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RESEARCH ARTICLE
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A Targeted Fungal Bioconversion Strategy for Renewable Plant Waste: Solid-State Fermentation with *Pleurotus ostreatus* (MBI-2022) and Residual Biomass Valorization with *Trichoderma* spp.

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ABSTRACT

Agricultural processing generates large volumes of lignocellulosic residues (wheat and barley straw, cotton stalks, corn cobs/husks, sunflower husks, and sugar beet waste) that are often under-utilized. We evaluated a circular bioconversion pipeline that (i) upgrades renewable plant waste (RPW) to food and feed products via solid-state fermentation (SSF) with the white-rot fungus Pleurotus ostreatus (strain MBI-2022) and (ii) valorizes post-fruiting residual biomass via short SSF with *Trichoderma citrinoviride* AEF-2024 and *T. harzianum* AEF-2024. RPW was pre-moistened (1:1w/w water), sterilized (1atm, 30 min), inoculated at 0.3 kg spawn per 10 kg substrate and incubated at 28°C for 10 days (n=10 containers per substrate; analytical subsamples n=5 unless stated). Cellulose (Kürschner), lignin (Komarov-modified H_2SO_4), protein (Kjeldahl, N×6.25), lipids (Soxhlet), and nucleic acids (A260/A280) were quantified. Fungal performance was assessed as weight loss, cellulose/lignin degradation, and protein enrichment. Spent substrate was re-inoculated with Trichoderma spp. (5 days) to formulate a biopreparation that was tested in open-field vegetables. Across RPW types, cellulose and lignin were present at levels conducive to bioconversion. After 10 days, representative fungi achieved 31–38% cellulose and 33–40% lignin degradation in wheat straw and cotton stalks, with protein increases from ~2.1–2.7% to ~6.0–8.4% ($P \leq 0.05$). *P. ostreatus* MBI-2022 supported edible fruiting on all substrates; cumulative yield distribution favored wheat straw and sunflower husks. Residual biomass-derived Trichoderma biopreparation reduced disease prevalence by ~20%, increased yield by up to 12%, and improved seedling morphometrics by ~17% in field tests. Conclusion: SSF with P. ostreatus MBI-2022 upgrades RPW to food and nutrient-enriched feed while enabling circular reuse of residual biomass for crop protection. The approach provides a reproducible, low-waste route to valorize agricultural residues within a circular bioeconomy framework.

Keywords: Plant waste, Bioconversion, Lignocellulose valorization, Solid-state fermentation, White-rot fungi, Biological efficiency, Circular bioeconomy, Trichoderma biopreparation.

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INTRODUCTION

Plants are abundant sources of bioactive compounds that are essential for human nutrition, health and disease prevention (Astudillo et al., 2023). These bioactive compounds, which include polyphenols, flavonoids, alkaloids, terpenoids, and vitamins, are synthesized as part of the plant's natural defense mechanisms and physiological processes. However, many of these bioactive compounds persist in the byproducts that remain after primary processing.

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If strategically harnessed, these residual bioactive constituents could be transformed into valuable resources, enhancing sustainability and adding economic value to what is often labeled as agricultural waste. The agricultural sector is a significant producer of plant-derived byproducts, often categorized as agricultural waste. These materials, including straw, husks, stems, shells, peels, and pulp, are frequently overlooked despite their rich chemical composition. Historically, these agricultural byproducts have been underutilized or mismanaged, often disposed of through open-field burning or unregulated dumping (Kennes, 2018; Lackner & Besharati, 2025). Despite being considered waste, these byproducts can contain a wide range of chemical constituents, including cellulose, lignin, hemicellulose, and a variety of secondary metabolites that offer potential for diverse applications (Zeng et al., 2017; Blasi et al., 2023). The challenges faced by the agricultural sector today are compounded by increasing global pressures, including climate change, biodiversity loss, land desertification, water scarcity and soil salinization (Muluneh, 2021; Bhatla & Lal, 2023). These challenges are further compounded by the degradation of arable land, increasing the difficulty of securing food resources and bioenergy production, thus highlighting the need for innovative strategies to enhance the sustainability and resilience of agricultural systems (Hussain et al., 2021). The urgency of these efforts becomes even clearer when viewed in the context of global demographic trends. The United Nations has projected that the global population will reach 9.4 to 10.1 billion by 2050, and 9.4 to 12.7 billion by the end of the century (United Nations, 2019). This unprecedented demographic growth placed an immense burden on global food production systems, requiring a significant increase in agricultural output. To meet these demands, it is essential to adopt sustainable agricultural practices that not only increase yield but also minimize environmental impacts. In this context, the valorization of agricultural byproducts presents a promising avenue to improve resource use efficiency and reduce waste.

Over 550 billion tons of carbon-based green biomass are generated annually through photosynthesis worldwide (Ho et al., 2020). However, only approximately 10 % of this biomass is directly utilized for food, energy, and industrial raw materials (Giller & Delaune, 2018; Bar-On et al., 2018). The remainder, which is often considered waste, represents a largely untapped resource. While this residual biomass, such as agricultural residues from cereal crops (e.g., wheat and barley straw), cotton stalks, corn husks, sugar beet waste, and sunflower husks, is conventionally classified as waste, it still retains a significant proportion of its original biochemical constituents, making it an excellent candidate for value-added biotechnological processes (Kumar et al., 2015; Lee et al., 2019; Ravindran et al., 2018). When left unmanaged, these residues contribute to environmental burdens, but when effectively utilized, they can serve as feedstock for multiple biotechnological applications.

The management of agricultural residues poses a dual challenge. On the one hand, the improper disposal of these materials leads to environmental degradation, and on the other hand, their potential as renewable resources remains largely underexploited. To address this challenge, the concept of renewable plant waste (RPW) has emerged, focusing on agricultural byproducts as a resource pool for bioenergy, biochemicals, bioplastics, functional foods, animal feed and organic fertilizers (Zeng et al., 2017; Sadh et al., 2018; Pal et al., 2024). Among the many strategies proposed, biological recycling methods, particularly fungal bioconversion, have attracted growing attention due to their sustainability, scalability, and environmental compatibility. The conversion of RPW into high-value products using biological methods such as fungal bioconversion has been identified as an innovative and sustainable solution. By utilizing microorganisms such as fungi for the degradation of lignocellulosic material, it is possible to repurpose these residues into nutritionally enriched feed, biofertilizers, or even biofuels, contributing to circular economy models within agriculture.

In addition to fungal bioconversion, other innovative biotechnological approaches are being explored for agricultural waste valorization. These include microbial fermentation, enzymatic hydrolysis, anaerobic digestion, and composting, all of which contribute to transforming waste into resources. Advances in synthetic biology, metabolic engineering, and systems biology are also expanding the potential of microorganisms to convert plant waste into high-value compounds with applications in food, pharmaceuticals, agriculture, and materials science. Importantly, integrating agricultural waste recycling into circular economy frameworks offers multiple benefits (Singh et al., 2020). It enhances resource efficiency by maximizing the use of biomass generated within agricultural systems. It reduces dependence on fossil resources by providing renewable alternatives for chemicals and materials. It mitigates environmental impacts by lowering greenhouse gas emissions, reducing landfill use, and preventing nutrient runoff. Furthermore, it creates new economic opportunities, particularly in rural areas, by generating additional income streams and employment through the establishment of biorefineries, decentralized processing units, and waste-to-resource enterprises. Agricultural value chains generate abundant lignocellulosic by-products (straws, stalks, cobs, husks, pulp) that are frequently under-utilized despite their rich composition in cellulose, hemicellulose, lignin, and bioactive metabolites. Unchecked disposal (e.g., open burning) exacerbates air pollution and forfeits opportunities for resource recovery. Within circular bioeconomy frameworks, renewable plant waste (RPW) can be repurposed into high-value products via biological processing. Solid-state fermentation (SSF) with white-rot fungi is particularly attractive because these organisms secrete complementary hydrolytic and oxidative enzymes capable of deconstructing recalcitrant lignocellulose while synthesizing nutritionally and pharmacologically relevant metabolites. Here, we target two coupled aims: (i) to evaluate RPW upgrading to edible mushrooms and nutrient-enriched biomass using Pleurotus ostreatus (MBI-2022) under SSF; and (ii) to valorize post-fruiting residual biomass by short SSF with Trichoderma citrinoviride and T. harzianum to yield an agronomically active biopreparation. We also standardize reporting of replication, controls and statistics to enhance reproducibility.

