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DEVELOPMENT AND PRELIMINARY CHARACTERIZATION OF A CARRIER-BASED *TRICHODERMA HARZIANUM* BIOFERTILIZER FORMULATION USING AGRO-INDUSTRIAL RESIDUES THROUGH SOLID-STATE FERMENTATION

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Abstract

Biofertilizers based on beneficial fungi offer a sustainable complement to synthetic agrochemicals, yet their use in Pakistan remains limited, partly because locally produced formulations are scarce. This technical note describes the development and preliminary characterization of a carrier-based Trichoderma harzianum formulation produced by tray solid-state fermentation (SSF) on a three-component agro-industrial residue substrate at the PCSIR Laboratories Complex, Lahore. A substrate of sawdust, wheat bran and rice husk (500:250:250 g; 2:1:1, w/w) was moistened with distilled water to the hand-squeeze end-point (estimated 55–65% moisture), maintained at pH 5.0–6.0 as measured with a digital pH meter, autoclaved at 121 °C (≈15 psi) for 30 min and inoculated aseptically with a T. harzianum culture maintained at PCSIR. Fermentation was carried out in foil-covered trays in

a growth chamber at 25–28 °C until dense green sporulation was observed. The fungus colonised the substrate with white mycelium that progressed to yellow-green and green conidiation, giving a granular, olive-brown product with an earthy odour. The final formulation had a measured viable count of 1×10^8 CFU g^{-1}, determined by serial-dilution plate counting. As a qualitative observation, the formulation retained viability for approximately three months when stored in sealed containers at room temperature; viable counts at monthly intervals were not determined. Process parameters that were not recorded during the experiment are reported as clearly identified estimates. The results demonstrate the feasibility of producing a viable T. harzianum formulation from locally available residues, while microbial purity, extended shelf life under improved storage conditions and agronomic efficacy remain to be evaluated.

1. Introduction

1.1. Need for sustainable fertility inputs in Pakistan

Agriculture in Pakistan depends heavily on synthetic fertilizers and pesticides, whose excessive and imbalanced use contributes to declining soil health, environmental pollution and rising production costs (Naveed et al., 2015; Yao et al., 2023). Biofertilizers, which are products containing living microorganisms that improve nutrient availability and plant growth, offer a sustainable complement to these inputs. However, biofertilizers account for less than 2% of the fertilizers used in Pakistan, and their wider adoption is limited by inconsistent product quality, weak regulation and the scarcity of locally produced formulations (Naveed et al., 2015).

At the same time, Pakistan's cereal-milling and timber industries generate residues such as wheat bran, rice husk and sawdust, much of which is under-used. Converting these residues into microbial inputs offers a practical way to add value to agricultural waste while providing farmers with a locally produced biological input.

1.2. *Trichoderma harzianum* as a biofertilizer and biocontrol agent

Species of *Trichoderma* are free-living filamentous fungi that are common in soil and the rhizosphere, and more than 370 species have been described (Yao et al., 2023). Selected strains can improve crop performance through several complementary mechanisms. They solubilise phosphate and micronutrients, produce phytohormones such as indole-3-acetic acid as well as the enzyme ACC deaminase, and stimulate root development (Li et al., 2015; Tyśkiewicz et al., 2022). They also protect plants by competing with pathogens for space and nutrients, producing antimicrobial compounds, parasitising pathogenic fungi and priming the plant's own defence responses (Guzmán-Guzmán et al., 2023; Woo et al., 2023).

T. harzianum is one of the most widely studied species. For example, a strain that produced indole-3-acetic acid and siderophores and solubilised phosphate reduced *Fusarium* wilt of pepper by 50% when applied alone (Abdelaziz et al., 2023). *Trichoderma*-based products account for a large share of the biological fungicides registered worldwide (Mulatu et al., 2021). Their effects are, however, strain-dependent and strongly influenced by formulation quality, which makes reliable production, viable-count determination and storage-stability testing essential (Sood et al., 2020; Martínez et al., 2023).

1.3. Solid-state fermentation on agricultural residues

Mass production of *Trichoderma* is commonly achieved by solid-state fermentation (SSF), in which the fungus grows on moist solid particles in the near absence of free water. SSF yields aerial conidia, which survive formulation and storage better than mycelium, and allows agricultural residues to act simultaneously as physical support and nutrient source (Sala et al., 2019; Kumar et al., 2023; Martínez et al., 2023). Substrate choice matters economically, because organic substrates can account for 35–40% of total production costs (Mulatu et al., 2021). Tray fermentation is the simplest SSF system

and requires only basic equipment, although oxygen and heat transfer limit the depth of the substrate bed.

