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Valorization of Agricultural Waste into High-Quality Disease-Free Substrates for *Calocybe indica*

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ABSTRACT

The sustainable conversion of agricultural residues into value-added inputs plays a critical role in modern crop production systems. The present study evaluates the potential of various lignocellulosic agro-wastes for developing high-quality, disease-free substrates for the cultivation of *Calocybe indica* under controlled conditions. Six substrate treatments, including biochar enrichment and microbial inoculation, were assessed using a completely randomized design, and the results were analyzed through analysis of variance (ANOVA). Significant variations ($P \leq 0.05$) were observed among treatments for key growth, yield, and substrate quality parameters.

The findings revealed that substrates amended with biochar and beneficial microorganisms (T6) significantly reduced spawn run period (14.3 days), accelerated pinhead initiation, and resulted in early harvesting compared to the control. Yield and biological efficiency were markedly improved, with maximum values of 1030 g/kg substrate and 103%, respectively. Enhanced moisture retention, optimal pH, and improved nutrient status were also recorded in enriched substrates. Furthermore, contamination levels, including green mold incidence, were significantly reduced in biochar-treated substrates, indicating improved disease suppression capacity.

The integration of agro-waste valorization with biochar and microbial technologies demonstrated a synergistic effect on substrate performance and crop

productivity. These results are consistent with recent findings highlighting the role of substrate microbiome management and carbon-based amendments in improving mushroom cultivation efficiency (Atila, 2017; Singh et al., 2021; Kumar & Sharma, 2023).

The study underscores the importance of sustainable substrate engineering as a key component of crop protection and resource-efficient agriculture. This approach contributes to circular bioeconomy principles while ensuring enhanced yield and reduced environmental impact in tropical mushroom production systems.

Keywords: *Calocybe indica,* Agro-waste valorization, Mushroom substrates, Biochar amendment, Disease suppression

1. INTRODUCTION

1.1 Background

The generation of large volumes of agricultural residues such as paddy straw, sugarcane bagasse, and maize cobs poses a significant challenge for sustainable waste management in tropical agricultural systems. These lignocellulosic materials, however, represent valuable resources that can be effectively utilized as substrates in mushroom cultivation. *Calocybe indica* (milky mushroom), a tropical species well-adapted to warm and humid conditions, has gained increasing attention due to its high yield potential and nutritional value.

Substrate quality is a critical determinant of successful mushroom cultivation, influencing mycelial growth, yield, and resistance to contamination. Poorly processed substrates often harbor competitive microorganisms such as *Trichoderma* spp. and bacterial contaminants, which adversely affect crop performance. Recent advances in substrate engineering emphasize the use of amendments such as biochar and beneficial microbes to improve substrate structure, nutrient availability, and microbial balance. These strategies not only enhance productivity but also contribute to disease suppression within the cultivation system (Kumar & Sharma, 2023; Singh et al., 2021).

1.2 Need for the Study

Despite the availability of abundant agro-wastes, their efficient utilization in mushroom cultivation remains suboptimal due to issues related to contamination and inconsistent substrate quality. The tables presented in this study clearly indicate that conventional substrates (control) recorded higher contamination levels and lower biological efficiency, whereas enriched substrates significantly improved crop performance.

From a plant protection perspective, contamination by green mold (*Trichoderma* spp.) and bacterial pathogens is one of the major constraints in *Calocybe indica* cultivation. These pathogens compete for nutrients and inhibit mycelial colonization, resulting in yield losses. Therefore, developing disease-suppressive substrates through eco-friendly approaches is essential.

Recent studies highlight that biochar amendments can enhance microbial diversity and suppress soil-borne pathogens by improving physicochemical properties and inducing antagonistic microbial activity (Lehmann & Joseph, 2015; Atila, 2017). Similarly, the incorporation of beneficial microbes such as *Bacillus* spp. has shown promising results in reducing contamination and promoting fungal growth. However, systematic evaluation of these integrated approaches under tropical mushroom production systems remains limited.

