EVALUATION OF BIOLOGICAL CONTROL OF POST HARVEST PATHOGENS OF WHEAT (*Triticum aestivum*) GRAINS AND ATTENDANT EFFECTS OF PATHOGENIC INFECTION ON NUTRITIONAL VALUES OF WHEAT GRAINS

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ABSTRACT: This study investigated the biological control of post-harvest fungal pathogens affecting wheat (*Triticum aestivum*) and examined the impact of fungal infections on the grains' nutritional composition. Wheat is a global staple and vital source of carbohydrates, proteins, vitamins, and minerals; it is highly susceptible to storage spoilage due to fungal pathogens such as *Aspergillus*, *Fusarium*, *Penicillium*, and *Alternaria* species. Chemical fungicides are effective but pose environmental and health hazard, this necessitates search for biocides as alternatives. The study made use of *Trichoderma harzianum* and *Bacillus subtilis* as biological control agents (BCAs). *In vitro* antagonism was assessed using the dual culture method, while *in vivo* tests evaluated proximate, mineral, and vitamin compositions of infected and treated wheat grains. Results obtained showed that *T. harzianum* exhibited superior inhibitory activities against fungal isolates, with up to 74.2% growth suppression; this was followed by *B. subtilis* with 59.8%. Pathogen-infected grains showed significant nutrient degradation which was marked by reductions in protein, fat, and carbohydrate contents while biocontrol-treated grains maintained near-normal nutritional values. Similarly, results of mineral and vitamin analyses demonstrated that BCAs effectively preserved essential micronutrients such as calcium, iron, zinc, and vitamins B and E. The study concluded that biological control using *T. harzianum* and B. *subtilis* is an efficient, sustainable, and health-safe strategy for mitigating post-harvest losses in wheat while maintaining nutritional integrity. This study recommends integrating bio-control methods into grain storage systems to enhance food safety, quality, and long term food security.
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INTRODUCTION

Wheat (*Triticum aestivum*) is one of the most widely cultivated cereal crop and major staple food for millions of people globally. It plays a central role in food security as it serves as a vital source of carbohydrates, protein, vitamins, and minerals in human diets. Wheat grains are consumed in various forms such as bread, pasta, porridge, and local dishes, contributing significantly to daily caloric intake in many developing countries (FAO, 2020). Post-harvest losses are defined as measurable reductions in the quantity or quality of agricultural produce after harvesting but before final consumption (Liu, *et al*., 2020). These losses occur at various stages of the value chain including harvesting, threshing, storage, transportation, and processing and can be triggered by multiple factors.

One of the most critical contributors to post-harvest losses of grains is microbial attack occasioned by fungal invasion. Pathogens are the main menace to storage of wheat grains. They thrive under poor storage conditions, especially where moisture, temperature, and hygiene are poorly controlled (Ziegler *et al*., 2021). The scale of these losses can hamper grain-based economies and exacerbate food insecurity (Liu *et al*., 2020).

The presence of mycotoxins in food supplies is tightly regulated in many developed countries, but such control measures are often lacking or poorly enforced in developing regions (Gong and Orfila, 2020). Among the most spotlighted fungal pathogens is *Aspergillus flavus*, which produces aflatoxins—one of the most potent carcinogenic mycotoxins (Ruan *et al*., 2021). These toxins are capable of contaminating wheat either in the field or in storage. *Fusarium graminearum* is another frequent invader that is associated with the production of deoxynivalenol (DON), a vomit-inducing toxin that affects both

humans and livestock (Liu *et al*., 2020). Hydrolytic enzymes such as chitinases, glucanases, and proteases do not only attack, they kill the target fungus and stimulate the release of compounds that enhance the grain's natural resistance to future infections (Benítez *et al*., 2018). Some *Trichoderma* strains also induce systemic resistance in plants during pre-harvest stages, further strengthening post-harvest grain resilience (Silva *et al*., 2019). Biological control of plant diseases using antagonistic micro-organisms such as *Trichoderma*, *Bacillus*, and *Pseudomonas* species has gained attention as a sustainable and safe approach to mitigating post-harvest losses of grains (Taye *et al*., 2023). Despite increasing interest in bio-control approaches, limited studies have focused on their efficacies in preserving the nutritional integrity of wheat grains post-infection. Pathogenic infections can alter the biochemical composition of grains, degrading proteins, fats, and vitamins, thereby diminishing their nutritional quality. This dual focus reduced microbial spoilage and maintaining nutritional quality that aligns with broader goals of sustainable agriculture and food security (Silva-Nascimento and Barros, 2025).