The choice of substrate strongly influences yield. Wheat bran is among the most productive substrates: Sargin et al. (2013) obtained $(1.30 \pm 0.68) \times 10^{10}$ CFU per gram of dry substrate from *T. harzianum* EGE-K38 on a wheat bran–malt sprout mixture (3:2), and Mulatu et al. (2021) selected a wheat bran–white rice mixture (2:1) as the best of 14 substrates. Rice husk also supports good growth; Naeimi et al. (2020) recorded 3.1×10^9 CFU g^{-1} on rice husk and recommended it for industrial-scale production, while Sala et al. (2020) optimised *T. harzianum* spore production on rice husk at 55–60% moisture and 25 °C. In contrast, sawdust alone is a poor growth substrate (Singh et al., 2007; Naeimi et al., 2020), although a wheat bran–sawdust mixture gave the longest shelf life of all the substrates tested by Singh et al. (2007). In Pakistan, Rajput et al. (2014) found that cereal grains supported the highest *T. harzianum* populations among nine substrates, with rice husk performing moderately well.

1.4. Research gap and objectives

Most previous studies have used single substrates or two-component mixtures, and the three-component combination of sawdust, wheat bran and rice husk has received little attention in the literature reviewed for this study. Few reports describe kilogram-scale tray SSF of *Trichoderma* in Pakistan, where earlier work has focused mainly on small-scale substrate screening (Rajput et al., 2014). In addition, Pakistan's national biofertilizer standard, PS 5330:2014, specifies requirements for bacterial products such as plant-growth-promoting rhizobacteria and *Rhizobium* and contains no category for fungal formulations (PSQCA, 2014).

This study therefore investigated the production of a carrier-based *T. harzianum* formulation on locally available agro-industrial residues using tray SSF. The specific objectives were to (i) formulate a sawdust–wheat bran–rice husk substrate in a 2:1:1 ratio by mass; (ii) produce *T. harzianum* on this substrate by tray SSF; (iii) document fungal colonisation, sporulation and the physical characteristics of the final product; (iv) determine the viable count of the final formulation and its storage stability at room temperature; and (v) place the results in the context of published yields and existing quality standards.

2. Materials and Methods

2.1. Study site and period

The study was carried out at the PCSIR Laboratories Complex, Lahore, Pakistan, from 21 April to 13 June 2026. The production process is summarised in Figure 1. Process parameters that were recorded or measured during the experiment are reported as such; parameters for which no record was kept are given as estimates based on standard practice and the literature, and are identified as estimates in the text and in Table 2.

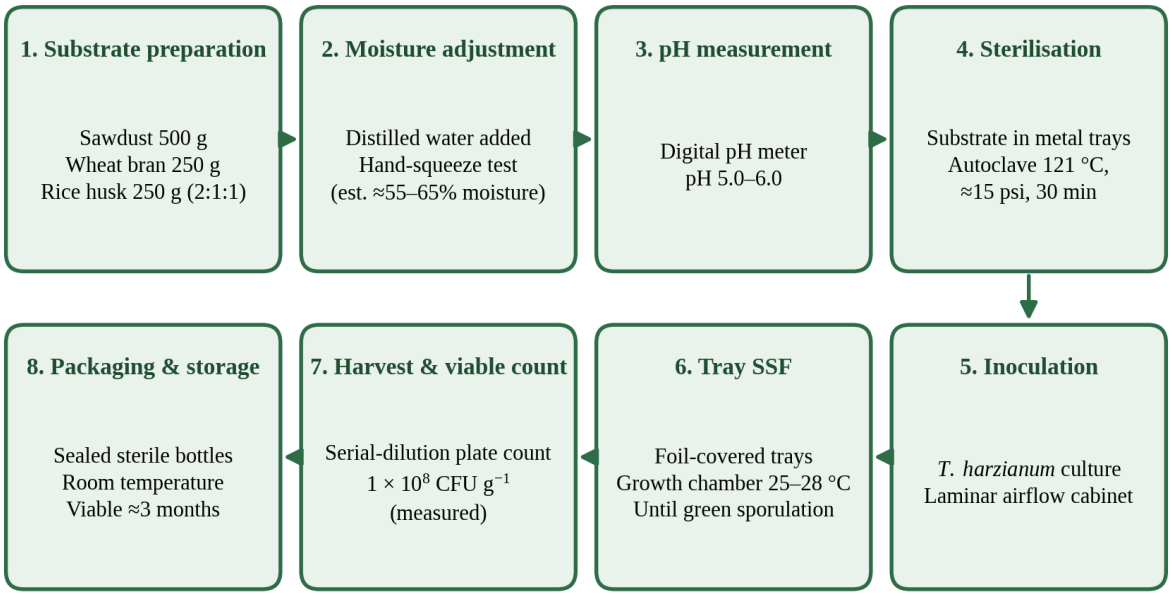


Figure 1. Flowchart of the production process for the carrier-based *Trichoderma harzianum* formulation by tray solid-state fermentation (SSF).

