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Sustainable Bioconversion Of Lignocellulosic Wastes Into High-Value Mycofertilizer By A White Rot Fungal Consortium: Enhancing Growth And Yield Of Triticum Aestivum

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Abstract
India generates approximately 998 million metric tonnes of lignocellulosic waste each year, which poses serious environmental challenges if left untreated or mismanaged. However, this vast quantity of agricultural and agro-industrial residue offers a valuable opportunity for sustainable bioconversion into value-added products. In this study, a mycofertilizer was developed using a consortium of three white rot fungi-Trametes hirsuta, Ganoderma gibbosum, and Ganoderma multipileum grown on cost-effective lignocellulosic substrates, including leaf litter, paddy straw, sugarcane bagasse, corn cobs, and corn stover. Four different formulations (F1–F4) were prepared by varying the proportions of these substrates and subjected to fungal degradation to select the most efficient composition. Among these, Formulation no. F2 proved to be optimal, achieving complete substrate degradation and producing a nutrient-rich mycofertilizer. Compositional analysis of the final product revealed N-P-K contents of 1.6–1.1–1.4% and a C:N ratio of 17.51, indicating its suitability for enhancing soil fertility and supporting plant growth. To assess its agronomic potential, field trials were conducted on Triticum aestivum (wheat), a widely cultivated crop of significant economic and nutritional value. Results showed that wheat plants treated with the mycofertilizer exhibited significantly higher yields compared to those treated with conventional fertilizers such as vermicompost. In contrast, plants grown in unfertilized soil demonstrated the slowest growth rates and the lowest yields, underscoring the importance of soil amendments for optimal crop productivity. This study highlights the promising potential of white rot fungal consortia for the bioconversion of lignocellulosic waste into an eco-friendly, cost-effective mycofertilizer that supports sustainable agriculture.
Keywords: *Eco-Friendly, Lignocellulosic Substrate, Mycofertilizer, Triticum aestivum, White Rot Fungal Consortium*

1. INTRODUCTION

Lignocellulosic biomass, composed primarily of lignin, cellulose, and hemicellulose, represents one of the most abundant renewable resources on Earth [1]. Major sources include trees, grasses, and agricultural residues, with an estimated global production of 181.5 billion tonnes annually. Yet, only about 8.2 billion tonnes are effectively utilized [2, 3]. In India, agriculture, forestry, and agro-industrial activities generate nearly 998 million tonnes of lignocellulosic biomass every year [4]. Despite its immense potential, a large proportion remains underutilized or is simply burned, contributing significantly to air pollution-ranking third globally after industrial and vehicular emissions[5, 6, 7]. The open-field burning of leaf litter and paddy straw is especially detrimental, releasing toxic pollutants into the atmosphere [8, 9].

Sustainable strategies for biomass valorization are, therefore, urgently required. Microbial bioconversion provides a promising pathway, transforming lignocellulosic residues into high-value products such as bioethanol, biofertilizers, biosorbents, and eco-friendly biomaterials [10,11]. White rot fungi (WRF), equipped with highly efficient lignocellulolytic enzyme systems, stand out as some of the most effective microorganisms for this purpose. They have been successfully employed in diverse applications, including biopulping, forage enrichment, and bioremediation [12-18]. The present study emphasizes three key species-*Trametes hirsuta*, *Ganoderma gibbosum*, and *Ganoderma multipileum*, selected for their potent enzymatic capabilities in degrading complex, lignin-rich substrates. *T. hirsuta* is particularly effective in producing lignin-degrading enzymes such as laccases and manganese peroxidases, whereas G. *gibbosum* and G. *multipileum* excel in cellulose and hemicellulose breakdown, thereby enhancing nutrient availability and stimulating microbial activity in soils. When combined in a fungal consortium, their synergistic action markedly accelerates biomass decomposition, leading to the generation of nutrient-rich, eco-friendly mycofertilizers.

Environmental sustainability constitutes a central motivation for this work. Excessive reliance on chemical fertilizers has resulted in soil degradation, nitrate leaching, and biodiversity loss [19]. Mycofertilizers, derived

from fungal bioconversion processes, offer a sustainable alternative capable of restoring soil health and promoting eco-friendly agricultural practices. By valorizing agro-industrial residues as growth substrates, this approach simultaneously addresses waste management and reduces the carbon footprint associated with synthetic fertilizer production [20]. Moreover, mycofertilizers enhance soil organic matter, support beneficial microbial communities, and improve nutrient cycling [21].

Economic viability is another significant advantage. The rising costs of conventional fertilizers, driven by resource depletion and energy-intensive manufacturing processes, place increasing pressure on smallholder farmers [22]. Mycofertilizers, produced from inexpensive lignocellulosic residues, provide an affordable and sustainable alternative. The use of multi-species fungal consortia enhances enzymatic efficiency, yielding biofertilizers with superior nutrient profiles and soil-conditioning properties [23]. Multi-strain inoculation has consistently outperformed single-species applications due to the complementary and synergistic interactions among fungal enzymes [24].

This study investigates the potential of white rot fungal consortia for the cost-effective production of mycelia-based mycofertilizers and evaluates their application to *Triticum aestivum* (wheat), one of India's most important staple crops. With the global population continuing to rise, achieving sustainable increases in food production is imperative [25]. Mycofertilizers contribute to food security by enhancing soil fertility, reducing dependency on synthetic inputs, and supporting the growth of organic farming systems [21, 26]. Although research on biofertilizers is extensive, relatively few studies have focused on the application of white rot fungal consortia for lignocellulosic biomass valorization [27]. This research therefore advances current understanding of fungal-mediated biodegradation and contributes to the development of scalable, sustainable, and economically feasible mycofertilizer technologies that benefit farmers, agronomists, and environmental stakeholders. The present investigation specifically assesses the impact of such mycofertilizers on wheat cultivation, a critical crop for India's food and economic security.

2. MATERIALS & METHODS

Procurement of Fungal Samples

White rot fungal isolates were obtained from the Department of Microbiology, Panjab University, Chandigarh. The cultures were maintained on Potato Dextrose Agar (PDA) under standard aseptic conditions to ensure purity and long-term viability.

Preparation of Inoculum

The fungal inoculum was prepared in the form of spawn for subsequent inoculation of lignocellulosic substrates. Sorghum grains were used as the carrier medium. The grains were first soaked in water, boiled for 30 minutes, and air-dried for one hour. To enhance texture and prevent clumping, 0.5% calcium carbonate ($CaCO_3$) and 0.5% calcium sulfate ($CaSO_4$) were added. The water-to-grain ratio was standardized at 1:2 (12.5 mL water per 25 g of grains). Approximately 25 g of prepared grains were dispensed into 250 mL Erlenmeyer flasks and sterilized by autoclaving at 121°C (15 psi) for 15 minutes. After cooling to room temperature, each flask was aseptically inoculated with five mycelial discs from actively growing pure cultures. The inoculated flasks were incubated at 28°C for seven days to ensure complete colonization of the grains by fungal mycelium [28].