MATERIALS & METHODS

In this study, renewable plant waste (RPW) derived from the agricultural sector was selected for bioconversion. The RPW included materials such as wheat and barley straw, cotton stalks, corn cobs, sunflower husks, and sugar beet waste (n=10 in each group). Unless noted, experiments used n=10 containers per substrate (≈3kg dry matter each), randomized on shelves in a controlled room. Uninoculated, sterilized substrates served as negative controls for analytical baselines. These residual by-products are typically considered waste, but they possess high concentrations of cellulose, lignin, and hemicellulose, making them valuable for bioconversion into high-value products. The transformation process was carried out under solid-state fermentation (SSF) conditions (Perwez and Al Asheh, 2024) according to the following sequence: The air-dried substrate (initial moisture content 12–15%, particle size 2–5mm) is moistened with plain water at a 1:1 ratio, and 10g (based on the initial substrate weight) is added to glass flasks in at least four replicates, followed by sterilization at 1 atm pressure for 30minutes. The sterile substrate is inoculated with fungal inoculum (at a rate of 3g of inoculum per 1kg of substrate on a dry weight basis) and incubated at 28°C for 10 days. After the incubation period, the resulting biomass is dried at a temperature not exceeding 45°C and analyzed according to the parameters specified below. To assess the efficiency of the bioconversion process, various parameters were evaluated, including weight reduction, cellulose and lignin degradation, and protein enrichment in the resulting biomass. Weight loss is calculated based on the dry weight of the initially taken substrate and the biomass obtained after bioconversion, and is expressed as a percentage. The cellulose content of both the original waste and the resulting biomass was quantified using the Kürschner method, which is based on treating the original objects of study with nitric acid and ethyl alcohol (1:4) during boiling and removing lignin, extractives and hemicelluloses. The lignin content was estimated by the sulfuric acid method modified by Komarov, who used 72% sulfuric acid. Protein concentration was determined by the Kjeldahl method, a standard procedure for analyzing nitrogen content and recalculating its protein levels using 6.25 (Noskova & Sinyaev, 2016). The concentrations of nucleic acids in the studied materials were determined by the spectrophotometric method. This method relies on measuring the absorbance of light at specific wavelengths to determine the concentration of nucleic acids (DNA and RNA) present in the sample. The absorbance ratio at 260nm and 280nm (A260/A280) is commonly used to assess the purity of nucleic acid samples (Galliano et al., 2021). To determine the amount of lipids, the materials were extracted in a Soxhlet apparatus with petroleum ether, and the lipid content was determined gravimetrically (Vostrikova et al., 2018).

An in vitro digestion method to assess the quality of proteins based on enzymatic hydrolysis is presented. The method consists of a two-step proteolysis at 37^0C, a 30-minute incubation of the protein with pepsin at pH 1.9, followed by pancreatin digestion at pH 8 for 24 hours in a dialysis bag with a 1000 molecular weight cutoff for the continuous elimination of the digested products into a replaceable buffer (Gauthier et al., 1982). The bioconversion process was mediated by xylotrophic macromycetes, a group of fungi known for their ability to degrade lignocellulosic materials. The fungal cultures used for this study were supplied by the Microbiological Biotechnology Laboratory of the Institute of Microbiology under the Ministry of Science and Education of Azerbaijan. The laboratory provided pure cultures of *Pleurotus ostreatus* MBI-2022, a white-rot fungus, which is known for its ability to break down complex plant materials.

For the cultivation of *Pleurotus ostreatus* MBI-2022, a standardized inoculum preparation procedure was followed. Wheat grains were chosen as the propagation medium for the fungal inoculum. The grains were initially boiled to sterilize them, then dried to a moisture content of 20 %. After drying, the grains were placed in containers and sterilized at 1 atm pressure for 30 minutes. Following sterilization, the wheat grains were aseptically inoculated with *Pleurotus ostreatus* mycelium to ensure sterility. The inoculated grains were incubated at 28°C for 10 days, with periodic agitation on days 2, 5, and 8 to promote uniform fungal colonization. The resulting mycelial biomass was then used as spawn at an inoculation rate of 0.3kg per 10kg of substrate.

For the bioconversion of the RPW, a pre-treatment process was carried out to enhance the degradation of lignocellulosic materials. The substrate underwent a hot water pre-treatment at temperatures between 65–70°C to soften the plant material and facilitate fungal colonization. After the pre-treatment, the substrate was boiled to further break down the plant material, thoroughly mixed with the fungal inoculum, and packed into perforated cellophane bags (50×100cm). These bags were sealed and incubated under controlled conditions at temperatures ranging from 25–28°C. The mycelium grew throughout the substrate, and once full colonization was achieved, larger perforations (4–5 cm in diameter) were made to stimulate fruiting body formation. Fruiting occurred in three sequential waves, each producing a distinct batch of fruiting bodies. The total yield was calculated as the cumulative mass of the fruiting bodies harvested across all three waves. Through these methods, the study aimed to optimize the bioconversion of RPW into high-value products such as edible mushrooms and nutrient-enriched biomass for animal feed. This process not only offers a sustainable approach to managing agricultural waste but also provides an alternative source of protein and other valuable compounds for use in food and feed industries.

Statistical Analysis

Analyses were conducted in standard statistical software. Group differences were tested by one-way ANOVA followed by Tukey's HSD; paired or independent-samples t-tests were applied where appropriate. Significance threshold $\alpha=0.05$. Normality and homoscedasticity were checked using Shapiro–Wilk and Levene tests, respectively.

RESULTS

Empirical Data Analysis

Empirical data analysis indicates that, despite variations in the quantitative composition of their constituents, all selected waste materials investigated in this study exhibit substantial levels of cellulose and lignin. The data in Table 1 summarizes the cellulose and lignin content in the different types of renewable plant waste (RPW) analyzed. These materials, which include wheat straw, barley straw, cotton stalks, corn cobs, sunflower husks, and sugar beet waste, were all found to contain significant amounts of cellulose and lignin, key components for bioconversion processes. Cellulose is the primary structural component of plant cell walls and is a polysaccharide composed of glucose units linked by β-1,4-glycosidic bonds. Lignin, on the other hand, is a complex polyphenolic structure that provides rigidity and waterproofing to the plant cell walls. The presence of these two components in substantial amounts makes these RPWs ideal candidates for bioconversion into value-added products, such as edible fungi, biofuels, and nutrient-enriched biomass. The quantitative analysis of the cellulose and lignin content was performed using standard analytical methods, including the Kürschner method for cellulose and the Komarov-modified sulfuric acid method for lignin, as detailed previously. Table 1 provides a detailed breakdown of the cellulose and lignin content for each of the RPW types.