2.2. Fungal culture: identity and maintenance

The fungus used was a pure culture of *Trichoderma harzianum* maintained in the culture collection of the PCSIR Laboratories Complex, Lahore. Its identity was based on the laboratory culture record and on colony morphology, namely rapid growth with dense green conidiation on agar (Figure 2C). No strain code or culture-collection accession number was available, and molecular identification was not performed in this study. Potato dextrose broth (PDB) and potato dextrose agar (PDA) plates were prepared and sterilised by autoclaving, and all culture work was carried out aseptically in a laminar airflow cabinet (Figure 2A, B).

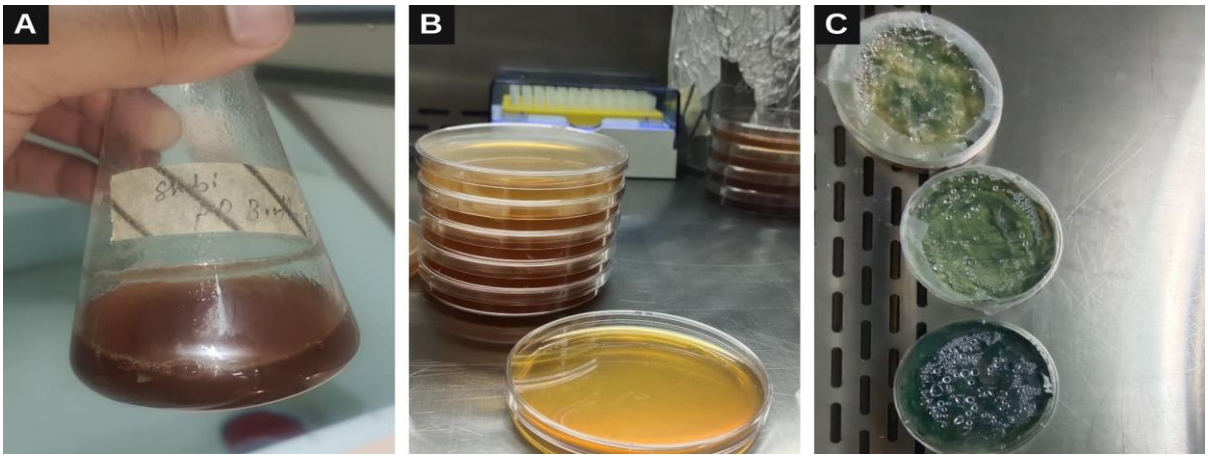


Figure 2. Culture media and fungal culture. (A) Potato dextrose broth (PDB) after autoclaving; the darkened stripes on the autoclave indicator tape show that the flask was exposed to the sterilisation cycle. (B) Prepared agar plates in the laminar airflow cabinet. (C) *Trichoderma harzianum* cultures showing dense green conidiation.

2.3. Substrate materials and composition

Three agro-industrial residues were used: sawdust, wheat bran and rice husk. Each batch consisted of 500 g sawdust, 250 g wheat bran and 250 g rice husk, giving a 2:1:1 ratio by mass (Table 1). Based on the literature, wheat bran was included as the main nutrient source, rice husk to improve porosity, aeration and structure, and sawdust as a lignocellulosic bulking agent and carrier. The components were weighed separately and mixed thoroughly.

Table 1. Composition of the solid substrate (per batch, before addition of water and inoculum).

Component	Mass (g)	Proportion (%)	Intended function (literature-based)
Sawdust	500	50	Lignocellulosic bulking agent, carrier and water retention
Wheat bran	250	25	Main nutrient source (carbohydrates, protein, minerals)
Rice husk	250	25	Porosity, aeration and substrate structure
Total	**1,000**	**100**	

2.4. Moisture adjustment

Distilled water was added gradually to the mixed substrate with continuous mixing, and moisture was assessed qualitatively using the hand-squeeze test. A handful of substrate was squeezed firmly, and water was added until a few drops were released between the fingers without a continuous trickle. Moisture content was not determined gravimetrically. In extension guidance, this end-point corresponds to approximately 55–65% moisture on a wet-weight basis (University of Missouri Extension, n.d.; USDA AMS, n.d.), and this range is therefore reported as an estimate. For reference, the water required to bring 1 kg of dry substrate to a target moisture fraction M (wet basis) can be estimated as $W = D \times M / (1 - M)$, where D is the dry substrate mass; for 60% moisture this gives approximately 1.5 L of water per kilogram, less the moisture already present in the residues. The actual volume of water added was not recorded.

