J.Mycopathol.Res. 64(3) : 499-515, 2026, ISSN : 0971-3719 (Print), 2583-6315 (Online) doi.10.57023/JMycR.64.3.2026.499
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REVIEW

Emerging research trends and technological innovations in edible mushroom cultivation for enhanced productivity

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Received : 19.06.2026 Accepted : 31.07.2026 Published : 28.09.2026

The global edible mushroom industry is undergoing a profound paradigm shift, evolving from a historically labor-intensive, environmentally dependent agricultural practice into a highly automated, data-driven, and biotechnologically advanced biomanufacturing sector. Driven by shifting global dietary preferences, the imperative for sustainable plant-based proteins, and the expanding pharmacological applications of fungal metabolites, mushroom cultivation has emerged as a cornerstone of the modern circular bioeconomy. This comprehensive scientific review examines the current global and Indian scenarios of mushroom production, consumption patterns, and underlying economic constraints. It explores cutting-edge diagnostic modalities for taxonomic identification and toxicity assessment, emphasizing DNA mini-barcoding, Fourier-transform infrared (FTIR) spectroscopy, and deep learning convolutional neural networks. Furthermore, the analysis delineates the fundamental transition in strain engineering, moving from conventional recombination to precise CRISPR-Cas9 multiplex genomic editing. Technological innovations, including Edge-computing IoT architectures, liquid spawn technology, automated robotic harvesting, and nanomaterial biofortification, are systematically evaluated for their impact on biological efficiency and yield optimization. Finally, the report investigates the domestication parameters of novel high-value species such as *Morchella*, alongside advanced post-harvest preservation techniques and the robust valorization of spent mushroom substrate (SMS) to achieve closed-loop agricultural sustainability.

Keywords : Biological efficiency, edible mushroom, hybridization, technological innovation

INTRODUCTION

The commercial cultivation of mushrooms represents one of the most rapidly accelerating segments of global agriculture. Mushroom farming is one such technology in India having potential to process the waste plant materials/crop residues(620 million tons) into best valuable food items with good protein conversion efficiency and productivity per unit area and time (Thakur and Singh, 2020).

Edible mushrooms are highly valued for their exceptional nutritional profile, providing a rich, bioavailable source of high-quality proteins, essential amino acids, crude fibre, and micronutrients such as Vitamin D, while remaining uniquely low in fat and carbohydrates (Thakur and Singh, 2013; Barman *et al.* 2018; Sangeeta *et al.*2024; Thakur *et al.* 2025).

Mushroom cultivation has the potential to transform the rural economy by fostering inclusive and sustainable economic growth. As a distinctive group of macrofungi, mushrooms offer India an opportunity to initiate a “non-green revolution” by creating new avenues for rural employment and income generation. Their cultivation holds significant potential to bring about positive socio-economic changes and improve livelihoods at the local, national, and regional levels.This comprehensive scientific review examines the current global and Indian scenarios of mushroom production, consumption patterns, and underlying economic constraints.

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18th Prof. S.B.Chattopadhyay Memorial Award Lecture organized by Indian Mycological Society delivered on 16th March 2026 at the Department of Botany, University of Calcutta

Market Valuation and Prospective Growth Strategies

The global mushroom market is experiencing unprecedented financial growth, reflecting a broader macro-economic transition toward functional and sustainable foods (Table 1). In 2025, the global market size was valued at USD 71.9 billion, with conservative projections estimating an expansion to USD 156.3 billion by 2033, registering a compound annual growth rate (CAGR) of 10.2% (Sharma *et al.*2017). Alternative predictive models utilizing extended forecasting horizons suggest the industry could reach a valuation of USD 191.0 billion by 2036, driven by a 10% CAGR(Anonymous,2026 a). The rapid growth of the mushroom industry is primarily attributed to heightened health consciousness among consumers, increasing demand for plant-based dietary alternatives, and the widespread utilization of mushroom-derived functional ingredients in nutraceutical, pharmaceutical, and cosmetic sectors (Anonymous,2026c).