Despite the inherent variability in the lignocellulosic content across the different types of waste materials, the general trend observed across all materials indicates a sufficient presence of these two key components to support effective bioconversion. This observation underscores the potential of agricultural residues as valuable resources for the production of both food and feed products, as well as bioenergy. The next phase of the study will involve investigating the extent of cellulose and lignin degradation during the bioconversion process and evaluating the resulting products for their nutritional and feed quality properties Empirical data analysis indicates that, despite variations in the quantitative composition of their constituents, all selected for investigation waste materials exhibit substantial levels of cellulose and lignin (Table 1).

The primary constituents of all analyzed waste materials, including lignin, cellulose, and hemicellulose, are indicative of their potential for bioconversion. From a theoretical perspective, these components suggest that these agricultural residues possess the necessary chemical characteristics to undergo biotechnological conversion. However, extensive research has demonstrated that, in their native form, the reactivity of natural materials remains relatively low. This inherent low reactivity poses a significant barrier to their effective bioconversion into high-value products. Consequently, various pre-treatment strategies are employed to modify their physicochemical properties, thereby increasing their overall reactivity and responsiveness to microbial and enzymatic degradation. Despite advancements in these pre-treatment methods, the widespread utilization of agricultural waste for bioconversion remains limited due to several unresolved challenges. These challenges warrant further investigation and innovation in the field of biotechnological waste valorization. The predominant constituents of most agricultural residues — cellulose, lignin, and hemicellulose — interact to form a molecular framework that is inherently resistant to microbial degradation. This recalcitrant structure is the primary reason for the slow and incomplete conversion of these materials into valuable products such as biofuels, edible fungi, or other value-added products (Bomble et al., 2017).

Moreover, the intricate polymeric structure and robust cellular architecture of these materials contribute to their low reactivity and difficulty in breaking down by natural microorganisms (Akhundova et al., 2019). As such, diverse preliminary processing methods (physical, chemical, and biological) are necessary to enhance the commercially viable yield of the end products. While these processing techniques improve the bioconversion process, they also often result in elevated costs associated with the final products derived from waste conversion (Fu et al., 2021). In response to these challenges, a cost-effective physical pre-treatment was deemed appropriate for the waste materials under investigation. This pre-treatment involved grinding the waste in a laboratory mill to achieve a particle size ranging from 0.2 to 0.5 cm. The shredded waste materials were visually differentiated by their color, as shown in Fig. 1. This preliminary grinding process facilitates the disruption of the lignocellulosic matrix, making the cellulose and hemicellulose more accessible for enzymatic and microbial degradation during the subsequent bioconversion stages. The shredded materials, having undergone the physical pretreatment, were then subjected to solid-state fermentation (SSF) with microbial inoculation for bioconversion analysis.

Table 1: Physicochemical and structural parameters of renewable plant waste (RPW) substrates prior to bioconversion

Parameter	Cotton stalks (n = 10)	Corn husk (n = 10)	Barley straw (n = 10)	Wheat straw (n = 10)	Sunflower husk (n = 10)	Sugar beet waste (n = 10)
Extracted water (%)	9.3±2.2 (7–11)	15.7±2.1 (13–17)	6.5±0.7 (6–7)	4.8±0.5 (4–5)	5.3±0.8 (5–6)	14.5±3.5 (14–17)
Cellulose (%)	38.1±4.5 (34–42)	36.5±2.5 (34–39)	35.5±1.5 (34–37)	35.5±2.5 (33–38)	23.5±2.5 (21–26)	35.7±4.2 (31–40)
Hemicellulose (%)	27.5±5.5 (22–33)	25.3±7.0 (18–32)	25.6±2.2 (23–27)	30.6±5.1 (25–35)	19.2±2.7 (17–21)	23.8±3.5 (21–26)
Lignin (%)	23.1±2.5 (21–25)	13.2±3.3 (10–16)	20.6±3.1 (17–23)	23.3±4.2 (19–27)	28.7±2.3 (26–30)	13.8±2.5 (12–17)
Protein (%)	3.3±0.7 (2.7–3.9)	4.0±0.3 (3.4–4.6)	3.6±0.5 (3.2–4.0)	3.6±0.8 (3.0–4.1)	3.3±0.8 (2.9–3.7)	3.6±0.1 (2.7–4.0)
Nucleic acids (%)	0.86±0.31 (0.75–0.97)	0.75±0.08 (0.63–0.86)	0.72±0.20 (0.50–0.73)	0.65±0.23 (0.47–0.72)	0.59±0.23 (0.36–0.62)	0.92±0.35 (0.67–0.96)
Ash (mineral elements, %)	6.0±1.0 (5–7)	6.0±2.0 (4–8)	5.0±1.0 (4–6)	6.0±1.0 (5–7)	5.2±1.4 (4–6)	5.7±0.2 (5–6)
Crystallization coefficient	55±2 (53–57)	43.5±2.5 (41–46)	49±2 (47–51)	52±2 (50–54)	43.6±1.1 (42–44)	38.2±0.3 (38–39)

Values (mean±SD) with range in parentheses, based on dry matter basis (%) from n=10 containers (3 kg each). Analytical methods: Cellulose (Kürschner method); Lignin (Komarov-modified sulfuric acid method); Hemicellulose (standard gravimetric procedures); Protein (Kjeldahl method; Hostettmann, 2014); Nucleic acids (UV spectrophotometry); Ash (gravimetric incineration); Crystallization coefficient (X-ray diffraction). Extracted water (%) refers to the proportion of water removed upon drying.

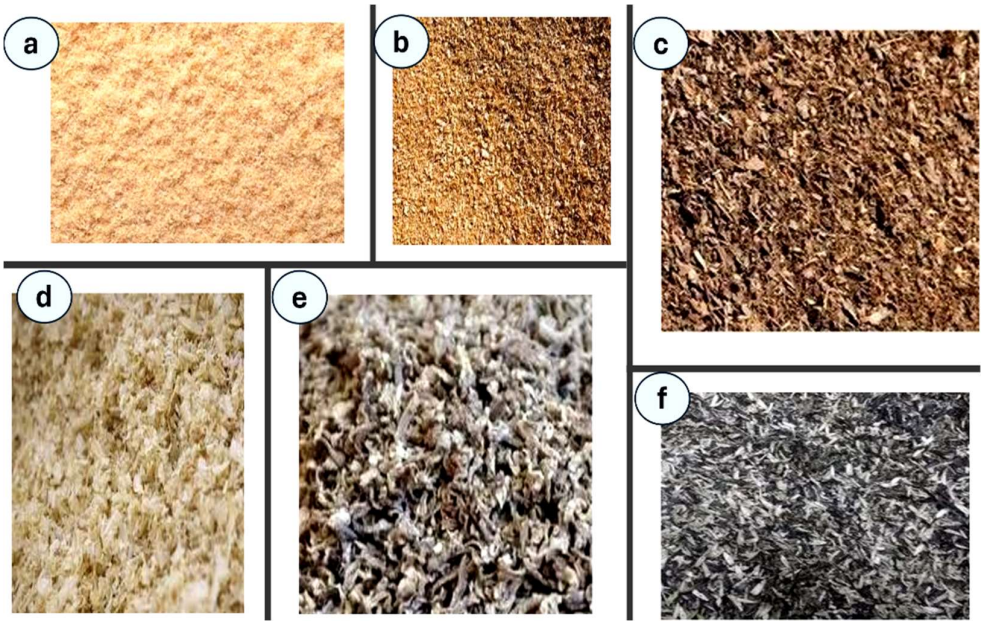

Fig. 1: Macroscopic appearance of renewable plant waste (RPW) substrates following grinding. (a) Wheat straw; (b) Corn husk; (c) Cotton stalks (richest in cellulose: 38.1±4.5%, n = 10); (d) Barley straw; (e) Sugar beet residues; (f) Sunflower husk (richest in lignin: 28.7±2.3%, n = 10). Scale bars = 2cm.