2.5. pH measurement

The pH of the moistened substrate was measured with a digital pH meter before inoculation and was within the range of 5.0–6.0. Individual readings, the sample preparation used for measurement and calibration records were not logged, and no pH-adjusting reagent was added. A mildly acidic initial pH is favourable for *T. harzianum* in SSF; Sargin et al. (2013) used an initial pH of 5.8 ± 0.3, and Serna-Díaz et al. (2020) reported that pH had little influence on sporulation within the mildly acidic range.

2.6. Sterilisation

The moistened substrate was spread in metal trays covered with aluminium foil, and the trays and substrate were sterilised in a large autoclave at 121 °C (approximately 15 psi) for 30 min. Bulky solid substrates heat slowly at the core, so tray loads generally require longer holding times than the small volumes used in laboratory flasks.

2.7. Inoculum preparation and inoculation

The inoculum was prepared from actively sporulating *T. harzianum* cultures grown on potato dextrose medium (Figure 2). After the sterilised substrate had cooled to room temperature, it was inoculated aseptically inside a laminar airflow cabinet and mixed to distribute the inoculum. The inoculum type, volume and concentration were not recorded. Based on standard practice for tray SSF, the inoculum is estimated to have been a conidial suspension of approximately 10^7 conidia mL^{-1} applied at about 100 mL per kilogram of substrate, corresponding to roughly 10^6 conidia per gram of substrate; these values are estimates and not recorded measurements.

2.8. Tray solid-state fermentation

The inoculated substrate was spread in a shallow, even layer in metal trays, with an estimated bed depth of 2–3 cm, and the trays were covered with aluminium foil. The trays were incubated in a growth chamber set at 25–28 °C, the range reported as favourable for *T. harzianum* growth and sporulation (Sargin et al., 2013; Sala et al., 2020; Hamrouni et al., 2026). Relative humidity was kept high to prevent the substrate from drying; it was not measured and is estimated at approximately 85–90%. The tray contents were not mixed during incubation. Fungal growth was monitored visually and photographed, and incubation was ended when the substrate was densely colonised and covered with green conidia. The exact start and end times were not recorded; the fermentation period is estimated at approximately 7 days.

2.9. Harvesting, packaging and storage

After fermentation, the colonised substrate was harvested, packed into sealed, sterile screw-capped bottles and labelled (Figure 4). The product was stored at room temperature. No separate drying step was recorded, and the final moisture content of the product was not determined.

2.10. Determination of viable fungal count

The viable count of the final formulation was determined by the serial-dilution plate method. A weighed sample of the formulation was suspended in sterile distilled water at a ratio of 1:10 (w/v) and mixed thoroughly to release the conidia. Tenfold serial dilutions were prepared in sterile distilled water, and a measured volume of suitable dilutions was spread on PDA plates. The plates were incubated at 25–28 °C until colonies could be counted, and plates bearing a countable number of colonies were used for the calculation. The viable count was calculated as CFU g^{-1} = (number of colonies × dilution factor) / volume plated (mL), and is expressed per gram of formulation on an as-is (wet-weight) basis. CFU g^{-1} represents viable colony-forming units and is distinct from a direct microscopic count of conidia.

2.11. Storage stability

Storage stability was assessed qualitatively. The packaged formulation was kept in sealed containers at room temperature and checked periodically for viable *T. harzianum* growth, and the storage period over which viable growth was obtained was recorded. Viable counts at defined time points (for example, 0, 1, 2 and 3 months) were not determined.

2.12. Data recording and analysis

Fungal colonisation, sporulation and the colour, texture and odour of the final product were recorded by visual and sensory assessment. Component proportions were calculated from the recorded masses. Results are reported as single determinations from the production run described; replicate statistics were not available, and no statistical tests were applied. The status of each process parameter (recorded, measured, observed or estimated) is given in Table 2.

3. Results

3.1. Substrate preparation

Each batch produced 1 kg of mixed substrate consisting of 50% sawdust, 25% wheat bran and 25% rice husk by mass (Table 1). After moistening, the substrate reached the hand-squeeze end-point, releasing a few drops of water when squeezed (estimated 55–65% moisture), and its pH before inoculation was within the range of 5.0–6.0.