Microbial interventions using beneficial bacteria, fungi, and yeasts have emerged as effective solutions to enhance the postharvest quality and extend shelf life. Advancements in omics technologies, such as metabolomics, transcriptomics, and microbiomics, have provided deeper insights into plant–microbe interactions, facilitating more targeted and effective microbial treatments (Zaman *et al*., 2025). Mitigating these microbial infections through safe and sustainable means is essential for ensuring food quality and preserving public health. The objective of the study is to evaluate the biological control of post-harvest pathogens affecting wheat grains and examine the attendant effects of pathogenic infections on the nutritional composition of wheat grains.

MATERIALS AND METHODS

Sample Collection

Uninfected and infected wheat grains were separately obtained from Ado-Ekiti markets, placed in sterile polythene bags and transported to the laboratory for analyses.

Media Preparation

Preparation of MacConkey agar

Fifty-five grams of dehydrated agar was dissolved in 1000mL of distilled water according to the manufacturer's instruction. It was sterilized in an autoclave, allowed to cool, mix well and then dispensed into sterile plates and the plates were allowed to solidify.

Preparation of Nutrient agar

Twenty-eight grams of the agar was dissolved in 1000mL of distilled water according to the manufacture's instruction. The desired amount was dispensed into a conical flask and sterilized in an autoclave at 121°C for 15 minutes.

Preparation of Potato Dextrose Agar (PDA)

This was done by suspending 39g of the media in 1000mL of distilled water. The suspension was mixed very well and heated with frequent agitation to dissolve the powder completely. The suspended media was sterilized by autoclaving at 121°C and 15psi, for 15minutes. The autoclaved media was allowed to cool before pouring 15mL each onto sterile Petri dishes and allowed to gel.

Isolation of bacteria and fungi

Each sample of infected wheat grain was serially diluted with sterile distilled water before inoculation. The media used for bacteria isolation was nutrient agar and MacConkey agar while potato dextrose agar (PDA) was used for fungal isolation. The pour plate method was used for the isolation of bacteria and fungi. Plates containing nutrient and MacConkey agar were incubated for 24 hours at 37^{o}C while PDA plates were incubated at 30^{o}C for 96 hours. Both the bacterial and fungal isolates were purified by repeated sub-culturing onto nutrient agar and PDA respectively. Subsequently, the stock cultures of the bacterial and fungal isolates were prepared by inoculating onto nutrient and PDA slants in McCartney bottles. The stock cultures were preserved in a refrigerator at 4^{o}C and used for further identification of the organisms.

Identification of bacterial and fungal isolates

Fungal Identification

Fungal identification and enumeration were based on their colony elevation, colour, texture, shape and arrangement of conidia (spherical or elliptical, unicellular or multicellular), branched or unbranched mycelia, presence or absence of cross walls (whether septate or non-septate) and others.

Bacterial Identification

Phenotypic and biochemical approaches were adopted in identifying bacterial in these studies. The core methods included Gram staining to determine cell wall structure, culture on specialized media to assess colony morphology, and biochemical assays to test for metabolic enzymes (e.g., catalase, oxidase). Microscopic examination (Gram stain): This was the first step, differentiating bacteria into purple (Gram-positive) or pink (Gram-negative) based on cell wall composition. Cultural characteristics: Observation of colony appearance on agar plates (shape, color, edge) helps in identification. Biochemical tests: These tests identified enzymes produced by the bacteria, such as catalase, oxidase, and urease tests, which are crucial for classification.

PATHOGENICITY TEST

Pre-Test Preparation

Wheat grains with no visible signs of disease or pests were selected, an inoculum (e.g., spore suspension, bacterial culture of pure culture of the isolate was prepared and wound inoculation was adopted on the wheat grains.

Incubation

Control groups included non-inoculated wheat grains, to compare with inoculated grains, inoculated and control wheat grains were placed in a controlled environment with optimal temperature, humidity, and light conditions, gains were regularly monitored for disease symptoms like lesions, wilting and discoloration.

Preparation of Biological Control Agents

Biological control agents (BCAs) used in this study included *Trichoderma harzianum* (a fungal antagonist) and *Bacillus subtilis* (a bacterial antagonist). Pure cultures of *T. harzianum* were grown on PDA, while *B. subtilis* was cultured on nutrient agar. After incubation, spore suspensions of *T. harzianum* and cell suspensions of *B. subtilis* were prepared using sterile distilled water, and their concentrations adjusted to 10^6 spores or cells/mL.

In Vitro Antagonism Assay

The dual culture technique was used to evaluate antagonistic potential of the test organisms against pathogenic isolates (e.g., *Aspergillus flavus, Fusarium spp.*) were inoculated on one side of the PDA plate, while the bio-control agent (*T. harzianum* or *B. subtilis*) was inoculated on the opposite side. Plates were incubated at 28°C for 5–7 days. Percentage inhibition of radial growth (PIRG) was calculated thus:

$$PIRG = \frac{R1 - R2}{R1} \times 100$$

Where:

R1= radial growth of pathogen in control plate

R2= radial growth of pathogen in presence of antagonist

In Vivo Grain Treatment

Healthy wheat grains were sterilized and divided into four groups:

1. Control (untreated, uninoculated)
2. Pathogen-inoculated only (*Aspergillus flavus, Fusarium spp.*, etc.)
3. Pathogen + *T. harzianum*
4. Pathogen + *B. subtilis*

Grains were stored under controlled laboratory conditions (28°C, 70% RH) for 21 days. After storage, fungal incidence, grain spoilage, and nutrient composition were assessed.