The food application sector overwhelmingly dominates the global mushroom market, contributing between 72% and 86.71% of total industry revenue. The White Button mushroom (*Agaricus bisporus*) commands the highest product volume share at 40%, driven by widespread global culinary familiarity, extensive historical domestication, and highly optimized year-round indoor cultivation stability (Anonymous, 2026 a). Conversely, the Shiitake mushroom (*Lentinula edodes*) captured the largest revenue share, accounting for 32.4% of the market in 2025,

largely due to its premium pricing structure, rich acceptable profile, and overwhelming demand in robust Asian markets. Oyster mushrooms (*Pleurotus* spp.) are similarly expanding at an accelerated pace, projected to grow at a significant CAGR of 11.5% due to their relatively simple cultivation requirements and high biological efficiency (Anonymous,2026).While fresh mushrooms constitute approximately 64% of the market volume—the processed segment is exhibiting the most aggressive growth. Processed mushrooms, encompassing dried, frozen, canned, pickled, and powdered forms, are projected to expand at a 10.7% CAGR (Anonymous,2026 c). This surge highlights a structural shift in consumer behaviour toward convenience foods and the industry's strategic response to the extreme perishability of fresh fruiting bodies.

Regional Production Ecosystems

The Asia-Pacific region remains the undisputed epicenter of global mushroom cultivation, commanding an overwhelming 78.7% of the global revenue share in 2025. China operates as the dominant global producer, accounting for 87% to 93% of the world's fresh mushroom output (Schmidt,2026). Cultivation in China has grown at a robust historical CAGR of 9.23% over the past two decades, heavily supported by proactive government agricultural reforms emphasizing food security, rapid urbanization, and massive investments in sustainable farming technologies (Anonymous, 2026a).

Beyond the Asian epicentre, European Union (EU) member states and North America maintain highly industrialized, technology-dense, albeit smaller, production scales. The EU-27 exhibits substantial intra-industry trade flow, importing €3.0 billion of mushroom products in 2022 to meet internal supply gaps (Schmidt,2026). Concurrently, nations possessing advanced agricultural infrastructure, such as the Netherlands, function as major processing and re-export hubs, generating €4.1 billion in export value (Schmidt, 2026). The United States remains a major player in large-scale *Agaricus bisporus* cultivation, though it faces increasing pressures from an aging agricultural workforce and rising wage inflation.

The Indian Scenario: Current Status of Mushroom Production in India, State-wiseDynamics, and Economic Realities

Historically, mushroom consumption in India was largely confined to wild foraging among tribal communities and localized, seasonal cultivation in northern hilly regions where cool ambient temperatures naturally supported *Agaricus bisporus*(Bijla and Sharma,2023). However, the introduction of climate-controlled technologies,

targeted institutional support, and localized strain development has catalyzed a nationwide agricultural paradigm shift.Effect of pruned tea leaves on the yield and nutritional quality of two species of *Pleurotus*(Roy and Chakraborty, 2018) and milky mushroom (*Calocybe indica*) in North Bengal have also been reported (Barman *et al.*2018).Besides, antioxidant properties such as DPPH scavenging activity, ferric reducing antioxidant power activity as well as flavanoid activity of four oyster (*Pleurotu*s) species (*P. djamor, P. sajorcaju, P. ostreatus and P. florida*) have been evaluated (Roy and Chakraborty, 2023).

Production Statistics and Agro-Climatic Distribution

India's total mushroom production has surged from a mere 40,600 metric tonnes in 2010 to an estimated 280,000 to 310,000 metric tonnes in 2022, registering an exceptional and sustained CAGR of 17.45% (Sangeeta *et al.* 2025). Despite this exponential supply- side growth, domestic per capita consumption remains remarkably low at less than 100 grams per year, indicating a massive untapped domestic market and heavy reliance on export channels(Bijla and Sharma,2023). The Indian cultivation landscape is predominantly structured around four key species: White Button mushroom (73%), Oyster mushroom (16%), Paddy Straw mushroom (7%), and Milky mushroom (3%)Bijla and Sharma(2023) (Fig. 1 A-D).