Preparation of Substrate Formulations

Lignocellulosic substrates including paddy straw, sugarcane bagasse, corn cobs, corn stalks, and rice husk were processed for formulation development. The substrates were chopped into uniform fragments (10–20 mm) using a hammer mill. To eliminate contaminants, they were treated with 4% sodium hypochlorite solution, thoroughly rinsed with boiling water to remove residual chemicals, and sterilized by autoclaving at 121°C (15 psi) for 15 minutes. Four different substrate formulations, each weighing 100 g, were prepared by combining these components in varying proportions:

- **Formulation 1 (F1):** Equal quantities of leaf litter, paddy straw, sugarcane bagasse, corn cob, corn stalk, and rice husk, each weighing 16.66 g.
- **Formulation 2 (F2):** 45 g leaf litter, 25 g paddy straw, 10 g sugarcane bagasse, 5 g corn cob, 10 g corn stalk, and 5 g rice husk.
- **Formulation 3 (F3):** 30 g leaf litter, 40 g paddy straw, 10 g sugarcane bagasse, 10 g corn cob, 5 g corn stalk, and 5 g rice husk.
- **Formulation 4 (F4):** 55 g leaf litter, 13 g paddy straw, 12 g sugarcane bagasse, 4 g corn cob, 10 g corn stalk, and 6 g rice husk.

These substrate formulations were designed to assess fungal degradation efficiency and identify the optimal composition for mycofertilizer production. Moisture content was adjusted to 75% prior to sterilization. After autoclaving and cooling, each formulation was inoculated with 5% (w/w) fungal spawn and incubated at 28°C for 14 days to allow complete colonization. Biodegradability was then evaluated by analyzing lignin, cellulose, and hemicellulose degradation, substrate weight loss, carbohydrate and reducing sugar content, and lignocellulolytic enzyme activity. The formulation exhibiting maximum degradation efficiency was selected for detailed characterization and subsequently tested as a mycofertilizer on *Triticum aestivum*.

Substrate Biodegradability Testing

The substrate formulations were designed to evaluate the efficiency of fungal degradation and to identify the optimal composition for mycofertilizer production. The moisture content of each formulation was adjusted to 75% prior to sterilization. After autoclaving, the substrates were cooled to room temperature, inoculated with 5% (w/w) fungal consortium spawn, and incubated at 28°C for 14 days to ensure complete colonization. Following colonization, biodegradability was assessed by evaluating the percentage degradation of lignin, cellulose, and hemicellulose, measuring weight loss, estimating carbohydrate and reducing sugar content, and analyzing the activity of lignocellulolytic enzymes. The formulation demonstrating the highest degradation efficiency was selected for further characterization and subsequent evaluation as a mycofertilizer on *Triticum aestivum*.

Determination of Enzyme Activity

Enzyme Extraction: Enzyme extraction was carried out from colonized lignocellulosic substrates after the incubation period. Mycelial biomass was macerated, and enzymes were extracted using buffers of different pH values: citrate buffer (pH 4.8), sodium acetate buffer (pH 5.0), phosphate buffer (pH 6.0), and distilled water (pH 7.0). For each extraction, 5 g of fermented biomass was mixed with 30 mL buffer and agitated at 120 rpm for 10 minutes. The suspension was centrifuged at 6000 rpm for 10 minutes at 4±1°C, and the resulting supernatant was collected as the crude enzyme extract [29]. Enzyme activity was determined using standard assays.

Enzyme Assays: The biodegradability of the formulations was assessed through the activity of key lignocellulolytic enzymes:

- **Laccase:** Determined spectrophotometrically by guaiacol oxidation at 420 nm for 10 minutes [30].
- **Xylanase:** Measured as the release of reducing sugars from 0.5% (w/v) xylan using the DNSA method [31].
- **Mannanase:** Evaluated with 0.5% guar gum in 0.1 M citrate buffer (pH 5.6) [32].
- **Cellulase:** Determined using the DNSA method with 1% CMC as substrate [29].

Estimation of Carbohydrate and Reducing Sugar:

- **Total Carbohydrates:** Quantified using the phenol-sulfuric acid method [33] before and after colonization to determine fungal degradation efficiency.
- **Reducing Sugars:** Estimated by the DNSA method [32].

Estimation of Lignocellulosic Components:

- **Lignin:** Lignin content was determined following [34]. One gram of extractive-free dried substrate was treated with 72% (v/v) H_2SO_4 for 2 hours, diluted, hydrolyzed, filtered, washed, dried, and weighed. Lignin was expressed as a percentage of dry weight.
- **Cellulose:** Cellulose content was determined using the [35] protocol. The sample was treated with acetic-nitric reagent, hydrolyzed with H_2SO_4, reacted with Anthrone's reagent, boiled, cooled, and absorbance was measured at 630 nm.
- **Hemicellulose:** Hemicellulose content was estimated from holocellulose prepared with sodium chlorite and acetic acid, followed by extraction with NaOH [35].
- **Pectin:** Pectin was extracted at pH 1.5-2.6 with HCl at 90°C, precipitated with ethanol, washed, dried, and weighed [36].

$$\% \text{ yield} = \frac{wi\ of\ compound\ extracted\ after\ drying}{wt\ of\ specimen\ (5g)} \times 100$$

Percent Degradation of Lignocellulosic Components: The percent degradation of different lignocellulosic components (lignin, cellulose, hemicellulose & pectin) of non-degraded and fungal degraded lignocellulose substrate was calculated using following equation as per [37]:

Degradation (%) = [(a−b)/a] ×100,

Where, 'a' is percentage of non-degraded lignocellulosic components & 'b' is percentage of fungal degraded lignocellulosic components

Percent Loss in Biomass and Bulk Density

- **Biomass Loss:** Determined after 14 days of incubation using weight differences [38, 39].

% **weight loss** = B_{W1}-B_{W2} /B_{W1}× 100,
Where, BW_1 = weight before degradation and BW_2 = weight after degradation

- **Bulk Density:** Calculated as per [40]:

Density = M /V,
Where, M is mass of lignocellulosic substrate; V is volume of the substrate

Characterization of Biodegraded Substrate

- **FTIR Analysis:** Conducted at the Central Instrumentation Laboratory, Panjab University, Chandigarh, to identify functional group modifications.
- **SEM Analysis:** Surface morphology examined at magnifications of 100X, 250X, and 500X using JSM 6100 (JEOL).
- **TGA:** Thermal stability analyzed up to 800°C.
- **Elemental Analysis:** C, H, N, P, and K determined using a CHNS analyzer.

