2–3 min each. The ground mixtures were filtered through Whatman No. 1 paper, and the filtrates were transferred into 250 mL separatory funnels. Extraction was then carried out with 25 mL methylene chloride, which was subsequently filtered through 40 g of anhydrous sodium sulfate to remove residual water. The extraction process was repeated twice, and the combined extracts were pooled in a polypropylene cup and evaporated to dryness in the dark under a fume hood. The evaporation residues were redissolved in 1 mL of the HPLC mobile phase (see below), diluted as required, and subjected to HPLC analysis to quantify AFB1, AFB2, AFG1, and AFG2, with mobile phase optimization to enhance chromatographic peak resolution. Analytical standards of AFB1, AFB2, AFG1, and AFG2 were obtained from Merck KGaA (Darmstadt, Germany) in prepared mixture solutions in acetonitrile: mixtures contained 1 µg/mL of AFB1 and AFG1, each, and 0.3 µg/mL of AFB2 and AFG2, each (Aflatoxin Mix kit in methanol solution #46304), or 20 µg/mL of AFB1, AFB2, AFG1, and AFG2, each (Aflatoxin Mix 4 solution #33415). Calibration curves were prepared with standard solutions at 0.01, 0.05, 0.10, 0.25, 0.50, 1.0, 2.0, 4.0, 10, and 20 µg/mL concentrations for all AFT types investigated. If the concentrations of given extracts appeared to be higher than the above concentration range, the extract was diluted ten-fold to allow precise quantification and to avoid saturation of signals.

An HPLC system comprising a quaternary pump, an autosampler, a degasser, and a UV detector (YL9100 HPLC system, Young Lin Instrument Co., Anyang, Republic of Ko-

rea) was used for analysis. A C18 column (Brisa LC2, Teknokroma, Barcelona, Spain, 4.6 × 150 mm, 5 µm) maintained at 30 °C was employed for separation. UV detection was performed at a wavelength of 365 nm. The mobile phase consisted of water/acetonitrile/methanol (60:20:20%). Samples were eluted at a flow rate of 1.0 mL/min, with an injection volume of 20 µL. YL Clarity software ver. 8.6.1 (Young Lin Instrument Co., Anyang, Republic of Korea) was used to capture and integrate the peak areas of AFTs [95,96]. Alternatively, samples were analyzed on a similar equipment (Shimadzu LC-20AT, Shimadzu Corp., Kyoto, Japan) equipped with a photodiode array detector using a slightly different C18 column (4.6 × 150 mm, 3.5 µm) maintained at 40 °C and a mobile phase of acetonitrile/methanol (70:30%) filtered through a 47 mm × 0.45 µm membrane and degassed before use, and LabSolutions software ver. 1.07 (Shimadzu Corp., Kyoto, Japan) for managing the chromatograms. It has to be noted that UV detection at 365 nm, although not being the most suitable method for AFT detection as detection by tandem mass spectroscopy would have offered more accurate detection, still provided LODs sufficiently low for the current study.

4.4. Evaluation of Antifungal Potential of T. harzianum and T. asperellum Against A. flavus

A 5 mm mycelial disk of the pathogen was placed 1 cm from the edge of a PDA plate using the dual culture technique, while the antagonistic fungus was positioned 1 cm from the opposite edge of the plate (Figure 7). A mycelial disk of the pathogen was inoculated alone on a PDA plate to serve as the control. Plates were incubated at 28 ± 2 °C for seven days. The assay was performed twice, each time in triplicate. The percentage of mycelial growth inhibition was then calculated [57,97].

$$\textit{Growth inhibition}\ (\%) = \frac{C - T}{C} \times 100$$

where C and T represent the radial growth (mm) of isolate in the control and treated plates.