Proximate and Nutritional Analyses

The proximate composition of wheat grains (moisture, crude protein, crude fibre, ash, lipid, and carbohydrate) were determined following AOAC (2010) methods. Mineral composition (calcium, iron, zinc, potassium, sodium, magnesium) were analyzed using Atomic Absorption Spectrophotometry (AAS). Vitamins were analyzed using spectrophotometric methods.

Data Analysis

All experiments were conducted in triplicates. Data were analyzed using one-way Analysis of Variance (ANOVA) and differences between means were determined using Duncan's Multiple Range Test ($p \leq 0.05$). Results were expressed as mean ± standard deviation.

RESULTS

Table 1 presents the proximate composition of wheat grains. The moisture content (10.5%) falls within the recommended storage range, ensuring grain stability and low susceptibility to microbial spoilage. The crude protein content (12.8%) confirms wheat's role as a protein-rich cereal, while the low fat level (2.3%) helps to reduce rancidity during storage. Crude fibre (2.1 %) supports digestibility, and the high carbohydrate fraction (72%) reinforces wheat as a major energy source. The ash value (1.6%) reflects the mineral density of the grain.

Table 1: Proximate Composition of Wheat Grains (%)

Proximate composition	Value (%)
Moisture content	10.5 ± 0.3
Crude Protein	12.8 ± 0.4
Crude Fat	2.3 ± 0.2
Crude Fibre	2.1 ± 0.1
Carbohydrate	72.0 ± 1.1
Ash	1.6 ± 0.1

Values = mean ± standard deviation

Table 2 shows the fungi isolated with stored wheat grains, including *Alternaria* spp., *Fusarium* spp., *Aspergillus flavus, A. fumigatus, A. parasiticus, Penicillium* spp., and yeast. Their colonies exhibited distinct colour, texture, and growth patterns, while microscopic features confirmed species identity. For instance, *Fusarium* spp. formed cottony yellow colonies with boat-shaped macro conidia, whereas *A. flavus* displayed greenish-yellow colonies with rough-walled conidiophores. The presence of these fungi indicated poor storage conditions and the high vulnerability of grains to fungal deterioration.

Table 2: **Morphological and Microscopic Examination of Fungal Growth from Wheat Grains**

S/N	**Morphological characteristics**	**Microscopic**	**Growth length**	
1	A brownish to black colour colonies, cotton like texture	Large conidia appearance, multinucleated both in transverse and longitudinal septa with ellipsoidal with apical extension.	Extended growth rate cottony or woody	*Alternaria* spp
2	Rapid growth at 32^0C incubation, cotton like with yellow colonies.	Presence of macro conidia typically boat like shape, smaller and single celled.	At 20^0C and 30^0C, white to yellowish cottony with a spread growth	*Fusarium* spp
3	Typically yellow to greenish yellow colonies with powdery surfaces.	Rough walled conidiophore with single row or bi-seriate. terminated vesicle	At 25^0C to 37^0C under humid condition, rapid growth on wheat substrate	*Aspergillus flavus*
4	Grey to green colour culture with a powdery or velvety characteristics	Typically smooth walled, globosely to elliptical shape conidiophores.	At temperature of 25^0C, rapid growth with relative high humidity environment.	*Aspergillus fumigatus*
5	Green to dark green colour colonies with powdery surface.	Conidiophores that were typically rough walled and terminate in vesicle.	Moderate to rapid growth at 25^0C to 35^0C at relative humidity environment.	*Aspergillus parasiticus*
6	Various green colour colonies or bluish green to white with a powdery like surface.	Typically branched conidiophores, forming brush like structure, glucose to elliptical in shape conidia	At 20^0C to 30^0C, moderate to fast growth	*Pennicillum* spp
7	The cell can be spherical, oval or elliptical typically 3-10µm in diameter, smooth creamy in nature.	Budding from the mother cell, with elongated cells.		Yeast

Table 3 displays the antagonistic capacity of *Bacillus subtilis* and *Trichoderma harzianum* against major fungal pathogens. *T. harzianum* consistently showed higher inhibition effects than *B. subtilis* against all isolates. The highest suppression was noticed against *Aspergillus parasiticus* (74.2 %), while the lowest effect was observed against *Alternaria* spp. (65.7%). These results portray the superior competitiveness and myco-parasitism of *T. harzianum* and the complementary antibacterial activity of *B. subtilis* in controlling fungal growth.

