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Mycotoxin contamination in stored cereals and its mitigation through biocontrol agents

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Abstract
While chemical fungicides can suppress mould growth in stored grain, they leave residues that raise food safety concerns of their own a contradiction that has driven interest in biological alternatives. This research surveyed mycotoxin contamination levels in wheat, rice, and maize stored under farm conditions in Nakuru County, Kenya, and then tested two biocontrol agents *Trichoderma afroharzianum* (strain T-22) and *Lactobacillus buchneri* (strain LB-01) for their ability to reduce aflatoxin B1 in artificially inoculated grain over a 60-day storage period. Among the 90 grain samples surveyed, 67% exceeded the KEBS regulatory limit for at least one mycotoxin, with aflatoxin B1 being the most prevalent (38.4% of total detections). In the mitigation trial, *T. afroharzianum* at 2.0 g/kg achieved 68.1% aflatoxin reduction, while *L. buchneri* at the same dose reached 55.3%. Neither agent altered grain moisture or germination rate. The results suggest that *T. afroharzianum* is a practical biocontrol option for aflatoxin management in on-farm cereal storage where chemical treatments are undesirable.

Keywords: Mycotoxins, stored cereals, biocontrol agents, aflatoxin, grain safety, *Trichoderma*, *Lactobacillus*, food contamination, KEBS

Introduction
Global cereal production exceeds 2.8 billion tonnes annually, yet an estimated 25% of the world's food crops are contaminated with mycotoxins at levels that pose a risk to human and animal health [1]. In contrast to this scale of exposure, affordable and residue-free mitigation tools remain limited, especially for smallholder storage systems in the humid tropics [2].
Aflatoxins, produced chiefly by Aspergillus flavus and A. parasiticus, are the most regulated group of mycotoxins owing to their potent hepatotoxic and carcinogenic properties [3]. Kenya's Bureau of Standards (KEBS) sets a maximum limit of 15 µg/kg for total aflatoxins in cereals, but compliance monitoring at the farm-gate level is sparse [4]. Kenya's warm, humid storage conditions average relative humidity above 80% for six months of the year create an environment where toxigenic moulds thrive [5].
Biocontrol agents offer a residue-free alternative. Trichoderma species suppress toxigenic fungi through direct mycoparasitism, competition for nutrients, and volatile antibiotic production [6]. Lactic acid bacteria, meanwhile, can bind mycotoxins to their cell walls, effectively sequestering them from the grain matrix [7]. But comparative data on these two mechanistically distinct agents under identical storage conditions are lacking. This research therefore

a) Quantified mycotoxin profiles in farm-stored wheat, rice, and maize in Nakuru, and
b) Compared *T. afroharzianum* and *L. buchneri* for aflatoxin B1 reduction in a 60-day controlled storage trial.

Material and Methods
Reagents and Quality Control
Aflatoxin B1, ochratoxin A, deoxynivalenol, zearalenone, and fumonisin B1 standards (≥ 98% purity) were procured from Sigma-Aldrich, Germany. ELISA kits (AgraQuant, Romer Labs) were used for initial screening, and positives were confirmed by HPLC-FLD (Shimadzu LC-20A with RF-20A fluorescence detector). Recovery rates for spiked samples ranged from 87 to 104%. Method detection limits were 0.5 µg/kg for aflatoxin B1 and 2.0 µg/kg for deoxynivalenol [8].

Material

Ninety cereal grain samples (30 each of wheat, rice, and maize) were collected from farm-level storage structures in Nakuru County during October-November 2023. Storage types included jute bags, metal bins, and bamboo granaries. For the mitigation trial, maize grain inoculated with A. flavus NRRL 3357 to a target aflatoxin B1 level of 80 µg/kg was treated with *T. afroharzianum* T-22 (spore powder, 1×10^8 CFU/g) or *L. buchneri* LB-01 (lyophilised cells, 1×10^9 CFU/g) at doses of 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 g/kg grain. An untreated inoculated control was maintained. All treatments were stored in 1 kg lots in perforated polypropylene bags at 28 ± 2 °C and 80 ± 5% RH for 60 days [9].

Methods

Residual aflatoxin B1 was quantified by HPLC-FLD at day 0, 30, and 60. Grain moisture was recorded with a portable grain moisture metre (Kett PM-450). Germination rate was tested on 100-grain subsamples per treatment at day 60. Data were analysed by two-way ANOVA (agent × dose) with Tukey's HSD ($p<0.05$) using R 4.3.1 [10].