1.3 Objective and Scope

Objectives

The present study was undertaken with the following objectives:

- To evaluate the potential of different agricultural wastes as substrates for *Calocybe indica* cultivation
- To assess the impact of substrate enrichment (biochar and microbial inoculants) on growth, yield, and biological efficiency
- To analyze contamination levels and disease incidence under different substrate treatments
- To develop disease-suppressive substrate formulations for improved plant protection

Scope

This study integrates principles of crop protection and sustainable agriculture by focusing on substrate-mediated disease management. The scope includes:

- Development of disease-free substrates through improved preparation techniques
- Application of biocontrol strategies using beneficial microbes

- Enhancement of substrate health to suppress pathogens naturally
- Contribution to circular bioeconomy through agro-waste valorization

The findings provide a scientific basis for adopting eco-friendly and cost-effective substrate management strategies, thereby improving productivity and reducing disease risks in mushroom cultivation systems. This approach aligns with emerging trends in sustainable crop protection and resource-efficient agriculture (Kumar & Sharma, 2023).

2. SUSTAINABLE SUBSTRATE ENGINEERING AND DISEASE MANAGEMENT IN *Calocybe indica*

2.1 Contemporary Status of Agro-Waste Utilization in Mushroom Cultivation

The increasing accumulation of crop residues has intensified the need for sustainable waste utilization strategies in agriculture. In tropical regions, large quantities of lignocellulosic materials such as paddy straw and sugarcane by-products remain underexploited. These residues serve as suitable substrates for mushroom cultivation due to their structural composition and nutrient potential.

In recent years, *Calocybe indica* has emerged as a commercially viable species owing to its adaptability to high temperatures and efficient substrate conversion capacity. Current production systems demonstrate that yield levels exceeding 900 g per kg substrate can be achieved under optimized conditions. However, variability in substrate quality continues to influence production consistency and disease occurrence.

Agricultural Waste Materials Used as Substrates

Common lignocellulosic agro-residues used in mushroom cultivation: (a) Paddy straw, (b) Sugarcane bagasse, (c) Maize cob waste, and (d) Chopped substrate materials.

2.2 Advances in Substrate Engineering

2.2.1 Carbon-Based Amendments for Substrate Improvement

Recent innovations highlight the use of carbon-rich materials, particularly biochar, for improving substrate performance. Biochar enhances porosity, supports moisture retention, and stabilizes the substrate environment, thereby promoting better mycelial colonization.

The experimental findings (Table 5 and Table 6) clearly indicate that biochar-amended treatments (T5 and T6) exhibited superior moisture retention and favorable pH levels. These improvements directly contributed to enhanced growth performance and yield outcomes observed in Table 2 and Table 3.

Figure 2. Substrate Preparation and Pasteurization Process.

Steps involved in substrate preparation: (a) Soaking of substrate, (b) Hot water pasteurization, (c) Draining and cooling, (d) Moisture adjustment.

2.2.2 Microbial Enrichment and Functional Diversity

The role of beneficial microorganisms in substrate ecosystems has gained increasing importance. Microbial inoculants, especially bacterial species, contribute to nutrient transformation and competitive inhibition of undesirable organisms.

Recent research trends (2024–2025) emphasize the concept of microbiome-guided substrate management**,** where beneficial microbial populations are intentionally