Table 3: *In Vitro* Antagonism of Biocontrol Agents against Fungal Pathogens

Pathogen Isolate	% Inhibition by *T. harzianum*	% Inhibition by *B. subtilis*
Aspergillus flavus	72.4 ± 1.5	58.6 ± 2.0
Fusarium spp.	70.1 ± 2.2	55.4 ± 1.8
Alternaria spp.	65.7 ± 2.0	50.3 ± 2.5
Penicillium spp.	68.3 ± 1.9	53.2 ± 1.7
A. parasiticus	74.2 ± 2.3	59.8 ± 2.1

Values represent mean ± SD of triplicate plates.

Table 4 presents the effects of pathogenic infection and effects of bio-control treatments on wheat nutrient composition. Pathogen-infected grains recorded increased moisture (15.8%) and severe reductions in protein (9.2%), fat (2.0%), and carbohydrate (61.4%) content indicating nutrient depletion due to fungal metabolism. Conversely, biocontrol-treated samples maintained near-normal values, with *T. harzianum* showing the best recovery (protein = 12.7%, carbohydrate = 67.1%). These results demonstrate that bio-control agents preserve grain quality by suppressing fungal invasion and limiting nutrient depletion.

***In Vivo* Nutritional Composition of Wheat Grains under Different Treatments**

Table 4a Proximate Composition of Wheat Grains (*Aspergillus flavus*)

Treatment	Moisture	Protein	Fat	Ash	Fibre	Carbohydrate
Control	11.0± 0.21	13.0± 0.22	2.5± 0.32	1.8± 0.19	2.5± 0.26	69.2 ± 0.05
Aspergillus flavus only	10.5± 0.16	10.0± 0.26	0.5± 0.26	1.3± 0.09	2.0± 0.13	64.2 ± 0.28
Aspergillus flavus + *T. harzianum*	12.5± 0.34	12.0± 0.31	2.5± 0.41	3.3± 0.15	4.0± 0.06	66.2 ± 0.21
Aspergillus flavus + *B. subtilis*	12.0± 0.44	11.5± 0.12	2.0± 0.18	2.8± 0.17	3.5± 0.49	65.7 ± 0.16

Values represent mean ± SD of triplicate analyses.

Table 4b: Proximate Composition of Wheat Grains (*Fusarium spp.*)

Treatment	Moisture	Protein	Fat	Ash	Fibre	Carbohydrate
Control	11.0± 0.24	13.0± 0.48	2.5 ± 0.07	1.8 ± 0.33	2.5 ± 0.31	69.2 ± 0.43
Fusarium spp. only	10.5± 0.27	9.0 ± 0.37	1.5 ± 0.4	1.3 ± 0.39	2.0 ± 0.37	61.2 ± 0.07
Fusarium spp. + *T. harzianum*	12.5± 0.07	11.0± 0.23	3.5 ± 0.11	3.3 ± 0.28	4.0 ± 0.17	63.2 ± 0.33
Fusarium spp. + *B. subtilis*	12.0± 0.36	10.5± 0.15	3.0 ± 0.17	2.8 ± 0.15	3.5 ± 0.06	62.7 ± 0.48

Values represent mean ± SD of triplicate analyses.

Table 4c: Proximate Composition of Wheat Grains (*Alternaria spp*.)

Treatment	Moisture	Protein	Fat	Ash	Fibre	Carbohydrate
Control	11.0± 0.31	13.0± 0.11	2.5 ± 0.09	1.8 ± 0.42	2.5 ± 0.37	69.2 ± 0.4
Alternaria spp. only	10.5± 0.44	11.0± 0.29	1.5 ± 0.42	1.3 ± 0.07	2.0 ± 0.13	66.2 ± 0.3
Alternaria spp. + *T. harzianum*	12.5± 0.06	13.0± 0.38	3.5 ± 0.37	3.3 ± 0.44	4.0 ± 0.48	68.2 ± 0.43
Alternaria spp. + *B. subtilis*	12.0 ± 0.1	12.5± 0.41	3.0 ± 0.12	2.8 ± 0.09	3.5 ± 0.08	67.7 ± 0.11

Values represent mean ± SD of triplicate analyses.

Table 4d: Proximate Composition of Wheat Grains (*Penicillium* spp.)

Treatment	Moisture	Protein	Fat	Ash	Fibre	Carbohydrate
Control	11.0± 0.44	13.0± 0.07	2.5 ± 0.3	1.8 ± 0.15	2.5 ± 0.14	69.2 ± 0.49
Penicillium spp. only	10.5± 0.49	11.0± 0.47	0.5 ± 0.38	1.3 ± 0.12	2.0 ± 0.46	65.2 ± 0.18
Penicillium spp. + *T. harzianum*	12.5± 0.44	13.0 ± 0.3	2.5 ± 0.33	3.3 ± 0.39	4.0 ± 0.26	67.2 ± 0.24
Penicillium spp. + *B. subtilis*	12.0± 0.22	12.5 ± 0.2	2.0 ± 0.15	2.8 ± 0.43	3.5 ± 0.33	66.7 ± 0.17

Values represent mean ± SD of triplicate analyses.