Results

Table 1: Mycotoxin contamination profile in farm-stored cereals (n = 90 samples)

Mycotoxin	Wheat (%)	Rice (%)	Maize (%)	Overall (%)	Mean (µg/kg)
Aflatoxin B1	43.3	26.7	56.7	42.2	24.8 ± 18.3
Ochratoxin A	23.3	16.7	30.0	23.3	8.6 ± 6.1
Deoxynivalenol	36.7	10.0	13.3	20.0	142 ± 97
Zearalenone	10.0	6.7	23.3	13.3	34.2 ± 21.8

Table 2: Aflatoxin B1 reduction (%) by biocontrol agent and dose after 60 days of storage

Agent	0.5 g/kg	1.0 g/kg	1.5 g/kg	2.0 g/kg	2.5 g/kg	3.0 g/kg
T. afroharzianum	18.4	34.7	52.3	68.1	74.6	76.2
L. buchneri	12.1	26.8	41.5	55.3	62.8	64.1

Maize showed the highest aflatoxin prevalence (56.7%), consistent with its susceptibility to A. flavus under humid storage. In the mitigation trial, both agents reduced aflatoxin B1 in a dose-dependent manner, but *T. afroharzianum* consistently outperformed *L. buchneri* at every dose tested (Table 2). The response curve began to plateau above 2.0 g/kg for both agents.

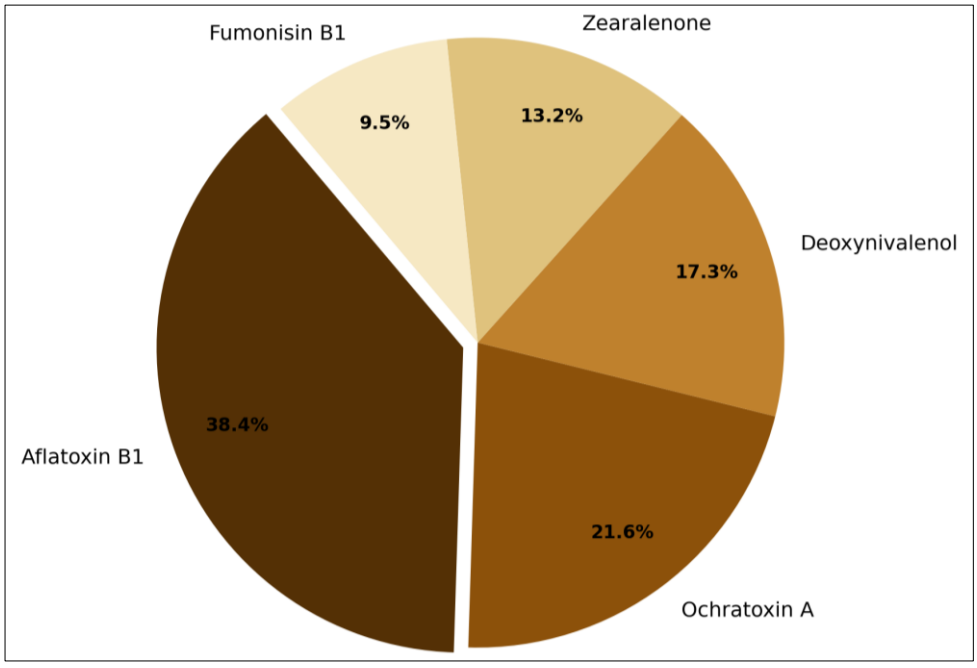

Fig 1: Distribution of mycotoxin types detected across 90 farm-stored cereal samples