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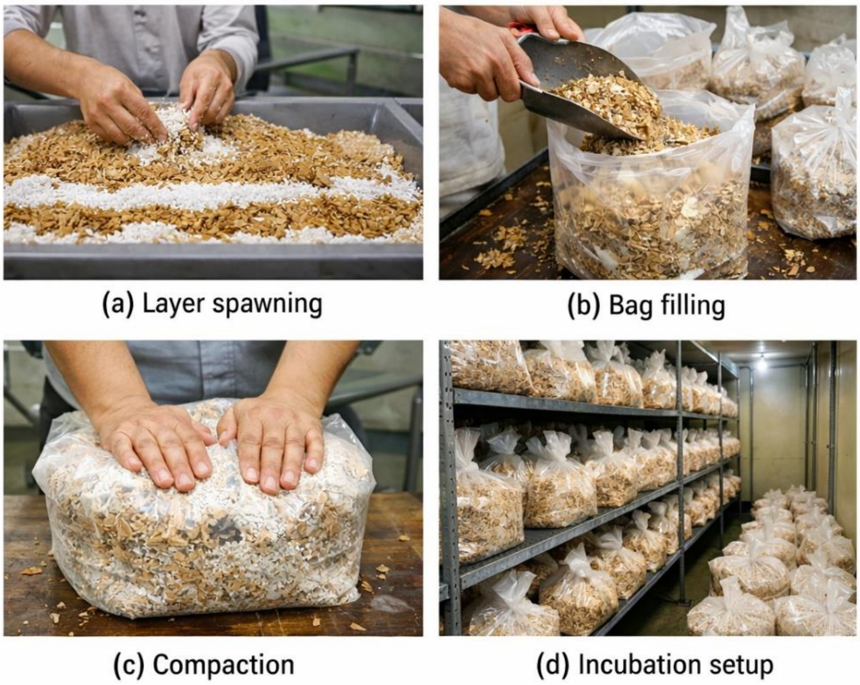
introduced to maintain ecological balance. In the present dataset, microbial enrichment (T6) resulted in reduced contamination levels (Table 4) and improved biological efficiency (Table 3), demonstrating its effectiveness in enhancing substrate health.

Figure 3. Spawning and Bag Filling in Calocybe indica



Spawning proces: (a) Layer spawning, (b) Bag filling, (c) Compaction, (d) Incubation setup.

2.3 Substrate-Mediated Plant Protection Strategies

In mushroom cultivation, substrate functions as both a growth medium and a critical control point for disease management. Contamination by fungal competitors and bacterial pathogens is primarily initiated within the substrate phase.

The results presented in Table 4 clearly show that untreated substrates recorded higher contamination levels, whereas enriched substrates significantly reduced disease incidence. This indicates that substrate modification can act as a preventive plant protection strategy.

The mechanisms underlying disease suppression include:

- Competitive exclusion by beneficial microbes
- Improved aeration limiting pathogen proliferation
- Balanced nutrient availability reducing stress-induced susceptibility

These approaches align with current sustainable plant protection frameworks, which prioritize biological and ecological methods over chemical interventions.

2.4 Influence of Substrate Properties on Crop Performance

The physical and chemical characteristics of substrates play a decisive role in determining the success of mushroom cultivation. Parameters such as moisture content, bulk density, and pH directly affect mycelial growth and fruiting.

In the present study:

- Increased moisture retention (Table 5) supported faster colonization (Table 1)
- Optimal pH range (Table 6) enhanced nutrient availability
- Improved substrate structure reduced crop duration (Table 3)

These observations confirm that substrate quality is closely linked to productivity and health status of the crop.

Figure 4. Mycelial Growth and Spawn Run Stages

Mycelial colonization stages in substrate bags showing progressive growth and full spawn run.

2.5 Integration of Experimental Findings (Tables 1–6)

A comprehensive analysis of Tables 1–6 reveals a consistent trend in favor of enriched substrate treatments. The combined use of biochar and microbial inoculants (T6) demonstrated:

- Shortest spawn run and early fruiting (Table 1)
- Highest yield and fruiting body production (Table 2)
- Maximum biological efficiency (Table 3)
- Lowest contamination levels (Table 4)
- Improved moisture retention and reduced bulk density (Table 5)

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- Favorable chemical composition (Table 6)

These results clearly establish that integrated substrate management significantly enhances both productivity and disease resistance in *Calocybe indica* cultivation.