Evaluation of Biodegraded Substrate as Mycofertilizer on *Triticum aestivum*

- **Soil Preparation**: Soil samples were sterilized at 121°C for 1 h at 15 psi to eliminate native microflora. The CFU count before and after sterilization was determined using the serial dilution and pour plate method [41]:

CFU/ml = no. of colonies x dilution factor / volume of culture plate

- **Plant Growth Assay:** Four treatments were applied to soil (2.5 kg per pot):

C1: Plain soil
C2: Soil + urea (0.5 g)
C3: Soil + vermicompost (50 g)
Test: Soil + biodegraded lignocellulosic substrate (50 g)
Soil salinity, pH, and electrical conductivity were analyzed at Punjab Agricultural University, Ludhiana, India.

Germination and Plant Growth Parameters

- **Germination Rate:** Calculated as:

$$\%\ Germination = \frac{Number\ of\ seeds\ germinated}{Total\ number\ of\ seeds\ sowed}\ x\ 100$$

Plant Growth: Parameters such as height, leaf number, branch number, and leaf size were recorded at regular intervals. Control were set with plain soil (C1), soil with urea (C2) and soil with vermicompost (C3).
Determination of Chlorophyll Content: Chlorophyll content was estimated spectrophotometrically following [42]. One gram of finely cut adult leaf tissue was ground with 20 mL acetone and 0.5 g $MgCO_3$ using a clean pestle and mortar. The extract was refrigerated at 4°C for 4 h, then centrifuged at 5000 rpm for 5 min. The supernatant was made up to 100 mL with 80% acetone, and absorbance was recorded at 645 and 663 nm against 80% acetone as blank.
Chl a= 11.75×$A_{662.6}$ – 2.35×$A_{645.6}$
Chl b=18.61×$A_{645.6}$ – 3.96× $A_{662.6}$
Where, Chl-a and Chl-b are the chlorophyll a and chlorophyll b, A is absorbance
Determination of Carbohydrate and Protein Content: Kernels of *Triticum aestivum* were dried, powdered, and digested in concentrated H_2SO_4.

- **Carbohydrates:** Estimated by phenol-sulfuric acid method [43].
- **Proteins:** Determined by [44].

3. RESULTS

Procurement of Fungal Samples

Three white rot fungi, *Trametes hirsuta* (MTCC 13584), *Ganoderma multipileum*, and *Ganoderma gibbosum* (MTCC 13375), were obtained from the Department of Microbiology, Panjab University, Chandigarh.

Inoculum Preparation

Spawn inocula (5%) were prepared on sorghum grains and employed for the inoculation of lignocellulosic formulations.

Substrate Biodegradability Testing

Four lignocellulosic formulations with varying compositions were assessed for degradability by the fungal consortium, using enzyme activity, carbohydrate release, lignocellulosic composition, weight and density loss, and moisture content as indicators.

Enzyme Activity

Formulation F2 supported the highest enzymatic activity, with laccase (1.45 U/ml), cellulase (0.69 U/ml), mannanase (0.78 U/ml), pectinase (4.03 U/ml), and xylanase (6.40 U/ml). Activities were comparatively lower in F3 (0.36, 0.35, 2.26, 2.35, 4.88 U/ml), F4 (0.13, 0.17, 2.19, 1.45, 6.40 U/ml), and F1 (0.14, 0.25, 0.39, 0.73, 2.16 U/ml). The results highlight F2 as the most degradable substrate, attributable to its favorable composition. Overall, degradation efficiency was driven primarily by pectinase, xylanase, and mannanase activities, with relatively lower contributions from laccase and cellulase.

Table 1. Enzyme activity profile of the fungal consortium during degradation of four lignocellulosic formulations.

Enzyme Activity (U/ml) Enzymes	F1	F2	F3	F4
Laccase	0.14 ± 0.41	1.45 ± 0.32	0.36 ± 0.32	0.13 ± 0.24
Cellulase	0.25 ± 0.32	0.69 ± 0.28	0.35 ± 0.21	0.17± 0.32
Mannanase	0.39 ± 0.76	2.78 ± 0.45	2.26 ± 0.43	2.19 ± 0.12
Pectinase	0.73 ± 0.56	4.03 ± 0.93	2.35 ± 0.56	1.45 ± 0.24
Xylanase	2.16 ± 0.46	6.40 ± 0.56	6.40 ± 0.56	6.40 ± 0.56

Values are mean ± SD of three observations.p <0.05

Enzyme activities were assayed on 14th day of incubation of each formulations (F1, F2, F3 and F4).

Formulation components: paddy straw, leaf litter, sugarcane bagasse, corn cob & rice husk in different quantities with fixed percentage of wheat bran (2%,w/w), glucose (1%, w/w) and yeast extract (0.5%,w/w) in all the four formulations.

Total Carbohydrate Content

Reduction in total carbohydrate content served as an indicator of substrate degradation by the fungal consortium. All formulations exhibited a significant decline compared to their controls, confirming effective biodegradation. The highest reduction was observed in F2 (52.17%; 9.2 → 4.4 mg/ml), followed by F3 (32.81%; 6.4 → 4.3 mg/ml), F4 (28.81%; 5.9 → 4.2 mg/ml), and F1 (11.86%; 5.9 → 5.2 mg/ml). The degradation efficiency thus followed the order: F2 > F3 > F4 > F1 (Fig. 1).

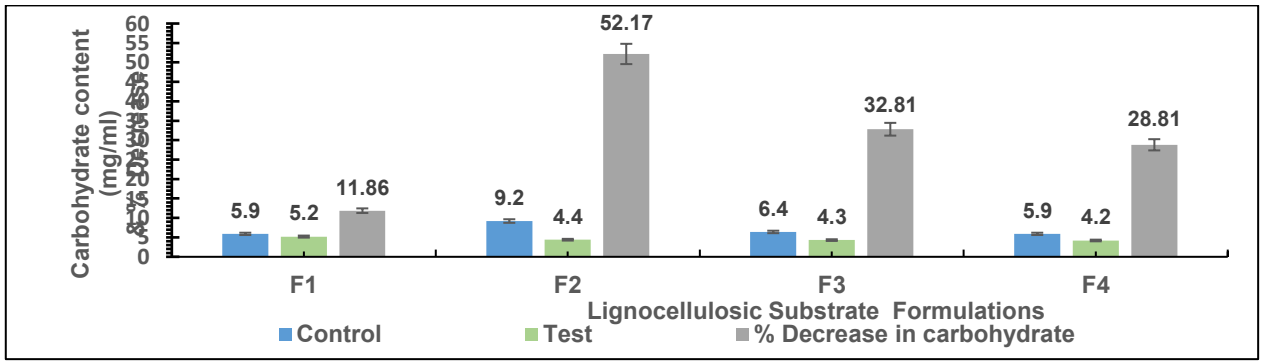

Fig. 1. Total carbohydrate content and percentage reduction in lignocellulosic formulations before (control) and after degradation by the fungal consortium. Values are Mean ± SD of three observations. p < 0.05