The geographical distribution of production has successfully decentralized from the traditional northern hill states (such as Himachal Pradesh and Uttarakhand) into the subtropical and tropical plains, leveraging varied agro-climatic zones. Currently, six states contribute approximately 50% of the national output (Pandya *et al*.2024) (Table 2).

Economic Dynamics, Institutional Intervention, and Systemic Constraints

The socio-economic impact of mushroom farming in India is profound, operating as a critical lever for poverty alleviation and women's empowerment. Demographic analyses reveal that 70% of mushroom growers are small-scale or marginal farmers holding less than 2 hectares of land, while 54.2% belong to the middle-age demographic i.e. in between 32–48 years. The integration of women through Self-Help Groups (SHGs)—such as the 300+ women operating in the Purba Bardhaman district of West Bengal has yielded measurable socio-economic improvements, with 64% reporting enhanced living standards and 60% securing better financial savings (Pandya *etal*.2024).For a standard 150–300 bag production unit, the average net return generated is Rs. 24,225, yielding a net income of Rs. 14.15 per kilogram. Fresh mushrooms generate a Benefit-Cost Ratio (BCR) of 1.75 to 2.00, whereas transitioning to value-added mushroom products dramatically elevates the BCR to 5.21, underscoring the critical need for processing infrastructure(Pandya *et al.*2024).

Despite these favorable micro-economics, the macro-industry faces severe systemic constraints. The Indian mushroom market remains highly unorganized and plagued by severe price fluctuations, particularly during summer months, exacerbated by the absence of a Minimum Support Price (MSP)(Sharma *etal.*2017). Furthermore, a critical supply-side bottleneck exists regarding high-quality spawn. The national spawn demand is estimated at 8,000 to 10,000 tons annually, yet public sector fulfilment covers merely 10% of this requirement, leaving growers reliant on fragmented private laboratories (Bijla and Sharma,2023). Institutional intervention is primarily spearheaded by the ICAR-Directorate of Mushroom Research (DMR) in Solan, Himachal Pradesh. Established to execute basic and applied research, the ICAR-DMR curates a germplasm bank of over 260 accessions and operates 32 All India Coordinated Research Project (AICRP) centers(ICAR-DMR Annual Report 2024). Alongside localized Krishi Vigyan Kendras (KVKs)—such as KVK Bilaspur, which promotes *Volvariella volvacea* in paddy-rich systems to deter crop residue burning—these institutions are the primary vectors for technology transfer and capacity building (Thakur *et al*.2025).

Advanced Diagnostic Approaches for Taxonomic Identification and Toxicological Evaluation

Traditional taxonomic identification relies heavily on macroscopic morphological characteristics

such as cap margins, spore print coloration, and gill attachment (Akshaya and Beegam, 2023). However, these phenotypic traits possess high phenotypic plasticity, rendering them highly susceptible to environmental variations, desiccation, and human error (Xing *et.al*. 2024). For instance, deadly *Amanita* species frequently emerge from volval sacs resembling edible "puffballs," while the toxic *Gyromitra* spp. is regularly mistaken for the prized *Morchella* (Anonymous, 2024). Consequently, robust molecular and computational technologies have become paramount in ensuring global myco-safety.Performance metrics and computational efficiency of deep learning models in mushroom toxicity classification have been laid out in Table 3.

DNA Barcoding

DNA sequence-based fungal identification has fundamentally revolutionized taxonomy, providing objective, genome-level differentiation. The Internal Transcribed Spacer (ITS) region of the nuclear ribosomal RNA gene cluster has been firmly established as the universal primary barcode for fungal species. While standard full-length DNA barcoding is highly successful for fresh, intact botanical specimens, its utility collapses when applied to cooked, highly processed, or gastric-digested clinical samples due to severe DNA fragmentation and thermal degradation (Xing *et al.*2024).