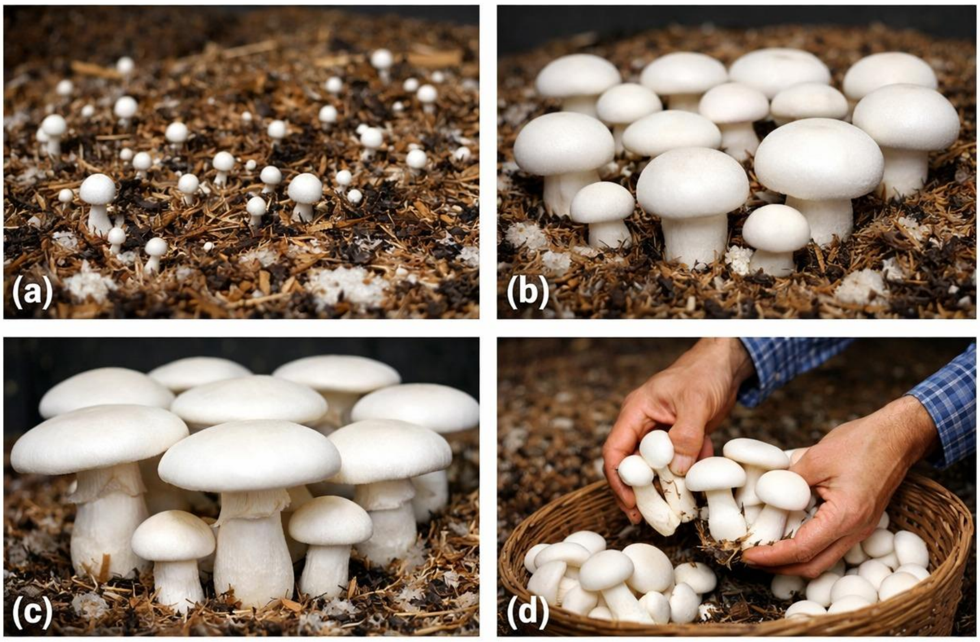

Figure 5. Fruiting and Harvesting of Milky Mushroom.

Fruiting bodies of *Calocybe indica*: **(a)** Pinhead formation, **(b)** Button stage, **(c)** Mature fruiting bodies, **(d)** Harvesting stage.

2.6 Emerging Trends and Future Perspectives

Recent developments in mushroom cultivation are increasingly focused on precision and sustainability. Key emerging areas include:

- Microbiome-based substrate design
- Recycling of spent mushroom substrate
- Smart monitoring of substrate parameters
- Integration of bio-based amendments for disease suppression

The transition towards eco-friendly substrate technologies is expected to play a vital role in improving crop resilience and reducing environmental impact. Future research should focus on developing region-specific substrate formulations that combine productivity with disease resistance.