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Total Reducing Sugar

An increase in reducing sugar content confirmed lignocellulosic degradation by the fungal consortium. F2 exhibited the highest rise (44.44%; 0.54 → 0.78 mg/ml), followed by F3 (30.61%; 0.34 → 0.49 mg/ml), F4 (21.95%; 0.32 → 0.41 mg/ml), and F1 (13.51%; 0.32 → 0.37 mg/ml). The trend of increase was F1 < F4 < F3 < F2 (Fig. 2).

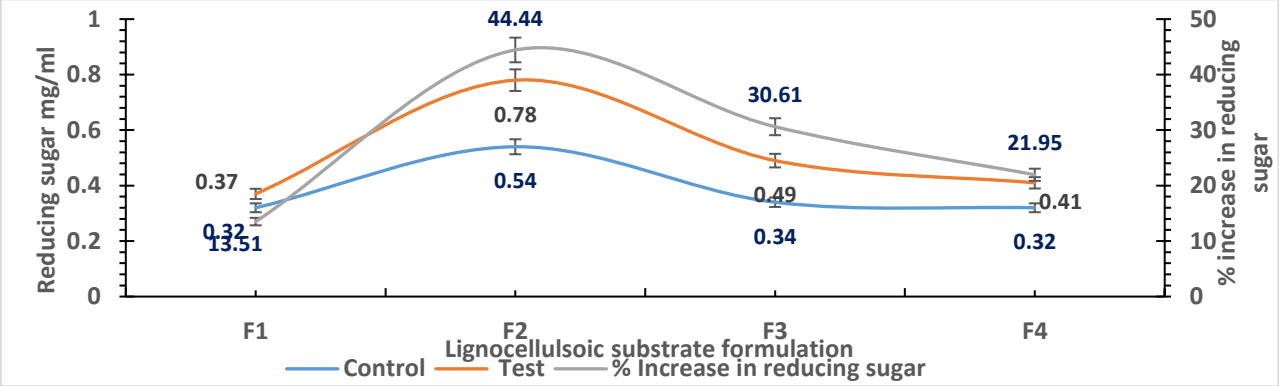

Fig. 2. Reducing sugar content and percentage increase in lignocellulosic formulations before (control) and after degradation by the fungal consortium Values are Mean ± SD of three observations. $p < 0.05$

Lignocellulosic Content

Degradation of lignocellulosic substrates was assessed through reductions in lignin, cellulose, hemicellulose, and pectin in fungal-treated biomass compared to controls (Table 2). Formulation F1 showed moderate decreases (23.80%, 18.97%, 25.0%, and 16.87%, respectively), while F2 exhibited the highest reductions (42.85%, 48.93%, 33.33%, and 45.0%). In F3, the respective decreases were 31.25%, 22.34%, 37.50%, and 42.0%, whereas F4 recorded 33.33%, 25.00%, 28.57%, and 40.0%. Overall, F2 demonstrated the greatest lignocellulosic degradation, underscoring its superior susceptibility to fungal bioconversion.

Table 2. Percent degradation of *lignocellulosic contents of four different lignocellulosic formulations by fungal consortium of white rot group.

Formulation	% Lignin Content		Degradation	% Cellulose Content		Degradation	% Hemicellulose Content		Degradation	% Pectin Content		Degradation
	* Control	* Test	(%)	*Control	*Test	(%)	*Control	*Test	(%)	*Control	* Test	(%)
F1	21.25 ± 1.25	16.0 ± 1.41	24.70 ±1.39	39.0 ± 1.0	31.6 ± 0.5	18.97 ± 0.52	8.0 ± 1.00	6.0 ± 0.18	25.00 ± 0.32	11.2±1.2	8.6±0.83	23±0.71
F2	28.50 ± 0.50	16.4± 1.59	42.45± 2.00	47.0 ± 0.8	24 ± 1.00	48.93 ± 0.78	18 ± 1.77	12 ± 2.0	33.33 ± 0.43	11.0±0.8	6.6±0.71	45±0.43
F3	32.00 ± 2.00	22.2 ± 2.12	31.25± 1.40	37.6 ± 0.5	29.2± 0.75	22.34 ± 0.43	8.0 ± 0.75	5.0 ± 0.95	37.50 ± 0.45	12.8±0.44	7.4±0.51	42±0.21
F4	33.00 ± 1.00	22.0 ± 1.41	33.33 ± 1.40	36.0±1.0	27.0±1.25	25.00 ±0.68	7.0 ± 0.68	5.0 ±1.00	28.57 ± 0.54	9.8±0.69	5.8±0.72	40±0.81

***Control:** Non-degraded lignocellulosic substrate; ***Test:** Degraded lignocellulosic substrate by fungal consortium of white rot group

***Lignocellulosic contents:** lignin, cellulose, hemicellulose & pectin

Values are Mean ± SD of three observations.

$p < 0.05$

Percent Weight and Density Loss

Biomass weight loss and the corresponding decrease in bulk density were measured before and after fungal degradation. As density is directly proportional to mass (D = M/V), reductions in weight resulted in proportional decreases in density. F1 showed 16.87% weight and 17.0% density loss, while F2 recorded the highest reductions (29.04% and 30.4%, respectively). F3 exhibited 22.28% and 23.0% losses, and F4 showed 21.82% and 22.0%

losses. Overall, degradation followed the order: F2 > F3 > F4 > F1, with F2 being the most susceptible formulation.

Characterisation of the Best Lignocellulosic Formulation

Based on maximum weight loss, F2 was identified as the most degradable formulation and was selected for detailed characterization.

Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR analysis of F2 on day 14 revealed clear structural modifications after fungal degradation. A new peak at 1648 cm^{-1} indicated C=C stretching and N–H bending, while peak shifts from 2853 to 2851 cm^{-1} and 2920 to 2918 cm^{-1} suggested perturbations in N–H and O–H stretching. Additional changes included a shift in the fingerprint region (732 → 724 cm^{-1}, C–N bending) and the appearance of new peaks at 1037 and 1082 cm^{-1}, corresponding to S=O and C–O stretching. These spectral alterations confirmed substantial biochemical transformations of lignocellulosic components by the fungal consortium (Fig. 3).