3.2. Fungal colonisation and sporulation

Fungal growth appeared on the substrate surface during incubation. White, cottony mycelium first spread across the substrate in patches and streaks, followed by yellow-green and then green conidial zones where growth was most advanced (Figure 3A, B). This sequence is typical of *Trichoderma* development on solid substrates. As incubation continued, colonisation became dense and green sporulation covered the substrate surface, at which point fermentation was ended. Visual appearance supports the description of fungal growth but does not by itself confirm species identity or freedom from contamination.

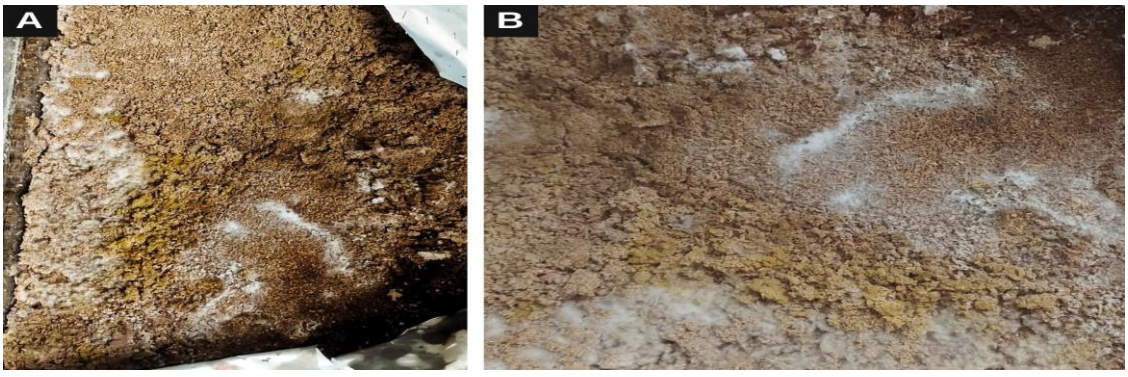


Figure 3. Tray solid-state fermentation of *Trichoderma harzianum* on the sawdust–wheat bran–rice husk (2:1:1) substrate during incubation. (A) Colonised substrate in a foil-covered metal tray, showing white mycelium and yellow-green conidiating zones. (B) Close-up of the advancing white mycelium and a yellow-green conidiating zone.

3.3. Viable count of the final formulation

The final formulation had a measured viable count of 1×10^8 CFU g^{-1}, determined by the serial-dilution plate method on PDA (Section 2.10) and expressed on an as-is basis. This value confirms that the substrate supported the production of a high number of viable propagules, but it does not by itself establish microbial purity or efficacy.

3.4. Characteristics of the final product

The harvested formulation was a granular, olive-brown material with a characteristic earthy odour. It was packed in sealed bottles bearing the PCSIR biofertilizer label (Figure 4).



Figure 4. Final carrier-based *Trichoderma harzianum* formulation packed in a sealed bottle bearing the PCSIR Laboratories Complex, Lahore, product label.

3.5. Storage stability

As a qualitative observation, the formulation remained viable for approximately three months when stored in sealed containers at room temperature. Because viable counts were not determined at monthly intervals, the extent of the decline in CFU g^{-1} over this period is not known.

3.6. Summary of process conditions and results

The process conditions and results of this study, together with the method used and the status of each value, are summarised in Table 2.

Table 2. Process conditions and results for the production of the *T. harzianum* formulation.

Parameter	Value	Method	Status
Substrate composition	Sawdust 500 g, wheat bran 250 g, rice husk 250 g (2:1:1)	Weighing	Recorded
Substrate moisture	≈ 55–65% (a few drops released on squeezing)	Hand-squeeze test	Estimated (qualitative test)
Water requirement	≈ 1.5 L per kg dry substrate for 60% moisture	$W = D \times M / (1 - M)$	Estimated (calculation)

Parameter	Value	Method	Status
Initial pH	5.0–6.0	Digital pH meter, before inoculation	Recorded range
Sterilisation	121 °C, ≈ 15 psi, 30 min	Autoclave	Recorded
Inoculum	Conidial suspension ≈ 10^7 conidia mL^{-1}, ≈ 100 mL kg^{-1} (≈ 10^6 conidia g^{-1})	Actively sporulating culture; aseptic	Estimated
Fermentation system	Foil-covered metal trays; bed depth ≈ 2–3 cm	Tray SSF	System recorded; depth estimated
Incubation temperature	25–28 °C	Growth chamber setting	Recorded range
Relative humidity	≈ 85–90% (high)	Growth chamber	Estimated
Fermentation duration	≈ 7 days	Ended at dense green sporulation	Estimated duration; end-point observed
Final product	Granular, olive-brown, earthy odour	Visual and sensory assessment	Observed
Viable count	1×10^8 CFU g^{-1} (as-is basis)	Serial-dilution plate count on PDA	Measured
Storage stability	Viable for ≈ 3 months	Sealed containers, room temperature; periodic checks	Observed (qualitative)
Final moisture; microbial purity	—	—	Not determined