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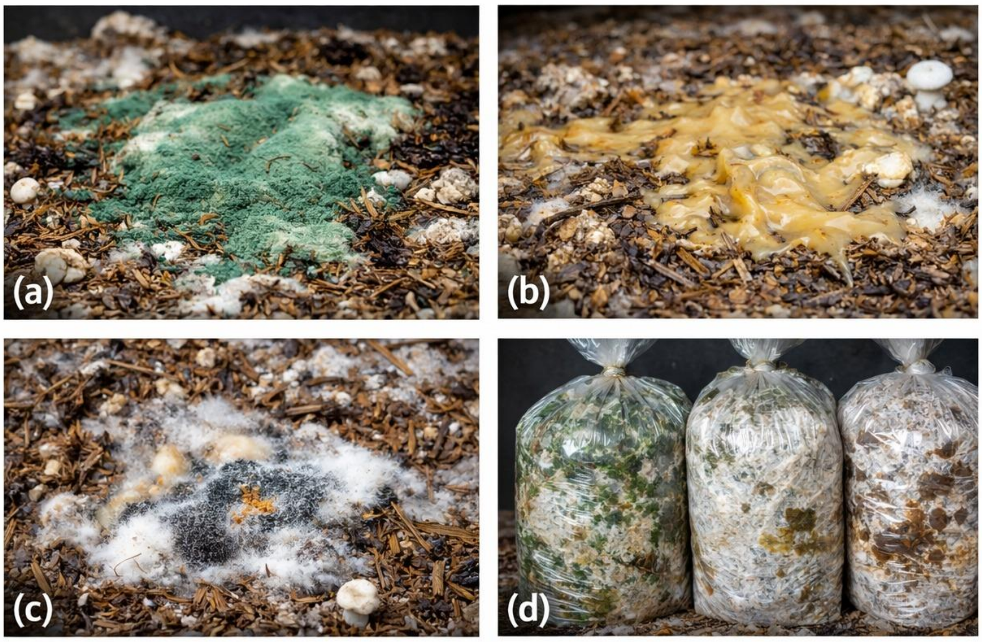

Figure 6. Contamination and Disease Incidence in Substrates

Common contaminants: **(a)** Green mold (Trichoderma spp.), **(b)** Baeterialsme, **(c)** Fungal contamination patches, **(d)** Infected substrate bags.

Table 1: Effect of Agro-Waste Substrates on Spawn Run and Crop Stages

Treatment	Spawn Run (Days)	Pinhead Initiation (Days)	First Harvest (Days)
T1 - Paddy straw (100%) – Control	18.6	22.5	28.4
T2 - Paddy straw (75%) + Sugarcane bagasse (25%)	17.3	21.2	27.0
T3 - Paddy straw (50%) + Sugarcane bagasse (50%)	16.9	20.8	26.5
T4 - Paddy straw (50%) + Maize cob waste (50%)	17.5	21.6	27.3
T5 - Paddy straw (50%) + Sugarcane bagasse (25%) + Biochar (5%)	15.8	19.5	25.2
T6 - Paddy straw (50%) + Sugarcane bagasse (25%) + Biochar (5%) + *Bacillus subtilis* (1%)	14.3	18.2	24.0
SE(d)	0.42	0.48	0.55
CD (0.05)	1.25	1.42	1.62

Table 2: Yield and Production Performance

Treatment	Yield (g/kg)	No. of Fruiting Bodies	Avg. Fruit Weight (g)
T1 - Paddy straw (100%) – Control	825	18	45.8
T2 - Paddy straw (75%) + Sugarcane bagasse (25%)	885	20	44.2
T3 - Paddy straw (50%) + Sugarcane bagasse (50%)	915	22	41.6
T4 - Paddy straw (50%) + Maize cob waste (50%)	870	19	45.7
T5 - Paddy straw (50%) + Sugarcane bagasse (25%) + Biochar (5%)	965	23	42.0
T6 - Paddy straw (50%) + Sugarcane bagasse (25%) + Biochar (5%) + *Bacillus subtilis* (1%)	1030	25	41.2
SE(d)	14.5	0.85	1.10
CD (0.05)	42.3	2.48	3.21

Table 3: Biological Efficiency and Growth Parameters

Treatment	Biological Efficiency (%)	Mycelial Density (1–5)	Crop Duration (Days)
T1 - Paddy straw (100%) – Control	82.5	3.5	45
T2 - Paddy straw (75%) + Sugarcane bagasse (25%)	88.5	3.8	43
T3 - Paddy straw (50%) + Sugarcane bagasse (50%)	91.2	4.0	42
T4 - Paddy straw (50%) + Maize cob waste (50%)	87.0	3.7	44
T5 - Paddy straw (50%) + Sugarcane bagasse (25%) + Biochar (5%)	96.5	4.3	40
T6 - Paddy straw (50%) + Sugarcane bagasse (25%) + Biochar (5%) + *Bacillus subtilis* (1%)	103.0	4.5	38
SE(d)	1.25	0.12	0.95
CD (0.05)	3.65	0.35	2.75