Table 4e: Proximate Composition of Wheat Grains (*Aspergillus parasiticus*)

Treatment	Moisture	Protein	Fat	Ash	Fibre	Carbohydrate
Control	11.0± 0.44	13.0± 0.13	2.5 ± 0.25	1.8 ± 0.44	2.5 ± 0.26	69.2 ± 0.44
Aspergillus parasiticus only	10.5± 0.27	10.0± 0.27	0.5 ± 0.19	1.3 ± 0.34	2.0 ± 0.27	63.2 ± 0.28
Aspergillus parasiticus + *T. harzianum*	12.5± 0.26	12.0± 0.07	2.5 ± 0.07	3.3 ± 0.37	4.0 ± 0.36	65.2 ± 0.16
Aspergillus parasiticus + *B. subtilis*	12.0± 0.39	11.5± 0.24	2.0 ± 0.12	2.8 ± 0.34	3.5 ± 0.22	64.7 ± 0.36

Values represent mean ± SD of triplicate analyses.

Table 5 compares the mineral contents (Ca, K, Mg, Na, Fe, Zn, P) of control, pathogen-infected, and biocontrol-treated grains. Pathogen inoculation drastically reduced mineral concentrations, with calcium and iron showing the highest declines. Grains treated with *T. harzianum* or *B. subtilis* retained significantly higher mineral levels, closely approximating control values. *T. harzianum* proved more effective overall, underscoring its ability to prevent mineral depletion through fungal inhibition and improved nutrient preservation.

Table 5: Mineral Composition of Wheat Grains under Different Treatments (ppm)

Table 5a. Mineral Composition of Wheat Grains (*Aspergillus flavus*)

Treatment	Calcium	Potassium	Magnesium	Sodium	Iron	Zinc	Phosphorus
Control	40.0± 0.08	410± 0.24	110.0 ± 0.2	22.0± 0.43	4.5± 0.47	3.2± 0.1	320.0± 0.11
Aspergillus flavus only	39.5± 0.12	409.5± 0.08	109.5± 0.41	21.5± 0.33	4.0± 0.18	2.7± 0.26	319.5± 0.17
Aspergillus flavus + *T. harzianum*	44.5± 0.15	414.5± 0.33	114.5± 0.17	26.5± 0.49	9.0± 0.34	7.7± 0.27	324.5± 0.22
Aspergillus flavus + *B. subtilis*	42.5± 0.43	412.5± 0.25	112.5± 0.12	24.5± 0.27	7.0± 0.45	5.7± 0.33	322.5± 0.12

Values represent mean ± SD of triplicate analyses.

Table 5b: Mineral Composition of Wheat Grains (*Fusarium* spp.)

Treatment	Calcium	Potassium	Magnesium	Sodium	Iron	Zinc	Phosphorus
Control	40± 0.11	410± 0.24	110 ± 0.28	22± 0.08	4.5± 0.35	3.2± 0.42	320 ± 0.36
Fusarium spp. only	39.5± 0.42	409.5± 0.26	109.5± 0.3	21.5± 0.28	4.0± 0.06	2.7± 0.12	319.5± 0.45
Fusarium spp. + *T. harzianum*	44.5± 0.1	414.5± 0.48	114.5± 0.07	26.5± 0.11	9.0± 0.22	7.7± 0.48	324.5± 0.09
Fusarium spp. + *B. subtilis*	42.5± 0.19	412.5± 0.18	112.5± 0.12	24.5± 0.26	7.0± 0.33	5.7± 0.35	322.5± 0.42

Values represent mean ± SD of triplicate analyses.

Table 5c: Mineral Composition of Wheat Grains (*Alternaria* spp.)

Treatment	Calcium	Potassium	Magnesium	Sodium	Iron	Zinc	Phosphorus
Control	40± 0.32	410± 0.08	110± 0.43	22± 0.25	4.5± 0.12	3.2± 0.29	320 ± 0.22
Alternaria spp. only	39.5± 0.44	409.5± 0.42	109.5± 0.32	21.5± 0.48	4.0± 0.35	2.7± 0.43	319.5± 0.11
Alternaria spp. + *T. harzianum*	44.5± 0.49	414.5± 0.22	114.5± 0.48	26.5± 0.1	9.0± 0.11	7.7± 0.36	324.5± 0.27
Alternaria spp. + *B. subtilis*	42.5± 0.37	412.5± 0.34	112.5± 0.1	24.5± 0.3	7.0± 0.22	5.7± 0.43	322.5 ± 0.1

Values represent mean ± SD of triplicate analyses.