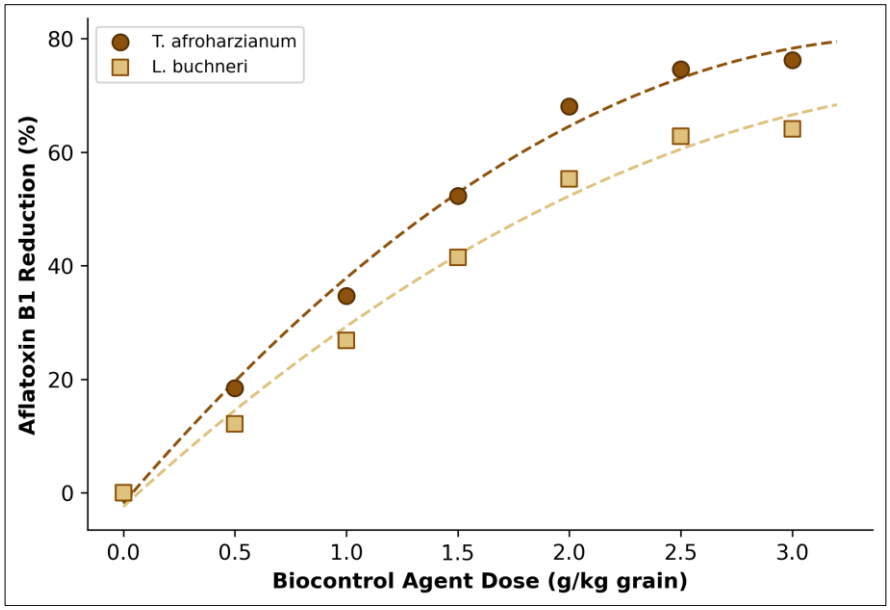

Fig 2: Dose-response curves for aflatoxin B1 reduction by *T. afroharzianum* and *L. buchneri* over 60 days

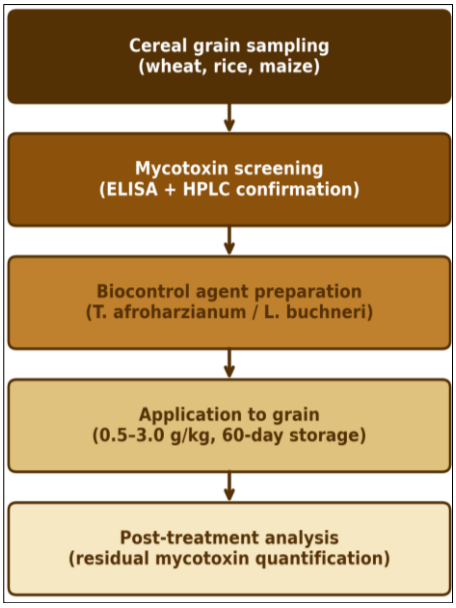

Fig 3: Biocontrol-based mycotoxin mitigation process used in this research

T. afroharzianum at 2.0 g/kg reduced aflatoxin B1 by more than two-thirds without affecting grain quality. The practical plateau beyond 2.5 g/kg suggests that 2.0 g/kg is the economically efficient application rate. *L. buchneri*, while less effective, still achieved over 55% reduction and may be preferred in settings where a bacterial agent is more readily available than a fungal one.

Discussion

The 67% overall mycotoxin exceedance rate in Nakuru farm stores is alarming but not unusual for humid sub-tropical storage. Reddy and colleagues [11] documented 58-72% exceedance in Tanzanian groundnut stores. That aflatoxin B1 dominated the contamination profile is expected given the favourable conditions for Aspergillus at 28-32 °C and above 80% relative humidity [12]. The higher prevalence in maize compared with rice may reflect maize's more porous endosperm, which permits deeper fungal penetration [13].

T. afroharzianum's superior performance aligns with its known dual mechanism: direct parasitism of Aspergillus hyphae and production of volatile organic compounds chiefly 6-pentyl-α-pyrone that inhibit spore germination [14]. *L. buchneri*, by contrast, acts mainly through mycotoxin adsorption to cell-wall peptidoglycans, a mechanism that is effective but saturable at lower toxin levels [15]. The absence of any effect on germination rate is reassuring for seed grain applications.

One limitation is that the trial used artificially inoculated grain at a single starting toxin level. Natural contamination involves mixed fungal communities and variable moisture pockets, which may modulate biocontrol efficacy [16]. On-farm validation with naturally contaminated grain lots is the logical next step.

Conclusion

This research set out to quantify mycotoxin contamination in farm-stored cereals in Kenya's Nakuru County and to evaluate two biocontrol agents for aflatoxin B1 reduction. The survey confirmed widespread contamination, with aflatoxin B1 as the dominant toxin in maize. In the mitigation trial, *T. afroharzianum* at 2.0 g/kg delivered 68% aflatoxin reduction over 60 days, outperforming *L. buchneri* at the same dose.

For smallholder cereal storage, a single application of *T. afroharzianum* powder at the time of binning offers a low-cost, residue-free defence against aflatoxin accumulation. Future work should extend the evaluation to mixed natural inocula, test shelf life of the biocontrol formulation under different humidity regimes, and assess consumer acceptability of treated grain.

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