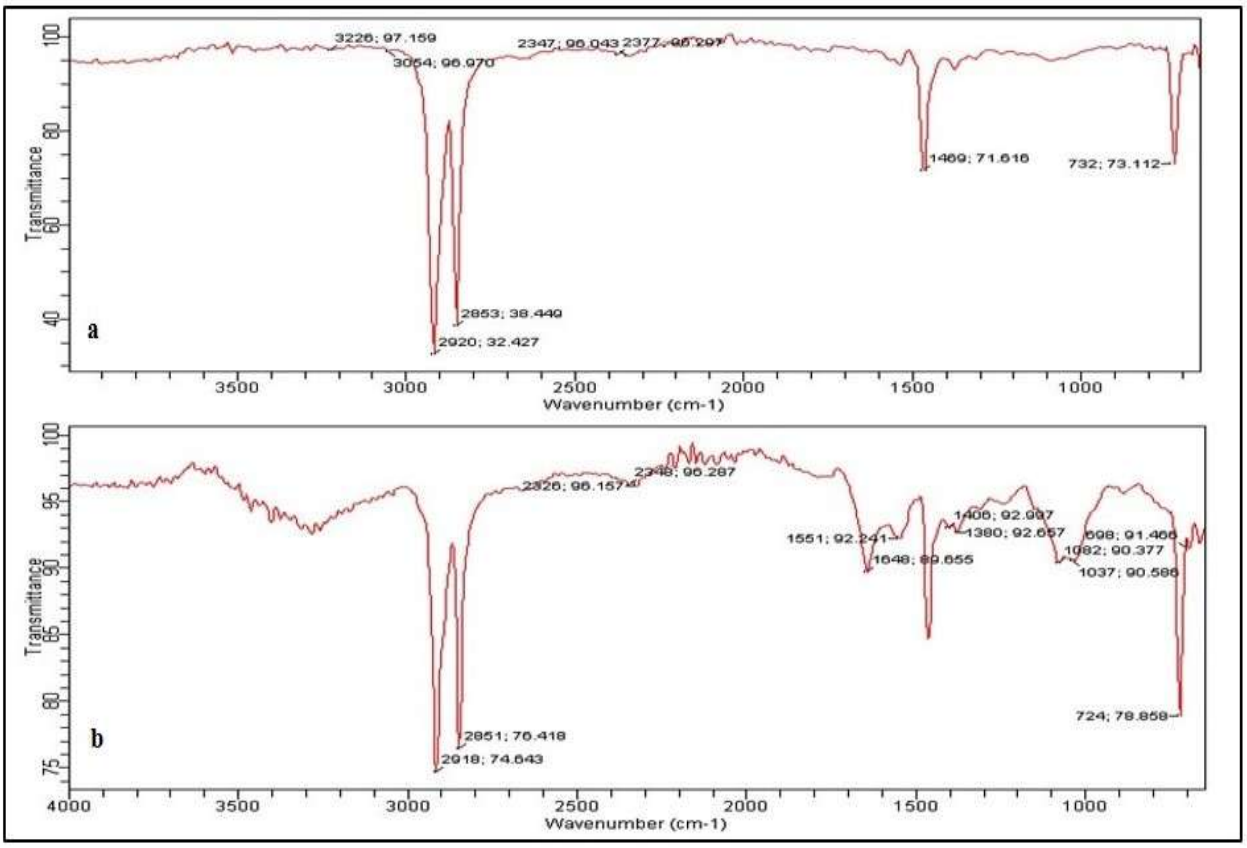

Fig. 3. FT-IR Micrographs of (a) Non-degraded lignocellulosic substrate; (b) Degraded substrate on 14th day of incubation by the fungal consortium.

Scanning electron microscopy (SEM)

SEM analysis of formulation F2 was conducted after 14 days of incubation to evaluate structural alterations induced by the white rot fungal consortium. Imaging at 10 kV and 250× magnification revealed intact surfaces in the control, whereas the degraded sample displayed pronounced structural disruptions, confirming extensive biomass degradation (Fig. 4).

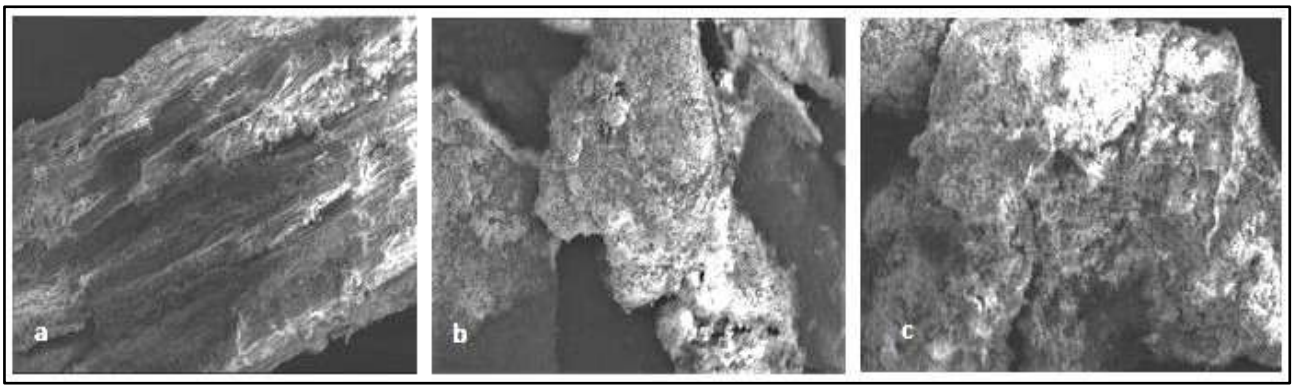

Fig. 4. SEM micrographs of formulation F2: (a) Control showing intact, non-degraded surface; (b) Test sample depicting colonization by the white rot fungal consortium; (c) Structural cracks and tearing of the lignocellulosic substrate after 14 days of incubation.

Thermogravimetric analysis (TGA)

Lignin, cellulose, and hemicellulose contribute to the inherent thermostability of lignocellulosic biomass, but fungal degradation disrupts these polymers, rendering the substrate more thermolabile. TGA revealed a 68% weight loss in the control at 600 °C, compared to 75% in the fungal-degraded sample. The higher weight loss in the test confirms extensive breakdown of polymeric constituents, indicating effective biomass degradation (Fig. 5).