The bioconversion of renewable plant waste (RPW) into value-added products has been explored through the application of various biological agents, specifically fungi and bacteria. Among these, fungi, particularly those that produce hydrolytic and oxidative enzymes, have gained significant attention for their potential to break down complex lignocellulosic materials. Micromycetes and macromycetes, as crucial components of the bioconversion process, play an essential role in catalyzing the degradation of complex polymers like cellulose, lignin, and hemicellulose. However, despite their recognized potential, the application of fungal agents in bioconversion is still not universally effective. This is largely due to the incomplete or inconsistent enzyme sets present in these microorganisms, which may not fully encompass the required range of enzymes needed to degrade the diverse polymers in agricultural waste.

One of the most significant challenges in using fungi for bioconversion arises from the heterogeneity of plant cell wall polymers. These polymers differ not only in their abundance but also in their degradation mechanisms. While some polymers are degraded via hydrolytic processes, others require oxidative mechanisms. The combination of these distinct degradation pathways complicates the overall efficiency of the bioconversion process. In particular, micromycetes, although highly efficient producers of hydrolytic enzymes, tend to have limitations when dealing with the oxidative breakdown of certain lignin structures. This often results in less effective waste degradation when compared to fungi that are capable of both hydrolytic and oxidative enzyme production.

Fungi, particularly those responsible for white rot, are well-documented for their ability to degrade lignocellulosic materials due to their broad-spectrum enzyme systems that include both hydrolytic and oxidative enzymes. White-rot fungi, such as *Pleurotus ostreatus* and *Trichoderma spp.*, have been identified as promising candidates for lignocellulose degradation, as they produce lignin peroxidases, manganese peroxidases, and laccases, which are particularly effective in breaking down lignin, one of the most recalcitrant components of plant biomass. Numerous studies have substantiated the potential of these fungi in bioconversion processes. For instance, *Pleurotus ostreatus*, a widely studied white-rot fungus, has been shown to efficiently degrade both cellulose and lignin, making it an excellent candidate for waste valorization (Fu et al., 2021).

In our study, we explored the bioconversion capabilities of several fungal strains, including those known for their white-rot capabilities (Bakhshaliyeva et al., 2021). The results from our experiments indicate that all the selected fungal strains were indeed capable of degrading the waste polymers present in the agricultural residues. However, significant variations were observed in their bioconversion efficiency, particularly in terms of weight loss, cellulose and lignin degradation, and protein accumulation. These variations were particularly evident in the degradation of wheat straw and Cotton stalks samples, as shown in Table 2. The data from Table 2 clearly demonstrate that while all fungal strains contributed to the breakdown of the waste polymers, some strains exhibited superior performance in specific degradation processes. For example, strains such as *Pleurotus ostreatus* demonstrated exceptional lignin degradation capabilities, which resulted in a higher weight loss and a more significant reduction in lignin content. In contrast, strains like *Trichoderma harzianum* exhibited higher cellulose degradation, though their lignin degradation was less pronounced. This difference in the performance of the strains can be attributed to the diverse enzyme systems they possess. Specifically, *Pleurotus ostreatus* produces a more comprehensive suite of enzymes that enable the breakdown of lignin through oxidative mechanisms, while *Trichoderma harzianum* focuses more on cellulose degradation through hydrolytic enzymes.

Furthermore, it is important to note that the overall success of bioconversion is not solely determined by the degree of polymer degradation but also by the subsequent enrichment of the waste with valuable nutrients. The accumulation of protein in the biomass, a critical parameter

Table 2: Microbiological conversion of studied plant waste after 10 days of fungal treatment

Fungi	Weight loss (%) Mean±SD	Cellulose degradation (%) Mean±SD	Lignin degradation (%) Mean±SD	Protein initial (% dry basis) Mean±SD	Protein final (% dry basis) Mean±SD	P (final vs. initial protein)
Wheat straw						
Bjerkandera adusta	22.1±2.3	37.9±3.4	37.4±4.6	2.7±0.3	7.9±1.2	0.0004
Cerrena unicolor	21.2±3.5	35.1±4.3	37.5±2.5	2.7±0.3	7.8±2.3	0.0073
Fomes fomentarius	19.2±2.5	34.5±4.5	34.8±8.1	2.7±0.3	7.5±3.4	0.0340
Ganoderma applanatum	20.1±3.1	35.2±3.9	35.1±7.2	2.7±0.3	7.9±0.9	0.0001
G. lucidum	19.3±4.1	34.7±2.1	33.8±5.4	2.7±0.3	7.7±1.1	0.0003
Phellinus igniarius	19.9±2.7	36.6±2.6	32.7±4.5	2.7±0.3	7.4±2.1	0.0070
Lentinus tigrinus	22.1±2.2	37.5±4.7	37.7±5.6	2.7±0.3	8.4±3.6	0.0237
Pleurotus ostreatus	23.2±3.5	38.0±5.6	38.2±5.2	2.7±0.3	8.1±4.3	0.0482
Trametes hirsuta	21.0±4.2	33.7±3.6	39.5±7.3	2.7±0.3	7.5±2.7	0.0160
T. versicolor	20.6±1.3	31.7±4.2	39.0±6.7	2.7±0.3	7.3±3.2	0.0321
Cotton stalks–						
Bjerkandera adusta	20.3±2.2	35.7±2.7	36.0±4.6	2.1±0.2	7.0±2.5	0.0116
Cerrena unicolor	19.0±3.6	34.5±3.5	34.9±5.3	2.1±0.2	6.9±2.1	0.0067
Fomes fomentarius	18.2±4.2	33.0±5.5	33.8±1.7	2.1±0.2	6.0±3.6	0.0725
Ganoderma applanatum	18.9±3.5	32.7±2.8	33.8±3.7	2.1±0.2	6.1±5.4	0.1730
G. lucidum	18.2±2.4	34.0±3.7	34.9±4.8	2.1±0.2	6.6±3.2	0.0345
Phellinus igniarius	18.2±4.2	35.1±5.3	36.9±6.3	2.1±0.2	6.9±1.1	0.0005
Lentinus tigrinus	20.1±3.5	36.5±2.0	37.0±3.6	2.1±0.2	8.3±3.7	0.0199
Pleurotus ostreatus	21.7±1.2	37.3±0.0	37.8±5.1	2.1±0.2	7.8±4.8	0.0566
Trametes hirsuta	22.8±3.2	32.1±1.8	38.3±4.2	2.1±0.2	6.6±3.8	0.0570
T. versicolor	22.6±3.9	31.1±4.1	38.0±5.7	2.1±0.2	6.3±3.2	0.0424

Data are expressed as mean±SD (n=5). Weight loss, cellulose degradation, and lignin degradation are given as percentages of the initial dry mass. Protein contents are expressed on a dry matter basis. *P*-values indicate statistical significance for differences between final and initial protein contents (independent-samples t-test).