4. Discussion

4.1. Roles of the substrate components

The roles of the three residues described below are based on the literature; their individual contributions were not tested separately in this study, and alternative ratios were not compared. Wheat bran supplies nitrogen and readily fermentable carbohydrates and is consistently among the most productive *Trichoderma* substrates (Sargin et al., 2013; Mulatu et al., 2021). Combining wheat bran with lignocellulosic residues is an established approach; Zhang and Yang (2015), for example, used corn stover with wheat bran and increased *T. harzianum* conidia yield 1.46-fold through optimisation.

Rice husk contributes porosity and structure, which favour oxygen transfer in static trays, and is itself a good *T. harzianum* substrate (Naeimi et al., 2020; Sala et al., 2020). Oxygen consumption, initial moisture and available sugars were the parameters most closely associated with *T. harzianum* conidia production across a range of wastes (Sala et al., 2021), which supports the use of a sugar-rich bran together with a porous husk in tray SSF.

Sawdust is a poor growth substrate on its own, yielding only 3.2×10^6 CFU g^{-1} in the study of Naeimi et al. (2020). However, it is widely available and improves water retention, and when combined with wheat bran it produced the longest shelf life among the substrates tested by Singh et al. (2007). The 2:1:1 mixture was the formulation investigated in this study, and the measured viable count of 1×10^8 CFU g^{-1} shows that it supported good fungal growth. Whether this ratio is optimal, and whether it offers a cost advantage, would require comparison with alternative ratios and a cost analysis.

4.2. Process conditions in relation to published studies

The process conditions used in this study are broadly consistent with those reported for *T. harzianum* SSF (Table 3). Moisture is a critical factor. Sala et al. (2020) identified moisture and temperature as the main determinants of spore yield on rice husk, with optima of 55–60% moisture and 25 °C, whereas wheat bran-based media performed best at about 70% moisture (Sargin et al., 2013). The hand-squeeze end-point used here, estimated at 55–65%, lies within this range, although it was not confirmed gravimetrically. The pH of 5.0–6.0 recorded before inoculation is close to the initial pH of 5.8 used by Sargin et al. (2013) and the pH of 6 found optimal for enzyme production by *T. harzianum* (Teo et al., 2025), and Serna-Díaz et al. (2020) showed that conidiation is not highly sensitive to pH within this range. The growth-chamber setting of 25–28 °C corresponds to the optima reported by Sala et al. (2020) and Hamrouni et al. (2026).

Published fermentation times vary widely with the production system. In controlled reactors with forced aeration, maximum spore production can be reached within 52–72 h (Hamrouni et al., 2026), whereas static, low-technology systems require longer incubation (Naeimi et al., 2020; Mulatu et al., 2021). The estimated fermentation period of about 7 days in this study is consistent with static tray SSF without forced aeration. Yields in Table 3 are reported on different bases (CFU, conidia or spores per gram of dry substrate, dry matter or product), so they are not directly equivalent and are presented for context only.

Table 3. Solid-state fermentation of *Trichoderma* on agricultural residues: published studies compared with the present work.

Study	Organism	Substrate	Conditions	Reported yield and basis
Sargin et al. (2013)	*T. harzianum* EGE-K38	Wheat bran–malt sprout (3:2)	28 °C; 70% moisture; pH 5.8; 4 days	1.30×10^{10} CFU g^{-1} dry substrate

Study	Organism	Substrate	Conditions	Reported yield and basis
Sala et al. (2020)	*T. harzianum*	Rice husk	25 °C; 55–60% moisture; mixing beneficial	Up to 2.0 × 10^9 conidia g^{-1} dry matter (as reported by Naeimi et al., 2020)
Naeimi et al. (2020)	*T. harzianum* AS12-2	13 substrates incl. rice husk, wheat bran, sawdust	30 days at room temperature; air-dried	Rice husk 3.1 × 10^9; wheat bran 7.0 × 10^8; sawdust 3.2 × 10^6 CFU g^{-1} substrate
Serna-Díaz et al. (2020)	*T. harzianum*	Barley straw	80% moisture (best of three levels); pH little effect	2.35 × 10^{10} conidia g^{-1} dry matter at 136 h
Mulatu et al. (2021)	*T. asperellum*; *T. longibrachiatum*	Wheat bran–white rice (2:1)	Temperature, moisture, inoculum and time significant	Up to ≈ 1.5 × 10^9 conidia g^{-1} substrate (optimised)
Hamrouni et al. (2026)	*T. harzianum* Rey 3; TF2	Solid substrate in controlled reactor	28 °C; forced aeration	8.9–10.1 × 10^9 spores g^{-1} dry matter (52–72 h)
Present study	*T. harzianum* (PCSIR culture)	Sawdust–wheat bran–rice husk (2:1:1)	25–28 °C; pH 5.0–6.0; est. 55–65% moisture; tray SSF	1 × 10^8 CFU g^{-1} formulation, as-is basis (measured)