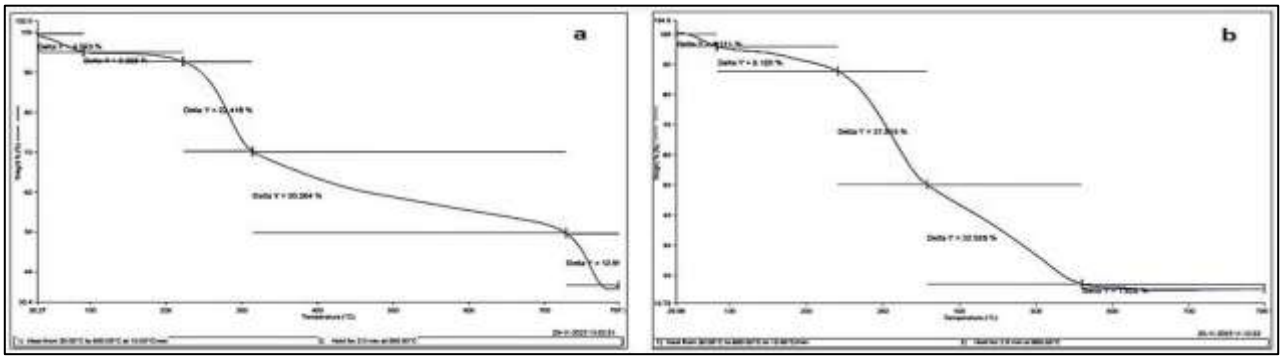

Fig. 5. TGA profiles of (a) control lignocellulosic substrate showing 68% weight loss at 600 °C and (b) fungal-degraded substrate showing 75% loss, indicating enhanced biomass degradation.

C:N Ratio in Biodegraded Lignocellulosic Substrate

CHNS analysis showed a marked reduction in the C:N ratio from 41.65 in the control to 17.51 in the degraded substrate (Fig. 6). A ratio below 20 is considered optimal for plant growth, and the value obtained here is consistent with earlier reports [45,46,47].

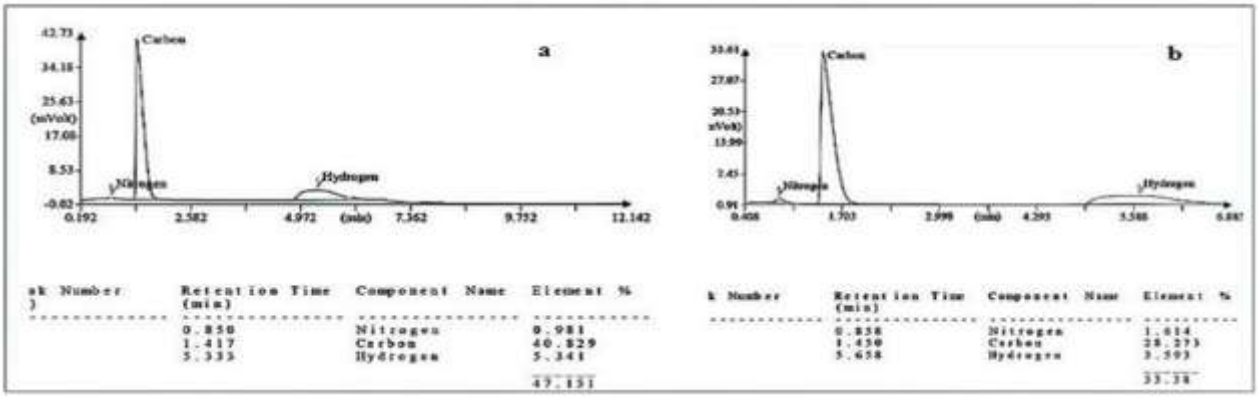

Fig. 6. CHNS analysis of lignocellulosic substrate: (a) Control-Carbon 40%, Nitrogen 0.98%; (b) Test-Carbon 28%, Nitrogen 1.6%.

Macroelements

ICP-MS analysis of the degraded substrate revealed 14,050 ppm potassium (1.4%) and 11,236 ppm phosphorus (1.1%). Together with nitrogen (1.6%), the substrate exhibited an N–P–K composition of 1.6–1.1–1.4%, highlighting its potential as a complete biofertilizer (Table 3).

Table 3. Percent macroelements in Formulation- F2 along with C: N ratio.

Test Sample	Carbon(C)	Nitrogen(N)	Phosphorus (P)	Potassium (K)	Hydrogen
Undegraded Biomass	40±1.78	0.98± 0.45	0.52±0.21	0.38±0.12	5.34± 0.82
C:N Ratio			40.81		
Mycofertilizer	28 ±1.5	1.6±0.8	1.12 ± 0.25	1.4 ± 0.88	3.59±1.2
C:N Ratio			17.51		

Values are Mean± SD of three observations.

Evaluation of Lignocellulosic Formulation F2 as a Mycofertilizer Candidate

The most degradable formulation, F2, was assessed for its potential as a mycofertilizer using *Triticum aestivum*. Seedlings were raised in pots (20 × 22 cm) containing 3 kg soil amended with 50 g finely ground F2 (Test). Two controls were maintained: C1 (soil only) and C2 (soil + 50 g vermicompost). Eight seeds were sown per pot, with germination monitored daily and growth parameters (plant height, leaf number, and yield) recorded every 20 days for 140 days.

Soil Quality Indicators

Prior to sowing, soil pH and electrical conductivity (EC) were analyzed to assess acidity, salinity, and nutrient status. EC values were 0.59 m mhos/cm in control soil (C1), 0.54 m mhos/cm in vermicompost-amended soil (C2), and 0.46 m mhos/cm in mycofertilizer-blended soil (Test), indicating the lowest salinity in the Test, favorable for plant growth. Since EC values below 2 are considered optimal (Hassani et al., 2021), the salinity order was Test > C2 > C1. pH values of 7.6 (C1), 7.4 (C2), and 7.8 (Test) all fell within the optimal range (5–8) for *Triticum aestivum* growth [48].

Soil Preparation

Soil microbial load was quantified using the serial dilution method. Raw soil contained 4×10^5 bacterial CFU/g and 3×10^4 fungal CFU/g (oven-dried basis). After sterilization for two hours, microbial counts dropped to zero, and this sterilized soil was used for plant experiments.

Percent Seed Germination

Seed germination varied significantly among treatments. Mycofertilizer-amended soil (Test) showed the highest germination (80%), followed by C2 (vermicompost, 50%) and C1 (plain soil, 30%).

Plant Height

Plant height was recorded at 20-day intervals for 140 days. Wheat plants in the Test soil attained a maximum height of 70 cm, compared to 60 cm in C2 and 48 cm in C1, establishing the growth order as Test > C2 > C1 (Fig. 7).