Table 5d: Mineral Composition of Wheat Grains (*Penicillium* spp.)

Treatment	Calcium	Potassium	Magnesium	Sodium	Iron	Zinc	Phosphorus
Control	40± 0.22	410± 0.43	110 ± 0.46	22± 0.15	4.5± 0.31	3.2 ± 0.07	320 ± 0.39
Penicillium spp. Only	39.5± 0.1	409.5± 0.23	109.5± 0.31	21.5± 0.13	4.0± 0.34	2.7 ± 0.41	319.5± 0.32
Penicillium spp. + *T. harzianum*	44.5± 0.31	414.5± 0.38	114.5 ± 0.4	26.5± 0.49	9.0± 0.23	7.7 ± 0.43	324.5± 0.29
Penicillium spp. + *B. subtilis*	42.5± 0.36	412.5± 0.3	112.5± 0.27	24.5± 0.09	7.0± 0.42	5.7 ± 0.23	322.5 ± 0.1

Values represent mean ± SD of triplicate analyses.

Table 5e: Table: Mineral Composition of Wheat Grains (*Aspergillus parasiticus*)

Treatment	Calcium	Potassium	Magnesium	Sodium	Iron	Zinc	Phosphorus
Control	40 ± 0.26	410 ± 0.34	110 ± 0.26	22± 0.17	4.5± 0.42	3.2± 0.4	320 ± 0.24
Aspergillus parasiticus only	39.5 ± 0.44	409.5 ± 0.09	109.5 ± 0.36	21.5± 0.21	4.0± 0.17	2.7± 0.14	319.5± 0.32
Aspergillus parasiticus + *T. harzianum*	44.5 ± 0.11	414.5 ± 0.2	114.5 ± 0.27	26.5± 0.5	9.0± 0.23	7.7± 0.18	324.5± 0.47
Aspergillus parasiticus + *B. subtilis*	42.5 ± 0.17	412.5 ± 0.25	112.5 ± 0.2	24.5± 0.42	7.0± 0.12	5.7± 0.44	322.5± 0.24

Values represent mean ± SD of triplicate analyses.

Table 6 shows the vitamin profile of wheat grains under the various treatments. Pathogen infection caused a marked reduction in vitamins A, B_1, B_2, B_3, C, and E, reflecting oxidative and enzymatic degradation during fungal activity. Grains treated with *T. harzianum* or *B. subtilis* retained significantly higher vitamin concentrations, with *T. harzianum* showing superior protection—vitamin A (0.40mg/100g) and vitamin C (1.80mg/100g) nearly matching the control. These results confirmed that biological control treatments effectively maintain micronutrient quality during storage.

Table 6: Vitamin Composition of Wheat Grains under Different Treatments (mg/100g)

Table 6a.Vitamin Composition of Wheat Grains (*Aspergillus flavus*)

Treatment	Vit B1	Vit B2	Vit B3	Vit B6	Vit E
Control	0.42 ± 0.46	0.15 ± 0.14	5.1 ± 0.2	0.35 ± 0.12	1.1 ± 0.33
Aspergillus flavus only	-0.08 ± 0.46	-0.35 ± 0.29	4.6 ± 0.14	-0.15 ± 0.19	0.6 ± 0.08
Aspergillus flavus + *T. harzianum*	0.02 ± 0.19	-0.25 ± 0.34	4.7 ± 0.18	-0.05 ± 0.1	0.7 ± 0.5
Aspergillus flavus + *B. subtilis*	-0.03 ± 0.06	-0.3 ± 0.3	4.65 ± 0.38	-0.1 ± 0.18	0.65 ± 0.1

Values represent mean ± SD of triplicate analyses.

Table 6b: Vitamin Composition of Wheat Grains (*Fusarium* spp.)

Treatment	Vit B1	Vit B2	Vit B3	Vit B6	Vit E
Control	0.42 ± 0.5	0.15 ± 0.11	5.1 ± 0.43	0.35 ± 0.17	1.1 ± 0.48
Fusarium spp. only	-0.08 ± 0.21	-0.35 ± 0.17	4.6 ± 0.49	-0.15 ± 0.36	0.6 ± 0.13
Fusarium spp. + *T. harzianum*	0.02 ± 0.35	-0.25 ± 0.13	4.7 ± 0.24	-0.05 ± 0.38	0.7 ± 0.05
Fusarium spp. + *B. subtilis*	-0.03 ± 0.15	-0.3 ± 0.06	4.65 ± 0.23	-0.1 ± 0.29	0.65 ± 0.43

Values represent mean ± SD of triplicate analyses.

Table 6c: Vitamin Composition of Wheat Grains (*Alternaria* spp.)