for evaluating the efficacy of bioconversion for feed purposes, varied significantly among the fungal strains. Fungi that excelled in lignin degradation, such as *Pleurotus ostreatus*, tended to show higher protein accumulation due to the release of bioavailable nitrogen from the breakdown of complex lignin structures. This enhancement in protein content is crucial for the development of nutrient-enriched feed products. The variation in performance across different fungal strains underscores the importance of selecting the appropriate microbial agent for specific bioconversion processes. While white-rot fungi like *Pleurotus ostreatus* and *Trichoderma spp.* show promise for the degradation of agricultural residues, further optimization of fungal cultivation conditions and enzyme production profiles is needed to improve the overall yield and cost-effectiveness of bioconversion. The combination of fungi capable of both hydrolytic and oxidative degradation may represent an ideal solution for overcoming the limitations of individual enzyme systems and achieving higher efficiency in waste valorization. Baseline composition of RPW confirmed substantial cellulose (≈31–38%) and lignin (≈13–29%) contents across substrates, with modest protein (≈3–4%) and variable hemicellulose. After 10 days of SSF, representative white-rot fungi achieved concurrent cellulose and lignin degradation (typically 31–40%), with weight loss concordant with polymer removal. Protein levels increased to approximately 6–8% dry basis ($P \leq 0.05$), indicating nutritional enrichment of the biomass. P. ostreatus MBI-2022 supported fruiting on all RPW substrates; wheat straw and sunflower husks showed the fastest onset and highest cumulative yields across three waves. Residual substrate, re-processed with Trichoderma spp., yielded a lyophilized biopreparation that in open-field vegetables reduced disease prevalence (~20%), increased morphometric parameters (~17%), and raised yield (≤12%) relative to untreated plots.

In the context of lignocellulosic degradation, fungal species exhibit distinct efficiencies in the breakdown of cellulose and lignin, alongside their capacity to enrich the substrate with proteins and other bioactive compounds. The variations in fungal performance can be attributed to differences in their enzymatic systems, which are responsible for catalyzing the hydrolytic and oxidative processes that break down the complex lignocellulosic structure. The results from our study reflect these differences and contribute to a deeper understanding of fungal bioconversion mechanisms. The fungal strains used in this study have been classified into three categories based on their lignocellulose degradation capabilities. These categories are defined by their efficiency in degrading cellulose and lignin:

First Category (Equal Degradation of Cellulose and Lignin)

Fungi in this category exhibit a balanced ability to degrade both cellulose and lignin. Species such as *Bjerkandera adusta*, *Fomes fomentarius*, and *Ganoderma applanatum* fall into this group. These fungi effectively break down both components of the lignocellulosic structure, resulting in a significant weight loss and enhanced substrate quality.

Second Category (Preferential Lignin Degradation)

Fungi in this group degrade lignin more efficiently than cellulose. *Cerrena unicolor*, *Trametes hirsuta*, and *Trametes versicolor* are examples of fungi that exhibit a higher degree of lignin degradation relative to cellulose. This selective degradation results in the preferential breakdown of lignin, which is more recalcitrant than cellulose, and contributes to the generation of bioactive compounds and protein enrichment in the substrate.

Third Category (Preferential Cellulose Degradation)

Some fungi, such as *Phellinus igniarius*, which showed different patterns for wheat straw and Cotton stalks, prefer to break down cellulose over lignin. This selective

degradation leads to varying degrees of weight loss and substrate modification depending on the type of agricultural waste. Interestingly, no single fungal strain demonstrated unambiguous superiority across all measured parameters, including cellulose and lignin degradation, weight loss, and protein enrichment. This highlights the complexity of fungal-mediated bioconversion, where individual species may excel in one aspect of degradation but not necessarily across all criteria. For example, *Trametes hirsuta* was found to be particularly efficient in lignin degradation, showing the highest lignin breakdown across both types of waste materials, wheat straw and Cotton stalks. However, it did not exhibit the same level of efficiency in cellulose degradation. In contrast, *Pleurotus ostreatus* demonstrated superior performance in the degradation of both cellulose and sugar components. This strain was highly effective in breaking down the primary polysaccharides in the waste, contributing to a significant reduction in cellulose content and an increase in the availability of sugars. Among the fungi evaluated, *Lentinus tigrinus* stood out for its ability to significantly enrich the substrate with proteins. The protein concentration in the substrate increased from 2.1% to 8.3%, making it a promising candidate for applications requiring nutrient-rich feedstocks. This ability to enhance protein content is a key advantage for using fungi in bioconversion processes aimed at producing animal feed or other high-value protein products. The findings from our study align with observations from other research that has classified fungal species based on their ability to degrade cellulose and lignin. The classification system, which includes fungi with varying xylolysis coefficients (Jc), further categorizes fungal species into three distinct groups:

1. Fungi with a Jc > 0.49, which preferentially degrade cellulose.
2. Fungi with a Jc < 0.49, which preferentially degrade lignin.
3. Fungi with a Jc = 0.50±0.01, which degrade both cellulose and lignin equally (Zeng et al., 2017; Andlar et al., 2018; Bakhshaliyeva et al., 2021).

Interestingly, white rot fungi, although widely recognized for their exceptional lignocellulose degradation capabilities, possess a far more expansive metabolic profile that extends beyond the mere breakdown of cellulose and lignin. These fungi are remarkable producers of a wide spectrum of secondary metabolites, many of which possess significant pharmacological and industrial value. Notably, species such as *Pleurotus ostreatus* are known to biosynthesize an array of bioactive compounds, including polysaccharides, terpenoids, polyketides, and phenolic derivatives. These metabolites have been documented to exhibit antimicrobial, antitumor, immunomodulatory, and antioxidant properties, making them attractive targets for biopharmaceutical development (Dicks et al., 2020). Additionally, the enzymatic arsenal of white rot fungi — comprising lignin peroxidases, manganese peroxidases, laccases, and cellulases — enables them to participate in a dual-action transformation: simultaneous decomposition of recalcitrant plant polymers and synthesis of high-value bioactive products. Given these multifaceted capabilities, *Pleurotus ostreatus* was chosen in this study as the primary biocatalyst for further experimentation. The strain's proven efficiency in degrading both cellulose and simple sugars, along with its robust enzymatic activity and secondary metabolite profile, supports its selection as a model organism for lignocellulosic bioconversion. Furthermore, its ease of cultivation, low pathogenic risk, and edibility enhance its suitability for integrated food-feed-bioproduct systems.

In combination with other strains like *Lentinus tigrinus*, which was shown to significantly enrich protein levels in the treated substrate, *Pleurotus ostreatus* represents a cornerstone of sustainable biotechnological solutions aimed at converting agricultural waste into nutritionally and economically valuable products (Yamasaki et al., 2021). These findings further highlight the need for strategic fungal strain selection based on target application — whether it be bioenergy, organic fertilizer, livestock nutrition, or pharmaceutical compound synthesis. Such precision in microbial resource utilization not only elevates the economic viability of bioconversion technologies but also contributes substantially to circular bioeconomy models and environmental remediation frameworks. The synergistic use of diverse fungal species offers the potential to optimize substrate degradation and maximize the spectrum of value-added outputs. Importantly, the ecological compatibility and low carbon footprint of fungal-based systems position them as eco-conscious alternatives to conventional chemical or thermal processing methods.

Beyond their enzymatic contributions, fungi also participate in the biosynthesis of biofertilizers by improving substrate digestibility and enriching it with nitrogenous compounds, thereby enhancing soil health upon application. In this regard, fungal mycelial residues post-harvest may be re-integrated into agricultural systems as bio-fertilizing agents. Additionally, the structural modification of lignocellulosic substrates by fungal enzymatic action improves the accessibility of nutrients, which is critical for the development of next-generation animal feeds. The implementation of such systems aligns with Sustainable Development Goals (SDGs), particularly those related to responsible production, climate action, and zero hunger. Fungal-derived feedstocks can potentially replace or reduce the use of synthetic additives in animal husbandry, offering safer and more natural alternatives. A deeper understanding of fungal genomics and metabolomics could pave the way for strain engineering, enhancing specificity and efficiency in substrate conversion (Sharma and Yadav 2022). Integrative 'omics' approaches, including transcriptomics and proteomics, are increasingly being applied to unravel the metabolic pathways governing fungal degradation and biosynthesis. Such insights could inform the rational design of bioprocesses tailored to diverse waste types and desired product outcomes. Furthermore, the adaptability of fungi to various environmental conditions allows for decentralized bioconversion systems, especially in low-resource or rural settings. This decentralization supports local economies and reduces dependency on globalized supply chains for feed and fertilizer inputs.