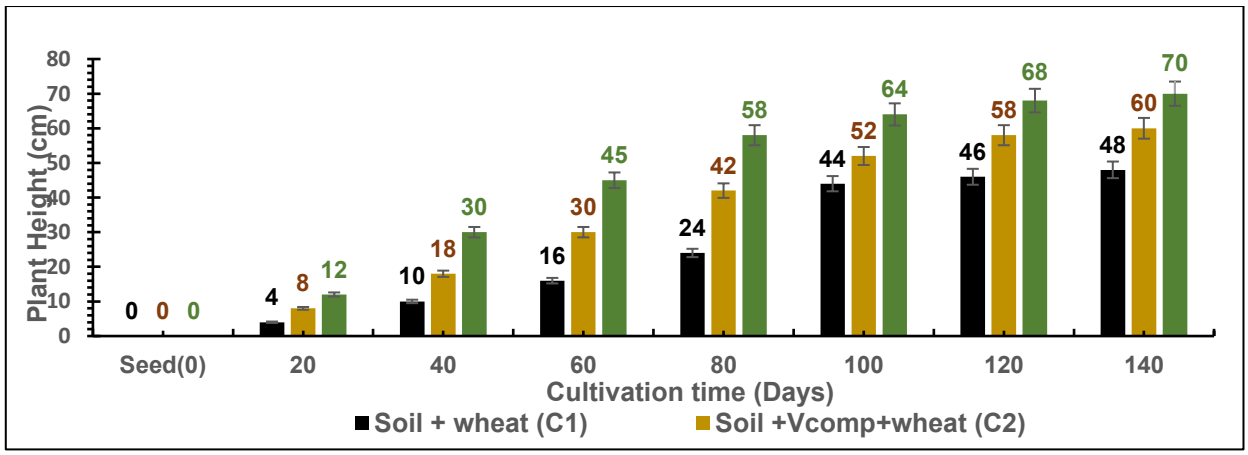

Fig. 7. Comparative height growth pattern of *Triticum aestivum* plants grown in the test mycofertilizer and in control (C1, C2) Values are Mean ± SD of three observations. P<0.05

Leaf Count

Leaf numbers were recorded at 20-day intervals for 140 days. Plants in mycofertilizer-amended soil (Test) produced the highest average leaf count (14), followed by vermicompost-amended soil (C2) with 11 leaves and plain soil (C1) with 7 leaves. The overall trend in leaf production was Test > C2 > C1 (Fig. 8).

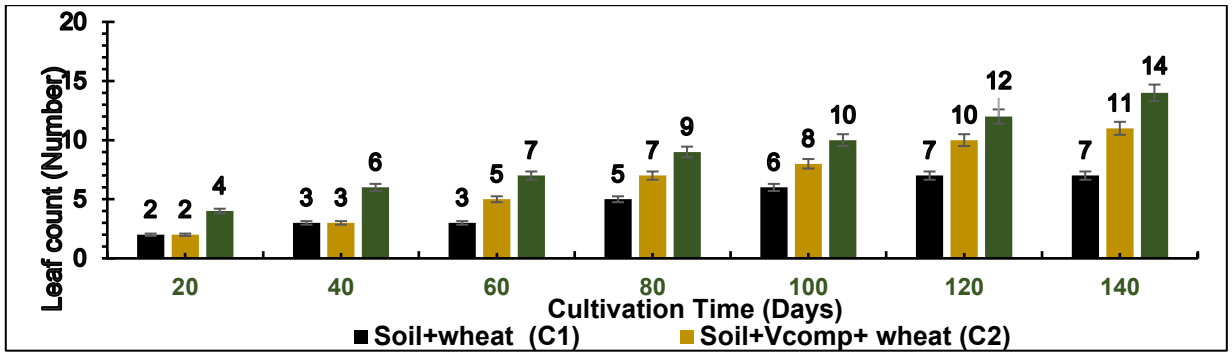

Fig. 8. Comparative leaf count pattern of *Triticum aestivum* plants grown in the test mycofertilizer and in control (C1, C2) Values are Mean ± SD of three observations. P<0.05

Number of Kernel Heads and Kernels in Wheat

Wheat plants grown in mycofertilizer-amended soil (Test) produced the highest yield attributes, with 8 kernel heads averaging 15.33 cm in length and 40 kernels per head. In comparison, vermicompost-amended soil (C2) produced 4 heads (10.66 cm, 33 kernels/head), while plain soil (C1) yielded only 2 heads (8.00 cm, 28 kernels/head) (Fig. 9; Table 4). These findings align with earlier reports on organic compost applications [49,50].

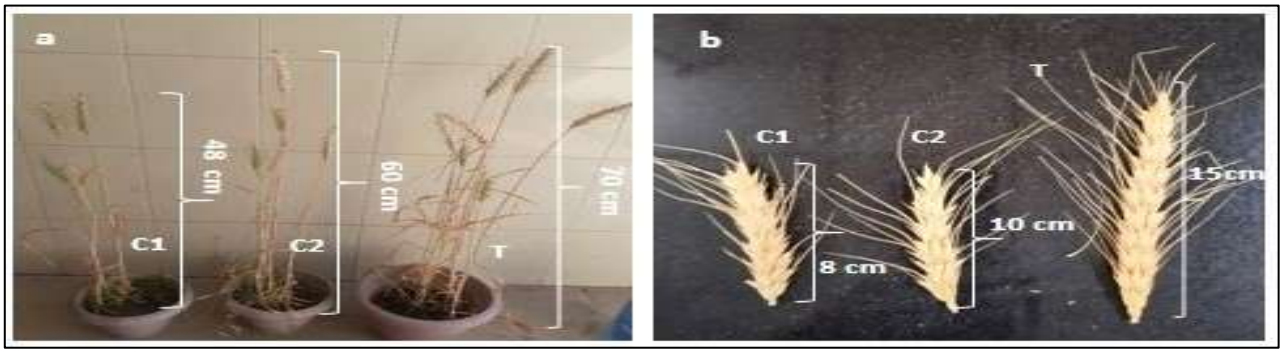

Fig. 9. Growth performance of *Triticum aestivum* in C1, C2, and T: (a) Kernel heads after 140 day growth cycle; (b) Kernel head length.

Agronomic Parameters

The different treatments markedly influenced the yield-attributing traits of *Triticum aestivum*. Plants grown in the test soil exhibited a significant improvement in both growth and yield compared to the control treatments (Table 4).