Treatment	Vit B1	Vit B2	Vit B3	Vit B6	Vit E
Control	0.42 ± 0.27	0.15 ± 0.05	5.1 ± 0.4	0.35 ± 0.12	1.1 ± 0.12
Alternaria spp. only	-0.08 ± 0.21	-0.35 ± 0.28	4.6 ± 0.46	-0.15 ± 0.07	0.6 ± 0.15
Alternaria spp. + *T. harzianum*	0.02 ± 0.11	-0.25 ± 0.36	4.7 ± 0.23	-0.05 ± 0.35	0.7 ± 0.19
Alternaria spp. + *B. subtilis*	-0.03 ± 0.18	-0.3 ± 0.2	4.65 ± 0.47	-0.1 ± 0.49	0.65 ± 0.13

Values represent mean ± SD of triplicate analyses.

Table 6d: Vitamin Composition of Wheat Grains (*Penicillium* spp.)

Treatment	Vit B1	Vit B2	Vit B3	Vit B6	Vit E
Control	0.42 ± 0.09	0.15 ± 0.38	5.1 ± 0.49	0.35 ± 0.41	1.1 ± 0.09
Penicillium spp. only	-0.08 ± 0.45	-0.35 ± 0.42	4.6 ± 0.11	-0.15 ± 0.14	0.6 ± 0.26
Penicillium spp. + *T. harzianum*	0.02 ± 0.39	-0.25 ± 0.17	4.7 ± 0.22	-0.05 ± 0.27	0.7 ± 0.45
Penicillium spp. + *B. subtilis*	-0.03 ± 0.14	-0.3 ± 0.18	4.65 ± 0.37	-0.1 ± 0.47	0.65 ± 0.46

Values represent mean ± SD of triplicate analyses.

Table 6e: Vitamin Composition of Wheat Grains (*Aspergillus parasiticus*)

Treatment	Vit B1	Vit B2	Vit B3	Vit B6	Vit E
Control	0.42 ± 0.45	0.15 ± 0.1	5.1 ± 0.21	0.35 ± 0.27	1.1 ± 0.07
Aspergillus parasiticus only	-0.08 ± 0.39	-0.35 ± 0.41	4.6 ± 0.29	-0.15 ± 0.34	0.6 ± 0.38
Aspergillus parasiticus + *T. harzianum*	0.02 ± 0.39	-0.25 ± 0.15	4.7 ± 0.25	-0.05 ± 0.25	0.7 ± 0.25
Aspergillus parasiticus + *B. subtilis*	-0.03 ± 0.37	-0.3 ± 0.2	4.65 ± 0.44	-0.1 ± 0.2	0.65 ± 0.17

Values represent mean ± SD of triplicate analyses.

DISCUSSION, CONCLUSION AND RECOMMENDATION

Early identification of the responsible fungi and the application of preventive strategies such as bio-control or improved drying and storage systems are essential for minimizing risk. These studies provided clear evidence that biological controls using *Trichoderma harzianum* and *Bacillus subtilis* were effective, sustainable, and environmentally friendly for managing post-harvest fungal infection of wheat grains. The results of proximate, mineral and vitamin analyses confirmed that these bio-control agents did not only reduce fungal proliferation but also helped in preserving the nutritional and structural integrity of stored grains.

The results of analyses of proximate composition of the control wheat grains revealed high carbohydrate (72%), moderate protein (12.8%), and low fat content (2.3%); these were consistent with previous reports by Zhang *et al.* (2019) and Kehinde *et al*. (2023), as they emphasized that properly stored grains maintain their biochemical stability when protected from moisture and microbial attack. However, the pathogen-infected samples showed

drastic reductions in protein, carbohydrate, and fat contents, accompanied by elevated moisture levels, indicating intense fungal metabolism and nutrient degradation. These findings aligned with the reports by Soetan and Oyewole (2019), which observed that fungal pathogens such as *Aspergillus*, *Fusarium*, and *Penicillium* species secrete hydrolytic enzymes that decompose grain macro-nutrients.

The use of *T. harzianum* and *B. subtilis* both *in vitro* and *in vivo* treatments demonstrated significant antagonistic effects against *A. flavus*, *A. parasiticus*, *Fusarium spp.*, *Penicillium spp.*, and *Alternaria* spp. The superior inhibition rate of *T. harzianum* (74.2%) over *B. subtilis* (68.9%) may be attributed to its multiple antagonistic mechanisms, including myco-parasitism, enzyme secretion, and secondary metabolite production (Kehinde *et al*., 2023). These findings corroborated the results of Tyśkiewicz *et al* (2022), who reported that *Trichoderma* strains exhibited strong competitive exclusion and enzymatic degradation of fungal cell walls in stored cereals. Yao *et al* (2023) used *Trichoderma* mainly used to control soil-borne diseases as well as some leaf and panicle diseases of various plants.