4.3. Viable count in relation to quality standards

The measured viable count can be placed in the context of existing standards (Table 4), although these comparisons are numerical only and do not by themselves establish regulatory compliance. In India, *Trichoderma* formulations are required to contain at least 2 × 10^6 CFU g^{-1}, with a water content of no more than 8% and non-pathogenic contaminants not exceeding 1 × 10^4 g^{-1} (CIBRC, 2022); the measured count of 1 × 10^8 CFU g^{-1} is numerically 50 times this minimum. Pakistan's standard PS 5330:2014 specifies at least 1 × 10^7 CFU g^{-1}, a carrier moisture of no more than 40% and a shelf life of 12 months, but its categories cover bacterial biofertilizers such as plant-growth-promoting rhizobacteria

and *Rhizobium* rather than fungal formulations (PSQCA, 2014); the measured count is numerically ten times this value. The count also lies within the range of 10^5–10^9 CFU g^{-1} reported for commercial *Trichoderma* products (Martínez et al., 2023). Because final moisture and microbial purity were not determined, and the shelf life observed was shorter than the 12 months specified in both standards, the formulation cannot be described as meeting these standards. The absence of a Pakistani standard for fungal biofertilizers is a regulatory gap that production data such as these may help to address.

Table 4. Numerical comparison of the present formulation with Indian and Pakistani quality standards.

Parameter	CIBRC, India (2022)	PSQCA PS 5330:2014, Pakistan	Present study
Products covered	*Trichoderma* and other biopesticides	Bacterial biofertilizers (e.g., PGPR, *Rhizobium*)	*T. harzianum* formulation
Minimum viable count	2×10^6 CFU g^{-1}	1×10^7 CFU g^{-1}	1×10^8 CFU g^{-1} (measured)
Moisture	≤ 8%	≤ 40% (carrier-based)	Not determined
Contamination	Pathogens absent; others ≤ 1×10^4 g^{-1}	No contamination at 10^5 dilution	Not determined
Shelf life	Up to 12 months	12 months	≈ 3 months at room temperature (qualitative observation)

PGPR, plant-growth-promoting rhizobacteria.

4.4. Storage stability of the formulation

The formulation remained viable for approximately three months in sealed containers at room temperature. This is a qualitative observation rather than a quantified shelf life, because viable counts were not determined at monthly intervals. The period is nevertheless shorter than the 6–12 months reported for dried *Trichoderma* preparations in several studies (Table 5). Naeimi et al. (2020) found that the population of dried *T. harzianum* on wheat bran fell from 7.0×10^8 to 5.8×10^5 CFU g^{-1} over 12 months at 25 °C but remained at 1.1×10^8 CFU g^{-1} at 4 °C. Mulatu et al. (2021) maintained more than 10^6 CFU g^{-1} in a wettable-powder formulation for eight months at 25 °C, and conidia stored on barley straw retained 71% viability after 12 months (Serna-Díaz et al., 2020). The observed three-month period is, however, consistent with the Pakistani study of Rajput et al. (2014), in which *T. harzianum* populations on several organic substrates peaked after 60–75 days of storage at room temperature before declining.

The most likely reasons for the shorter shelf life are the absence of a drying step and storage at room temperature. Residual moisture activates dormant conidia and accelerates the loss of viability, which is why carrier moisture must be kept to a minimum (Martínez et al., 2023), and high ambient temperatures during the Lahore summer would further reduce survival. Refrigerated storage markedly improves survival (Naeimi et al., 2020), and commercial products generally need to remain stable for at least one year (Yao et al., 2023). Drying the formulation before packaging and storing it at a lower temperature are therefore expected to extend its shelf life, although this should be confirmed experimentally.

Table 5. Storage stability of the present formulation compared with published data for *Trichoderma* on other carriers.