Table 4. Yield attributing traits of *Triticum aestivum* grown on soil supplemented with test mycofertilizer and in control soils (C1& C2)

Exp. model	% germination seeds	No. of kernel heads	No of kernels per head	Kernel head Length (cm)	Kernel head Weight (g)	Protein g/ 100g	Carbohydrate g/ 100g kernel
Wheat in plain soil (C1)	3/10=30%	2	28±4.10	8.0±0.94	2.46±0.45	5.1±1.8	28.7±1.78
Wheat in soil+ Vcomp (C2)	5/10=50%	4	33±4.72	10.66±0.72	2.48±0.57	6.8±1.39	32.0±2.15
Wheat in soil + mycofertilizer (T)	8/10=80%	8	40±3.31	15.33±0.72	3.13±0.11	8.3±1.48	48.0 ±3.02

Root, Shoot Biomass and Root Length of Wheat

In *Triticum aestivum*, root biomass (dry weight) was recorded as 0.83 ± 0.40 g in plain soil (C1), 1.03 ± 0.49 g in vermicompost-amended soil (C2), and 1.54 ± 0.24 g in mycobiofertilizer-treated soil (T). Corresponding root lengths were 7.83 cm, 11.0 cm, and 13.7 cm, respectively (Table 5). Shoot biomass (dry weight) was 4.57 ± 1.04 g in C1, 4.64 ± 1.30 g in C2, and 5.63 ± 0.79 g in T, while shoot lengths were 40 ± 3.5 cm, 50 ± 3.8 cm, and 65 ± 2.0 cm, respectively (Table 5). These results demonstrate a consistent enhancement in both biomass and growth parameters under mycobiofertilizer treatment, aligning with similar findings reported by [51] using organic compost amendments.

Table 5. Root and shoot biomass of wheat (*Triticum aestivum*)

Parameters	Shoot dry weight (g/plant)	Root dry weight (g/plant)	Root length (cm)	Shoot length (cm) (Wheat Plant)
Test (T) (Soil+ Mycofertilizer)	5.63 ± 0.79	1.54 ± 0.25	13.7 ± 0.88	65.0 ± 2.0
Control -1 (Only soil)	4.57 ± 1.04	0.83 ± 0.40	7.83 ± 0.88	40.0 ± 3.5
Control-2 (Soil+ Vermicompost)	4.64 ± 1.30	1.03 ± 0.49	11.**0** ±1.86	50.0 ± 3.8

Values are Mean± SD of three observations
P <0.05
Growth Pattern: T > C2 > C1

4. DISCUSSION

The present investigation provides novel insights into the rapid biodegradation of lignocellulosic biomasses such as leaf litter, paddy straw, corn stover, and sugarcane bagasse through a strategically designed fungal consortium comprising *Ganoderma gibbosum*, *G. multipileum*, and *Trametes hirsuta*. While individual strains of white rot fungi have been studied extensively, this is the first report demonstrating the synergistic action of this unique consortium across multiple substrates, resulting in highly efficient lignocellulolytic enzyme production. The consortium exhibited impressive enzymatic activities-laccase (1.45 U/ml), cellulase (0.69 U/ml), mannanase (0.78 U/ml), pectinase (4.03 U/ml), and xylanase (6.40 U/ml) on the 14th day of incubation. These activities not only exceeded those reported in earlier works but also significantly reduced the time required for substrate degradation. For instance, compared to the laccase activity of 1.18 IU/ml reported by [52] using *Pleurotus ostreatus*, and 0.08 IU/ml by [53] using *Lentinula edodes*, the present consortium exhibited superior catalytic potential. Similarly, the release of 39% glucose from cellulose within 14 days surpassed the 31% yield obtained by [54] after 60 days with *P. ostreatus*, underscoring the novelty and efficiency of the approach.

A remarkable achievement of this study is the formulation of a nutrient-enriched mycofertilizer with an N-P-K composition of 1.6–1.1–1.4% and a favorable C:N ratio of 17.51. This nutrient profile not only surpasses traditional biofertilizers such as vermicompost or spent mushroom substrate but also positions mycofertilizer as a high-value agricultural input. For instance, the values reported by [55] (1–1.4–0.9%) are considerably lower, highlighting the superior nutrient enrichment achieved in the present study. The bioefficacy trials with *Triticum aestivum* further validated the agronomic potential of the developed formulation. Wheat plants supplemented with mycofertilizer exhibited significantly enhanced growth and yield parameters, including the production of eight kernel heads per plant, compared to four in vermicompost-amended soil and only two in plain soil controls. Equally significant is the drastic reduction in decomposition time. Conventional organic fertilizers such as cow dung compost (6–9 months) or vermicompost (90–120 days) require prolonged maturation, whereas the mycofertilizer developed herein was ready within just 14 days, requiring minimal labour input. Furthermore, the consortium achieved 42% lignin degradation within 14 days, which is substantially higher than the 26% reported by [56] over 90 days using *Pleurotus* spp. This accelerated degradation, combined with higher nutrient recovery, highlights the dual benefits of waste management and soil fertility improvement within a remarkably short

timeframe. Collectively, these findings establish the present study as a pioneering advancement in fungal-mediated bioconversion strategies, demonstrating both scientific novelty and practical significance.

5. CONCLUSION

The eco-friendly and cost-effective mycofertilizer developed through fungal consortial degradation of lignocellulosic biomass holds immense promise as a sustainable alternative to chemical fertilizers and conventional biofertilizers. Its superior nutrient profile, rapid maturation, and ability to significantly enhance plant growth underscore its potential role in modern agriculture. Beyond nutrient enrichment, the formulation provides additional benefits through the release of fungal-derived hormones, vitamins, and anti-pathogenic metabolites, contributing to holistic soil and plant health.

By integrating such mycofertilizers into agricultural systems, this study demonstrates a sustainable pathway for addressing multiple global challenges: effective waste valorization, reduction in dependence on synthetic fertilizers, improved crop yields, and enhancement of soil fertility. The dual advantage of environmental remediation and agricultural productivity positions fungal consortia-based mycofertilizers as a transformative innovation in sustainable farming. Ultimately, these findings highlight the broader significance of fungal-mediated biodegradation of lignocellulosic waste into value-added products, thereby advancing ecological balance, promoting food security, and fostering a circular bioeconomy.

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Author's Contributions

DKR conceptualized the study, designed the experiments, supervised the research, analyzed the results, and critically revised the manuscript. DKS conducted the laboratory experiments, collected the data, contributed to data analysis, and drafted the manuscript. SR and IW performed additional data analysis and assisted in interpretation. All authors read and approved the final manuscript.

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Data Availability All data generated or analysed during this study are included in this manuscript.

Declarations

Ethical Approval: The current study did not include any human or animal subjects and thus requires no approval from ethical committee.

Conflict of Interests: The authors declare no conflict of interest.

Consent to publish: The research presented is an original study that has not been previously published. All authors consented to the submission of the manuscript for publication.

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