Given the diversity of agricultural residues worldwide, establishing a global fungal strain bank could facilitate cross-regional application of optimized bioconversion

protocols. Collaborative frameworks among research institutions, industry, and policymakers will be essential to scale these technologies and embed them into national waste management and agricultural policies. Ultimately, harnessing the full biotechnological potential of fungi like *Pleurotus ostreatus* and *Lentinus tigrinus* offers a transformative avenue for sustainable agriculture, circular economy integration, and bio-based innovation.

One of the primary justifications for the selection of *Pleurotus ostreatus* in this study lies in its dual functional relevance: firstly, as an edible mushroom species widely accepted in human diets across various cultures, and secondly, due to its well-documented capacity to biosynthesize a diverse array of bioactive compounds with pharmacological potential (Iram et al., 2021; Nakazawa et al., 2024). These bioactive metabolites include, but are not limited to, β-glucans, terpenoids, phenolics, and polysaccharide-protein complexes, which exhibit immunomodulatory, antitumor, antioxidant, antimicrobial, and cholesterol-lowering properties. This dual role enhances its applicability in integrated biorefinery systems where nutritional, therapeutic, and ecological benefits are prioritized simultaneously.

The specific strain utilized in this investigation—*Pleurotus ostreatus* MBI-2022—was initially isolated in pure culture from the mature fruiting body of a wild-growing specimen collected from the bark of *Morus alba* (white mulberry) in the Central Botanical Garden of the National Academy of Sciences of Azerbaijan in 2020. Following successful isolation and cultivation, the strain was characterized morphologically and genetically, and subsequently preserved within the microbial strain collection of the Institute of Microbiology under the official catalog number MBI-2022.

The morphological features of *P. ostreatus* MBI-2022, including fruiting body structure, colony formation on solid-state fermentation substrate, and the mycelial network under light microscopy, are shown in Fig. 2. The morphological characteristics of *P. ostreatus* MBI-2022 were evaluated to assess its growth and structural features. As shown in Fig. 2, the fruiting bodies displayed the typical oyster mushroom morphology (Fig. 2a). The colony structure on the solid-state fermentation substrate exhibited dense and uniform growth patterns (Fig. 2b). Examination of the mycelial network under light microscopy revealed an extensive hyphal network with interconnected filaments, indicating healthy mycelial development (Fig. 2c). Representative images from three independent experiments (n = 3) confirmed the consistency of these morphological traits.

Given its robust lignocellulolytic enzyme profile and promising bioactivity indicators, this strain was selected for the optimization of bioconversion parameters under the experimental model of "plant residues into feed products." The aim was to maximize both the nutritional enhancement of the substrate and the overall biomass yield. The optimization process focused on determining the ideal cultivation conditions (temperature, humidity, inoculation rate, and substrate composition) for efficient substrate colonization and fruiting, as well as for the accumulation of protein and bioavailable micronutrients in the post-bioconversion feed product (Table 3). These parameters were calibrated through iterative cultivation trials, each followed by quantitative assessment of key biochemical and biophysical indicators, ensuring that the selected fungal strain achieved consistent and reproducible performance under laboratory-scale solid-state fermentation (SSF) conditions.

Biochemical analysis of the fungal-treated biomass, cultivated under optimized conditions, revealed that the resulting product exhibits neither phytotoxic nor zootoxic effects, affirming its safety for agricultural and zootechnical applications. Notably, the bioconversion process resulted in a substantial degradation of recalcitrant lignocellulosic components: cellulose and lignin content in the treated substrate decreased by approximately 34–40% compared to the untreated control. Concurrently, the concentrations of soluble sugars and crude proteins increased markedly by factors ranging from 2.1 to 4.2 indicating not only efficient polymer breakdown but also significant biosynthetic activity by the fungal mycelium. As a result of these compositional changes, the digestibility of the final product improved considerably, reaching values between 62% and 75%, a notable enhancement compared to the raw lignocellulosic material. Thermal post-processing of the fungal biomass at temperatures not exceeding 40°C ensured the retention of heat-sensitive bioactive compounds. These include a suite of beneficial metabolites synthesized by *Pleurotus ostreatus* during substrate colonization and fruiting, such as polysaccharides (notably β-glucans), organic acids, hydrolases (e.g., cellulases, proteases), and oxidative enzymes (e.g., laccases, peroxidases). The persistence of these compounds post-drying suggests that the biological functionality of the product is largely preserved, reinforcing its suitability as a high-quality feed supplement with potential immunomodulatory and digestive benefits for livestock.

Further investigations are currently underway to assess the utility of this enriched biomass as a feed additive under controlled animal nutrition trials. Preliminary data suggest improved feed intake and growth performance in test groups, supporting the hypothesis of enhanced bioavailability and palatability. In parallel, exploratory research into the feasibility of producing a food-grade mycological product has also yielded promising insights. All tested lignocellulosic waste substrates demonstrated varying levels of suitability for supporting *Pleurotus ostreatus* fruiting body development. However, they differed significantly in key performance metrics, including the latency period to initial fruiting, cumulative yield and yield distribution across successive fruiting waves, as documented in Table 4. These variations underline the influence of substrate composition on fungal productivity and emphasize the need for substrate-specific cultivation protocols when scaling up for commercial applications.

Notably, among the tested lignocellulosic substrates, wheat straw and sunflower seed husks demonstrated superior performance in terms of overall fungal productivity and the rapidity of bioconversion process completion. These substrates not only supported earlier onset of fruiting body formation but also yielded a higher cumulative

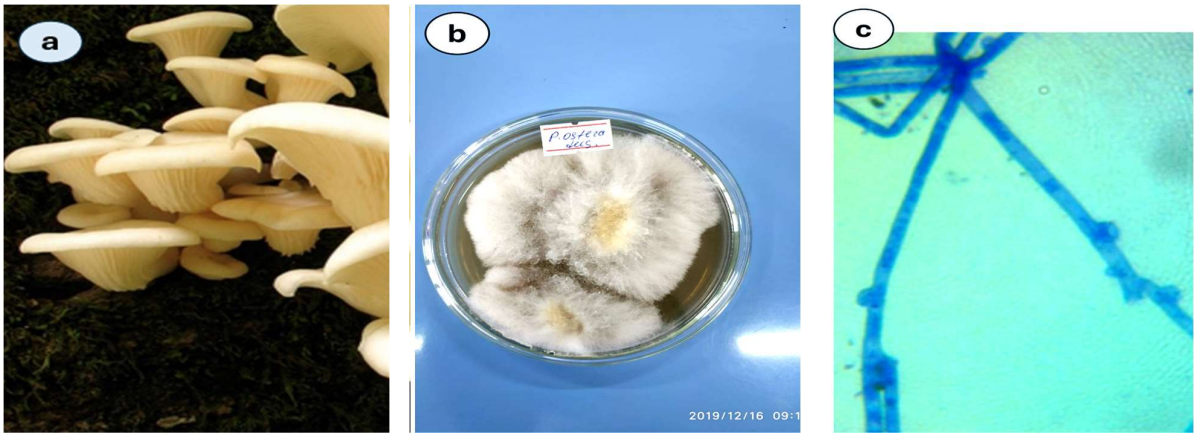

Fig. 2: Morphological characteristics of *P. ostreatus* MBI-2022. (a) Fruiting body morphology; (b) Colony structure on solid-state fermentation substrate; (c) Mycelial network under light microscopy (scale bar = 50 µm). Representative images from three independent experiments (n = 3).