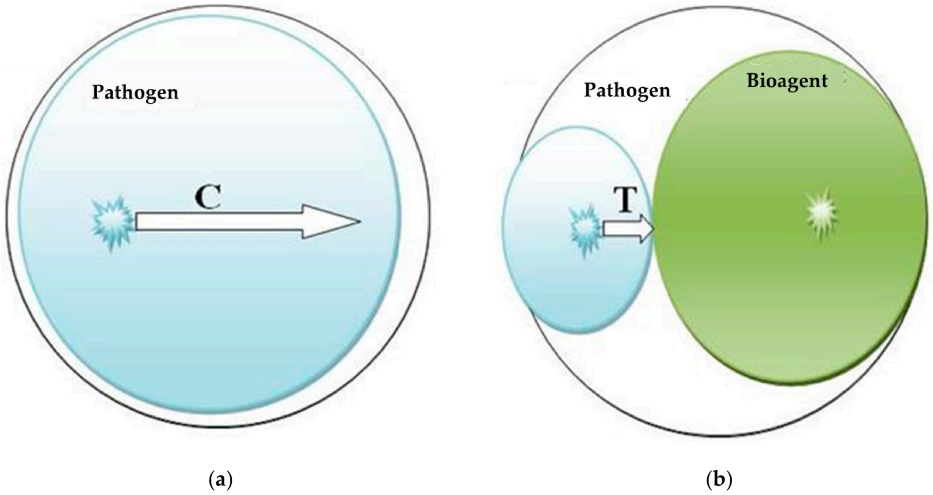


Figure 7. The antifungal activity of the investigated bioagent against the *A. flavus* pathogen evaluated using the dual culture method. Blue and green colors represent the pathogen and the bioagent (both fungal), respectively. (**a**) The control plate, (**b**) a plate with two cultures. Letter C indicates the average distance (in cm) of pathogen growth from the inoculation point to the edge of the colony. Letter T indicates average distance (in cm) of pathogen growth from the inoculation point to the edge of the colony in the direction of the antagonist bioagent.

4.5. Statistical Analysis

Results of the AFT concentrations and germination parameters determined were statistically analyzed using the Statistica 8.0 software (StatSoft, TIBCO Software Inc., Tulsa,

OK, USA). Various steps of statistical analysis were performed at significance level of 0.05. Normality of the distribution of the data was assessed by the Shapiro–Wilk test. Since all datasets were found to be of normal distribution, the homogeneity of their variance was determined using Bartlett's test. Significant effects of the experimental conditions and germination parameters were determined by *t*-test or Mann–Whitney U test for pairwise comparisons according to the results of normality and variance test. For multiple comparisons, one-way analysis of variance or the Kruskal–Wallis test were applied followed by Tukey's honest significant difference or Student–Newman–Keuls post hoc tests, respectively, according to the results of normality and variance tests.

5. Conclusions

Our research findings strongly support incorporating non-aflatoxigenic isolates such as *T. harzianum* and *T. asperellum* into an integrated AFT control program for maize. These isolates may complement existing cultural practices and post-harvest storage strategies to minimize the risk of mycotoxin contamination in regions with high AFT prevalence, such as Punjab, Pakistan. Furthermore, the use of native strains enhances environmental adaptability, thereby improving field performance. The intensive inoculation procedure applied in the present laboratory and greenhouse modeling of AFT production and its biocontrol resulted in very high levels of AFT production, more than 10 times higher than the permissible level in the European Union. The aim of the experiments has been to model worst-case fungal infection scenarios, and infection rates under field conditions are expected to remain substantially below the laboratory outcomes. Yet, biocontrol of strongly aflatoxigenic *Aspergillus* species by the use of competing non-aflatoxigenic microorganisms displayed superior suppression of AFT production even at these high toxin production levels; therefore, the extent of such suppressive efficacy is rather promising for applications under field conditions. Thus, future research should focus on multi-season field trials, optimization of formulation and delivery methods (e.g., seed coating, soil drenching), and evaluation of the long-term ecological and ecotoxicological impacts, positive or negative, of introducing aflatoxigenic fungal strains. Importantly, although the practice of competitive exclusion biocontrol demonstrated here has been extensively documented worldwide, this study is among the first ones in Pakistan to systematically assess endophytic *Trichoderma* species as AFT biocontrol agents, addressing a critical national challenge with locally adapted solutions. The dual benefits observed—effective AFT mitigation and improved germination and seedling vigor—underscore the potential of these isolates as sustainable, environmentally friendly biocontrol tools. Overall, the findings contribute new insights to the limited body of regional research and open pathways for developing scalable biological strategies to reduce AFT contamination in maize production systems.

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Abbreviations

The following abbreviations are used in this manuscript:

AFB1	Aflatoxin B1
AFB2	Aflatoxin B2
AFG1	Aflatoxin G1
AFG2	Aflatoxin G2
AFT	Aflatoxin
HPLC	High-performance liquid chromatography
IARC	International Agency for Research on Cancer
LOD	Limit of detection
PDA	Potato dextrose agar
spp.	Species
UV	Ultraviolet
VOCs	Volatile organic compounds

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