Study	Carrier / substrate	Storage condition	Viability reported
Present study	Sawdust–wheat bran–rice husk (not dried)	Sealed containers, room temperature	Viable for ≈ 3 months (qualitative; no monthly counts)
Rajput et al. (2014)	Organic substrates incl. rice husk	Room temperature	Peak at 60–75 days, then gradual decline
Naeimi et al. (2020)	Wheat bran (dried)	12 months at 25 °C and 4 °C	$7.0 \times 10^8 \rightarrow 5.8 \times 10^5$ CFU g^{-1} (25 °C); 1.1×10^8 CFU g^{-1} (4 °C)
Mulatu et al. (2021)	Wettable powder from SSF	8 months at 25 °C	$> 10^6$ CFU g^{-1}
Serna-Díaz et al. (2020)	Barley straw support	12 months	71% viability retained
Singh et al. (2007)	Wheat bran–sawdust	210 days	Longest shelf life among substrates tested

4.5. Contribution of the study

This study adds to the literature in four ways. First, it documents the use of a three-component sawdust–wheat bran–rice husk substrate for *T. harzianum* production by tray SSF, a combination that has received little attention in previous work. Second, it reports the viable count (1×10^8 CFU g^{-1}) and room-temperature storage behaviour (approximately three months) of the resulting formulation. Third, it describes a process based on simple laboratory equipment and locally available residues, which is suited to small-scale production in Pakistan. Fourth, by comparing the product with Indian and Pakistani standards, it highlights the absence of a specific fungal biofertilizer standard in Pakistan.

4.6. Limitations

Several limitations should be acknowledged. The strain was identified from the laboratory culture record and colony morphology; molecular identification was not performed, and no accession number was available. Moisture was assessed qualitatively by the hand-squeeze test rather than gravimetrically, and individual pH readings were not logged. The inoculum type and rate, tray bed depth, relative humidity and fermentation duration were not recorded and are reported as estimates. Results are single determinations from one production run, so replicate statistics are not available. Microbial purity and the final moisture content of the product were not determined, and storage stability was observed qualitatively without viable counts at 0, 1, 2 and 3 months. Finally, the efficacy of the formulation in promoting plant growth or controlling plant pathogens was not tested, so the product is described here as a biofertilizer formulation intended for agricultural use rather than one with demonstrated agronomic benefit. For these reasons, the work is presented as a technical note on formulation development and preliminary characterization rather than as a complete efficacy study.

5. Conclusion

This technical note shows that a carrier-based *Trichoderma harzianum* formulation can be produced by tray solid-state fermentation on a 2:1:1 mixture of sawdust, wheat bran and rice husk using basic laboratory equipment. The fungus colonised the substrate and sporulated, and the final granular, olive-brown product had a measured viable count of 1×10^8 CFU g^{-1}. As a qualitative observation, the product retained viability for approximately three months in sealed containers at room temperature.

These findings are preliminary. The results come from a single production run, several process parameters were estimated rather than recorded, and microbial purity, final moisture, quantitative shelf life and agronomic efficacy were not determined. Within these limits, the study demonstrates the feasibility of converting locally available agro-industrial residues in Pakistan into a viable *T. harzianum* formulation and provides a documented starting point for the further development and validation of a fungal biofertilizer.

6. Recommendations and Future Work

Building on this study, future work should:

1. identify the strain by ITS and *tef1* gene sequencing and deposit it in a recognised culture collection;
2. record all process parameters, including gravimetric moisture (oven-drying at 105 °C), pH readings, inoculum rate, bed depth, relative humidity and fermentation start and end times;
3. repeat the viable count on replicate plates from several independent production batches and report the mean ± standard deviation;
4. test the final product for microbial contaminants on general and selective media;
5. dry the product to 8% moisture or less and compare its shelf life at room temperature and 4 °C, with viable counts at 0, 1, 2 and 3 months and thereafter at regular intervals up to 12 months;

6. compare alternative substrate ratios and evaluate periodic mixing of the tray contents, which improved sporulation in earlier work (Sala et al., 2020);
7. test antagonistic activity against *Fusarium*, *Rhizoctonia* and *Pythium* in dual-culture assays, followed by pot and field trials; and
8. carry out a cost analysis of the production process.

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Author Contributions

S.U.H.S. carried out the experimental work, including substrate formulation, moisture and pH adjustment, sterilisation, inoculation, solid-state fermentation, viable-count determination, storage monitoring and photographic documentation, and wrote the original manuscript draft. M.S.R., U.F., J.J., L.S., S.S., S.Saq and B.A reviewed the manuscript, provided suggestions and recommended edits and revisions. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

Data Availability

Laboratory records, viable-count data and the original photographs are available from the corresponding author on reasonable request.

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