Table 3: Key parameters governing the conditions necessary for the direct bioconversion of waste by the fungus *P. ostreatus* MBI-2022

Substrate moisture (%)	Cultivation temperature	pH (initial)	Method and duration of inoculum preparation	Cultivation time (hours)
59.2-64.5	28 °C	6.5	7-day biomass of fungi in a nutrient medium containing 1 % of the relevant waste under deep cultivation conditions	240

Table 4: Direct conversion of waste to food products using *P. ostreatus* MBI-2022 fungi

Substrate	Duration of the first and ultimate (I/III) wave of fruiting body formation. days	Total mass of fruiting body produced. kg	Distribution of the fruiting body in waves.%		
			I	II	III
Cotton stalks	32/49	2.76	50	30	20
Corn husk	28/43	2.87	60	29	11
Barley straw	29/42	3.01	62	30	8
Wheat straw	27/40	3.12	68	26	6
Sunflower seed husk	24/37	3.54	72	20	8
Sugar beet waste	27/39	2.89	61	29	10

biomass across all fruiting cycles. In contrast, cotton waste exhibited the lowest mycological performance metrics, characterized by prolonged colonization periods and reduced total yield. The other tested substrates, including corn husks, barley straw, and sugar beet waste, displayed intermediate efficacy, with moderate yields and standard cultivation durations. Compositional analysis of the harvested *Pleurotus ostreatus* fruiting bodies confirmed their high nutritional quality and safety for consumption. The protein content ranged between 25.4 % and 31.6 %, positioning the fruiting bodies as a viable source of plant-based protein. Polysaccharide fractions comprised 56.7% to 65.4% of the dry mass, which is particularly significant due to the known immunomodulatory and antioxidant activities of fungal β-glucans. Fat content was relatively low (1.2–3.5%), aligning with dietary recommendations, while ash content, an indicator of mineral composition, ranged from 5.6% to 7.1%. Importantly, no toxic metabolites or heavy metals were detected in any of the analyzed samples, affirming the suitability of the product for both food and feed applications.

An additional critical component of the circular bioconversion approach presented in this study lies in the valorization of residual biomass post-harvest. After the third fruiting wave, the remaining biomass consisting of the spent substrate colonized by fungal mycelium was subjected to drying, sterilization, and reused as a nutrient-rich medium for secondary fermentation. This medium was inoculated with *Trichoderma citrinoviride* AEF-2024 and *Trichoderma harzianum* AEF-2024, fungal strains isolated from fruit and berry plants and taxonomically confirmed via molecular-genetic techniques (Muradov et al., 2009). Cultivation of these strains under solid-state fermentation conditions for five days yielded a biologically active preparation. Following lyophilization, the product was field-tested in open plots cultivated with tomato and cucumber crops. Application of the biopreparation at a rate of 1 kg (dry weight) per hectare led to a 20% reduction in the incidence of fungal and bacterial phyto-pathologies, in comparison to untreated controls. Moreover, crop yield increased by up to 12%, and notable improvements in plant vigor were observed. When applied during tomato seedling production, the preparation contributed to enhanced seedling development: morphometric parameters such as stem height, leaf number, and biomass increased by 17% compared to the current standard practices, as observed 15 days post-application (Fig. 3). These findings underscore the holistic nature of the bioconversion platform described herein, wherein both the primary fungal biomass and the post-cultivation residue are converted into high-value products. This closed-loop approach not only minimizes waste generation but also aligns with sustainable agricultural and circular bioeconomy paradigms. Future studies are warranted to optimize field-level application protocols, assess long-term ecological impacts, and explore potential scalability of the integrated biotechnological system.

Furthermore, specific residual substrates, particularly sunflower husks and corn cobs, remaining after the harvest of fungal fruiting bodies, have demonstrated favorable physicochemical and nutritional properties that render them suitable candidates for inclusion in poultry feed formulations. Preliminary compositional analyses revealed

an increase in digestible protein fractions, as well as the retention of bioactive fungal metabolites known to enhance gut health and immunity in poultry. These residues are currently undergoing systematic evaluation through in vivo feeding trials, aimed at assessing their digestibility, palatability, and potential effects on growth performance, feed conversion efficiency, and overall health status of poultry. The integration of such fungal-enriched agricultural residues into animal husbandry practices represents a promising strategy for promoting sustainable feed alternatives, reducing dependence on conventional feed resources, and contributing to waste minimization within the agricultural sector.

Fig. 3: Effect of residual substrate-derived material on tomato plant development. (a) Control group (no amendment); (b) Application of residual material (rate: 1 g kg^{-1} substrate). Photographs taken at 30 days post-planting; scale bars = 10 cm. Plant height and biomass data (mean±SD, n = 10) were compared using Student's t-test; different letters above bars indicate significant differences ($P<0.05$).

DISCUSSION

The present study provides compelling evidence for the promising role of renewable plant waste (RPW) as a feedstock for bioconversion into high-value products. Empirical analyses revealed that all examined agricultural residues; wheat straw, barley straw, cotton stalks, corn cobs, sunflower husks, and sugar beet waste—are rich in cellulose and lignin, key structural polymers fundamental to bioconversion processes. Despite quantitative variations, these residues consistently demonstrated sufficient lignocellulosic content to serve as robust substrates for microbial degradation and transformation. This finding is consistent with prior reports highlighting the abundant cellulose and lignin content in agricultural waste, which underpins their use in bioenergy, animal feed, and bioproduct applications (Bomble et al., 2017).

The use of standard analytical methods, including the Kürschner and Komarov-modified sulfuric acid assays, provided precise quantification of these polymers and established a solid foundation for evaluating the potential of RPW in biotechnological applications. However, as recognized in the literature, the inherent low reactivity of untreated lignocellulosic biomass poses a critical limitation to its direct bioconversion. This limitation stems from the complex, cross-linked molecular framework formed by cellulose, lignin, and hemicellulose, which confers resistance to enzymatic and microbial attack (Muradov et al., 2009; Akhundova et al., 2019). Therefore, pretreatment steps are indispensable to increase substrate accessibility and maximize downstream degradation efficiency. In this study, the application of a cost-effective physical pretreatment—grinding to a particle size of 0.2–0.5cm proved instrumental in disrupting the lignocellulosic matrix. This approach aligns with previous findings that mechanical pretreatment enhances the surface area and porosity of biomass, thus improving enzyme penetration and microbial colonization (Fu et al., 2021). By combining physical disruption with fungal inoculation under solid-state fermentation (SSF), the study established a practical strategy for advancing RPW bioconversion while maintaining economic feasibility.

The selection of fungal agents played a pivotal role in determining the bioconversion efficiency of the different waste materials. Fungi, particularly white-rot species such as *Pleurotus ostreatus*, are renowned for their broad enzymatic repertoire, which includes both hydrolytic and oxidative enzymes. The experimental results revealed significant inter-strain variability in degradation patterns and bioproduct yields. *Pleurotus ostreatus* emerged as an especially effective degrader, capable of simultaneously breaking down cellulose and lignin, thus achieving substantial weight loss and compositional improvement in the treated substrates. This dual capacity is attributed to its secretion of lignin peroxidases, manganese peroxidases, laccases, and cellulases, which together facilitate comprehensive polymer degradation (Fu et al., 2021).