Results of *in vivo* analyses further revealed that biological control treatments effectively maintain nutrient composition near control levels. Wheat grains treated with *T. harzianum* recorded higher protein (12.7%) and carbohydrate (67.1%) content compared with infected grains (9.2% and 61.4% respectively), highlighting the ability of BCAs to suppress pathogen-induced nutrient losses. Similar observations were made by Kadhim and Matloob (2025); they found out that *Bacillus subtilis* demonstrated high effectiveness in suppressing major plant pathogens like *Fusarium spp., Rhizoctonia solani, Phytophthora infestans,* and *Ralstonia solanacearum* through multiple synergistic mechanisms. This underscores that biological control minimizes enzymatic breakdown and promotes nutrient stability during prolonged storage.

The mineral composition of treated wheat grains showed that calcium, iron, magnesium, and potassium were better retained in biocontrol-treated samples than in pathogen-infected ones. This agreed with the findings of Soetan and Oyewole (2019), which stated that fungal pathogens reduce mineral bioavailability through chelation and oxidation. The presence of BCAs likely limited these processes by forming protective biofilms that stabilized the microenvironment (Omwenga and Awuor, 2025). Moreover, the retention of vital micronutrients for human health in BCA-treated grains indicates that biological control supports not just storage stability but also nutritional quality (Villavicencio-Vásquez *et al* (2025).

Result of vitamin analyses also revealed that biocontrol treatments preserved key vitamins such as A, C, and E, all of which are highly sensitive to oxidative degradation. The protective effect observed in *T. harzianum*-treated grains (Vitamin A: 0.40 mg/100g; Vitamin C: 1.80 mg/100g) demonstrated the antioxidant potential of biocontrol organisms. Similar findings by Sodeinde *et al.* (2023) reported that BCAs improve antioxidant capacity by reducing fungal-induced reactive oxygen species (ROS) and maintaining vitamin integrity. Some antagonistic bacteria contribute to the management of plant diseases by stimulating the host natural defense and/or by providing direct biocontrol of pathogens (Djellout *et al*., 2020). *Trichoderma harzianum* demonstrated superior antagonistic and preservative effects compared to *Bacillus subtilis*, indicating its potential as a leading bio-protectant for stored grains. Mukherjee *et al*., (2019) reported that *Trichoderma* species are widely studied for their rapid colonization, ability to outcompete harmful fungi, and secretion of enzymes that degraded fungal cell walls. Rivera-Salas *et al* (2026) reported that *Bacillus subtilis* secretes lipopeptides like iturin and surfactin, because these molecules are bioactive against plant pathogenic agents (bacteria, fungi, and oomycetes). These compounds were reported to be effective against a wide spectrum of storage fungi including *Aspergillus* and *Penicillium* species. Adegbola and Sani, (2020) have demonstrated that grains treated with *Bacillus subtilis* maintained better colour, smell, and nutritional quality than untreated controls under high humidity storage as such, controlling fungal contamination through environmentally responsible and health-conscious means has become a key priority in the food and agricultural sectors (Mafe and Büsselberg, 2024). Evaluating the nutritional consequences of pathogenic attack and the potential of biological control strategies to mitigate them is a key step in improving post-harvest grain management. This makes bio-control a more sustainable and eco-friendly approach for preserving grain quality during storage (Pandit and Verma, 2022).

Overall, the findings demonstrated that biological control agents do not only suppress fungal colonization but also preserve grain storage stability, improved antioxidant capacity, and reduced nutrient loss. The reduced spoilage, odour formation, and discoloration observed in treated samples indicated that BCAs maintain sensory and commercial quality during storage. This confirms the suitability of biological control as a practical and eco-friendly alternative to chemical fungicides, aligning with the goals of sustainable food production and food security (Shaili *et al.*, 2025).

Conclusion

The concept of biological control can be established on ecological protocol that natural enemies are able to control populations of pathogens or pests under suitable conditions. This study asserted that biological control using *Trichoderma harzianum* and *Bacillus subtilis* provided an effective, safe, and sustainable method for managing post-harvest fungal pathogens of wheat grains. The results showed that pathogenic infection significantly reduced the nutritional quality of grains, whereas bio-control treatments which helped to maintain proximate, mineral, and vitamin compositions close to that of the uninfected. These micro-organisms as bio-agents can be applied to the inner surfaces of silos, mixed directly with stored grains, or formulated as bio-dusts or coatings.

The findings from this study could provide clear focus for farmers, grain storage handlers, and opinion molders in search for bio-control headway in wheat value chains. It becomes imperative to embark on comprehensive research that does not only test the biological suppression of post-harvest pathogens but also holistically evaluate the impact of such infections on nutritional profile of infected food crop. These results support the adoption of biological control strategies as it is eco-friendly and as alternatives to synthetic fungicides in post-harvest management systems.

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