Mini-Barcoding

To circumvent this limitation, forensic mycology has pioneered DNA "mini-barcoding" which targets significantly shorter gene sequences (100–300 bp) nested within the broader ITS region (Xing *et al.* 2024). Recent optimizations have yielded the ITS-a primer set, which exhibits robust universality across *Amanita* samples, accurately amplifying a 290 bp target fragment. This methodology has proven highly effective in identifying deadly species—including *Amanita phalloides*, *A. virosa*, and *A. fuliginea*—directly from heavily degraded vomits and stomach extracts, providing critical, time-sensitive diagnostic data in acute poisoning cases(Xu, 2016). Furthermore, beyond the ITS region, the cytochrome oxidase 1 (CO1) gene is emerging as a superior secondary genetic marker. Studies demonstrate that insertion/deletion patterns within the CO1 sequence offer higher resolution than the standard rDNA cluster when discriminating the edible Japanese *Entoloma sarcopum* from its sympatric, highly toxic look-alikes, *E. rhodopolium* and *E. sinuatum* (Aoki *et al*.2020).

Spectroscopic Fingerprinting: FTIR and Raman Analysis

Fourier-Transform Infrared (FTIR) and Raman spectroscopy offer rapid, non-destructive chemical fingerprinting for taxonomic classification, bypassing the need for complex DNA extraction protocols. FTIR interrogates the molecular vibrations of specific functional groups—primarily lipids, proteins, and structural polysaccharides—based on mid-infrared photon absorption (Feher *et al.*2021). Spectroscopic mapping of basidiomycetes has isolated key diagnostic spectral windows across species: 3400-3200 cm^{-1} (O-H and N-H stretching), 2900-2850 cm^{-1}(aliphatic C-H stretching), and 1658-1625 cm^{-1} (amide I corresponding to protein structures) Liu *et al.* (2004).

Because spectroscopic signals are highly complex and non-selective, they mandate the integration of advanced chemometric models for interpretation. When coupled with mathematical modeling, FTIR achieves exceptional precision. For instance, Linear Discriminant Analysis (LDA) applied to FTIR spectra successfully classified 100% of wild *Armillaria mellea*, *Boletus edulis*, and *Cantharellus cibarius* specimens in test cohorts (Feher *etal.* 2021). Similarly, utilizing Partial Least Squares (PLS) for feature extraction alongside Support Vector Machines (SVM) achieved a remarkable identification accuracy of 99.82% when distinguishing high-value *Bachu* mushrooms from common sympatric fungi using specific stalk spectral data (Rui Gao *et al.* 2020).

Deep Learning and Computer Vision for Classification

Artificial Intelligence (AI), specifically the deployment of deep Convolutional Neural Networks (CNNs), represents the frontier of automated visual classification in mycology. By

training deep learning architectures on vast, augmented image datasets encompassing diverse visual features—such as cap geometry, hymenium structure, and stipe texture—AI systems can predict species toxicity with precision rates that exceed human expert capabilities (Akshaya and Beegam, 2023).

Recent architectural evaluations reveal that modified, pre-trained deep learning networks exhibit varying degrees of computational efficiency and accuracy in differentiating edible from poisonous species.Utilizing Gradient-weighted Class Activation Mapping (Grad-CAM and Grad-CAM++), AI (XAI) frameworks system generate heat maps that visually highlight the specific morphological regions (e.g., universal veil remnants, annulus structures, or specific cap margins) that dictate the algorithm's toxicity classification, thereby ensuring the model's decisions correspond to biologically meaningful mycological features[29]. Furthermore, ensemble strategies, employing soft voting consensus across disparate architectures like DenseNet121 and MobileNet-V3, have achieved robust real-world accuracies of 95.67% and a minimal log loss of 0.175, providing a highly reliable, field-deployable tool against accidental ingestion (Korkmaz *et al*. 2026).