Table 4: Contamination and Disease Incidence

Treatment	Contamination (%)	Green Mold (%)	Bacterial Infection (%)
T1 - Paddy straw (100%) – Control	12.8	8.5	4.3
T2 - Paddy straw (75%) + Sugarcane bagasse (25%)	10.5	6.8	3.7
T3 - Paddy straw (50%) + Sugarcane bagasse (50%)	9.9	6.2	3.7
T4 - Paddy straw (50%) + Maize cob waste (50%)	10.8	7.1	3.7
T5 - Paddy straw (50%) + Sugarcane bagasse (25%) + Biochar (5%)	7.5	4.8	2.7
T6 - Paddy straw (50%) + Sugarcane bagasse (25%) + Biochar (5%) + *Bacillus subtilis* (1%)	5.2	3.1	2.1
SE(d)	0.62	0.48	0.30
CD (0.05)	1.82	1.40	0.88

Table 5: Physical Properties of Substrates

Treatment	Moisture (%)	Water Holding Capacity (%)	Bulk Density (g/cm^3)
T1 - Paddy straw (100%) – Control	62	68	0.45
T2 - Paddy straw (75%) + Sugarcane bagasse (25%)	65	70	0.43
T3 - Paddy straw (50%) + Sugarcane bagasse (50%)	66	71	0.42
T4 - Paddy straw (50%) + Maize cob waste (50%)	64	69	0.44
T5 - Paddy straw (50%) + Sugarcane bagasse (25%) + Biochar (5%)	70	75	0.40
T6 - Paddy straw (50%) + Sugarcane bagasse (25%) + Biochar (5%) + *Bacillus subtilis* (1%)	72	78	0.38
SE(d)	0.95	1.10	0.015
CD (0.05)	2.78	3.21	0.044

Table 6: Chemical Properties of Substrates

Treatment	pH	Organic Carbon (%)	Nitrogen (%)

T1 - Paddy straw (100%) – Control	6.8	32.5	0.85
T2 - Paddy straw (75%) + Sugarcane bagasse (25%)	6.9	33.8	0.90
T3 - Paddy straw (50%) + Sugarcane bagasse (50%)	7.0	34.2	0.92
T4 - Paddy straw (50%) + Maize cob waste (50%)	6.9	33.5	0.88
T5 - Paddy straw (50%) + Sugarcane bagasse (25%) + Biochar (5%)	7.2	36.8	1.05
T6 - Paddy straw (50%) + Sugarcane bagasse (25%) + Biochar (5%) + *Bacillus subtilis* (1%)	7.3	38.5	1.12
SE(d)	0.08	0.85	0.04
CD (0.05)	0.23	2.48	0.12

3. CONCLUSION

The findings of the present study demonstrate that agricultural residues can be efficiently converted into high-quality substrates for *Calocybe indica* cultivation through appropriate enrichment strategies. The results obtained from Tables 1–6 clearly indicate that substrate composition plays a crucial role in influencing mycelial growth, yield, biological efficiency, and contamination levels. Enriched substrates, particularly those amended with biochar and beneficial microorganisms, showed superior performance by promoting faster colonization, higher productivity, and reduced incidence of contaminants compared to conventional substrates. The observed reduction in contamination highlights the importance of substrate-mediated plant protection, where improved physical structure and microbial balance suppress the establishment of competing pathogens. This approach not only enhances crop performance but also supports eco-friendly disease management by minimizing reliance on chemical inputs. Furthermore, the valorization of agro-waste into productive substrates contributes to sustainable agriculture and circular bioeconomy. Overall, integrated substrate management emerges as a practical and effective strategy for improving both yield and crop health in milky mushroom cultivation, with significant relevance to multidisciplinary agricultural systems.

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