By contrast, *Trichoderma harzianum*, although efficient in cellulose degradation, displayed limited ligninolytic activity. This enzymatic specialization resulted in selective polymer breakdown, highlighting the need for strain-specific or even consortia-based strategies to achieve optimal substrate utilization. The three-category classification adopted in this study—based on the preferential degradation of cellulose, lignin, or both-provided a useful framework for characterizing fungal performance. This approach aligns with prior research categorizing fungi by their xylolysis coefficient (Jc) and provides a practical tool for strain selection depending on the desired application (Zeng et al., 2017; Andlar et al., 2018; Bakhshaliyeva et al., 2021). Importantly, the discussion extends beyond polymer degradation to address the nutrient enrichment outcomes of bioconversion. The observed increase in protein content, particularly with *Lentinus tigrinus*, underscores the added value of fungal treatment in producing nutritionally fortified feedstocks. Protein enrichment, driven by fungal biomass accumulation and nitrogen liberation from lignin depolymerization, has

important implications for animal nutrition, especially in the context of developing sustainable and cost-effective feed alternatives.

The study also sheds light on the broader metabolic potential of white-rot fungi. Apart from their enzymatic roles in biomass degradation, species like *Pleurotus ostreatus* are prolific producers of secondary metabolites, including β-glucans, terpenoids and phenolic compounds, many of which exhibit immunomodulatory, antioxidant, and antimicrobial activities. This dual-functionality positions *Pleurotus ostreatus* as an attractive candidate for integrated biorefinery models targeting both nutritional and pharmaceutical markets. Optimization of bioconversion parameters using *Pleurotus ostreatus MBI-2022*—a locally sourced strain—was a key strength of this research. The strain's capacity to reduce cellulose and lignin by up to 40 %, coupled with a marked increase in digestibility and protein content, highlights its utility in sustainable agricultural applications. Importantly, the retention of heat-sensitive bioactive compounds post-processing reinforces the potential for the development of functional feed and food products. Preliminary animal trials further support the hypothesis that fungal-enriched biomass improves palatability and growth performance, although larger-scale validation is warranted.

A particularly innovative aspect of the study is the valorization of post-harvest residual biomass. By repurposing spent substrate as a growth medium for *Trichoderma* species, the study demonstrated the feasibility of a circular bioconversion model that extends value generation across multiple cycles. Field trials on tomato and cucumber crops demonstrated both plant health benefits and yield improvements, highlighting the agronomic potential of these fungal-derived biofertilizers. This approach exemplifies a holistic, closed-loop system aligned with circular economy principles and sustainable development goals (SDGs) related to responsible production, climate action, and food security (Nazir et al., 2024). In addition, the study's investigation of the nutritional suitability of residual substrates for poultry feed applications is particularly relevant in light of global efforts to reduce dependence on conventional protein sources. Initial findings suggest that fungal-enriched residues, notably from sunflower husks and corn cobs, could serve as digestible, protein-rich components in poultry diets, although rigorous in vivo trials are needed to confirm their efficacy and safety.

Overall, the findings underscore the critical importance of strategic fungal strain selection, substrate-specific optimization and integrative process design in advancing RPW bioconversion technologies. While challenges remain—including improving scalability, reducing processing costs, and addressing regulatory hurdles—this study makes a strong case for the broader adoption of fungal-based bioconversion as an eco-friendly and economically viable waste valorization strategy. Future research directions should focus on exploring fungal consortia, applying omics technologies (genomics, transcriptomics, and proteomics) to unravel the metabolic pathways underpinning degradation, and conducting comprehensive life cycle assessments to quantify environmental impacts. Furthermore, developing global fungal strain repositories and fostering public-private partnerships will be essential to translate laboratory-scale innovations into commercially viable solutions that support global bioeconomy goals. Our findings support SSF with P. ostreatus MBI-2022 as a practical route to upgrade RPW to edible mushrooms and nutrient-enriched biomass, while enabling circular reuse of spent substrates via Trichoderma-based biopreparations. Inter-strain variability in cellulose vs lignin degradation underscores the need for substrate- and goal-specific strain selection, or consortia. Strengths include multi-substrate comparison, standardized analytics, and preliminary field validation. Limitations include single-season field trials, absence of life-cycle assessment, and limited BE reporting for some substrates. Future work should incorporate multi-location trials, LCA, omics to optimize enzyme systems, and rigorous feed trials (palatability, health markers).

Conclusion

The present study confirms that agricultural plant residues—such as wheat and barley straw, cotton stalks, sunflower husks, sugar beet pulp, and corn cobs—can be effectively bioconverted into high-value food and feed products through fungal fermentation using the *Pleurotus ostreatus* MBI-2022 strain. This biotechnological approach not only offers a sustainable strategy for the valorization of lignocellulosic waste but also contributes to resolving critical global issues, including ecological degradation and protein malnutrition. SSF using locally isolated P. ostreatus (MBI-2022) upgrades agricultural residues into edible mushrooms and protein-enriched biomass, while enabling residual substrate valorization via Trichoderma-based biopreparation. By incorporating non-conventional and renewable raw materials into bioeconomic cycles, this method expands the raw material base for the biotechnology and agricultural industries while simultaneously reducing environmental burden. Furthermore, the integration of fungal strains *Trichoderma citrinoviride* AEF-2024 and *T. harzianum* AEF-2024 into the process enhances the utility of post-cultivation residual biomass, broadening the application spectrum of the resulting bio-products in crop protection and productivity enhancement.

Key Findings

1. Improved Nutritional Profile and Digestibility
The bioprocessing of lignocellulosic plant waste via solid-state fermentation with *P. ostreatus MBI-2022* significantly improved substrate digestibility and nutrient content. The resultant biomass is rich in protein and bioactive compounds, offering a promising, sustainable alternative for use in livestock feed formulations.
2. Agronomic Benefits of Residual Biomass Applications
A five-day cultivation of *T. citrinoviride AEF-2024* and *T. harzianum AEF-2024* on the post-fruiting biomass yielded a composition suitable for bioformulation application in vegetable crop protection (tomatoes and cucumbers). Field trials demonstrated a 20% reduction in disease prevalence,

a 17% increase in plant morphometric parameters, and an up to 12 % increase in crop yield compared to control treatments.

3. Poultry Feed Potential of Post-Cultivation Residues The residual fungal biomass from mushroom cultivation on sunflower husks and corn cobs retains significant nutritive value, confirming its viability as a feed supplement in poultry diets. Ongoing trials are currently evaluating its effects on poultry growth performance, immunity, and feed efficiency.

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Data availability: Datasets and analysis scripts are available from the corresponding author upon reasonable request.

Ethics Statement: This study did not involve human participants or animal testing. All experimental procedures involving microorganisms were conducted in accordance with institutional biosafety and ethical guidelines. The authors affirm that the research complies with national and international standards for ethical conduct in scientific research.

Author's Contribution: Bakhshaliyeva K.F.& Jafarzadeh S.A. – Experimental design, methodology development, and data analysis. Musayeva V.V. & Khonagova Sh.B. – Investigation, solid-state fermentation experiments, and data curation. Bunyatova L.N. – Statistical analysis, interpretation of results, and critical review of the manuscript. Iskender E.O. – Support in microbiological assays and validation of fungal strains. Muradov P.Z. – Data verification, resources management, and laboratory coordination. Amirova M.F. – Editing, language review, and quality assurance of the final manuscript. All authors have read and approved the final version of the manuscript.

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