Genomic Architecture and Strain Engineering

Maximizing biological efficiency (BE), enhancing the synthesis of targeted bioactive compounds, and overcoming the pervasive issue of strain degeneration require rigorous and precise genetic intervention (Dong *et al*.2022). The genetic complexity of basidiomycetes—characterized by unique dikaryotic life cycles, clamp connections, and highly complex multi-allelic mating systems—has historically impeded rapid agricultural breeding programs (Wang *et al*.2024).

Traditional Breeding, Mutagenesis, and Recombination

Conventional crop improvement in mycology relies heavily on phenotypic selection and intraspecific hybridization (Barh*et al.*2019; Akhtar *et al*.2022). Through arduous single-spore isolation and dual culture techniques, compatible monokaryons are manually crossed (monokaryon-monokaryon or di-mon mating) to generate novel dikaryotic mycelia exhibiting heterosis, or hybrid vigor (Fig. 2). While physical mutagens (such as low-power He-Ne lasers or Atmospheric and Room Temperature Plasma - ARTP) and chemical mutagens (ethyl methanesulfonate - EMS) can artificially induce genetic variability, these methodologies are inherently flawed (Akhtar *et al .* 2022). They induce random, untargeted chromosomal alterations, require massive screening efforts (utilizing isoenzyme electrophoresis or drug resistance markers), and suffer from exceptionally high spore mortality rates. It has been reported that, gamma irradiation of *Agaricus bisporus* basidiospores results in mortality rates ranging from 50.6% to 90.63% (Dong *et al.*2022).

Molecular Breeding: The CRISPR-Cas9 Revolution

The advent of the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated nuclease 9 (Cas9) system has fundamentally disrupted fungal genetics, superseding older technologies like Zinc-Finger Nucleases (ZFNs) and TALENs(Shanmugaraj *et al*.2025). CRISPR-Cas9 enables marker-free, highly targeted, and cost-effective genomic modifications, allowing researchers to rationally design strains directly addressing industrial bottlenecks (Dong *et al*. 2022) (Fig.3).

The mechanistic action of the CRISPR-Cas9 system involves a synthetic single guide RNA (sgRNA) directing the Cas9 endonuclease to a highly specific genomic locus. The HNH and RuvC active domains of the Cas9 protein induce a double-strand break (DSB) 3- to 4-bp upstream of a Protospacer Adjacent Motif (PAM) (Zhang *et al*.2023). The fungal cell subsequently attempts to repair the catastrophic DNA damage via one of two endogenous pathways. The error-prone Non-Homologous End Joining (NHEJ) pathway introduces insertions or deletions (indels), effectively knocking out target gene function. Alternatively, Homology-Directed Repair (HDR) utilizes an exogenously supplied donor DNA template to achieve precise point mutations or the insertion of entirely novel genetic

cassettes(Shanmugaraj *et.al*. 2025).Summary of advanced CRISPR-Cas9 genomic engineering applications in commercial fungi is presented in Table 4.The deployment of CRISPR-Cas9 across commercial fungal species has yielded transformative results as follows:

Agaricus bisporus

The *Agrobacterium*-mediated transformation (AMT) to target the *ppo4* gene. By knocking out polyphenol oxidase activity, the resulting strain exhibits delayed enzymatic browning, drastically extending post-harvest visual quality and shelf life (Zhang *et al*.2023).

***Pleurotus* Species**

In *P. ostreatus*, marker-free editing through transient expression has been utilized to elucidate the regulatory roles of functional genes like *pcc1* and *clp1*. In *P. eryngii*, highly efficient HDR and NHEJ systems targeting the *pyrG* locus have been established (Dong *et al.*2022).

Ganoderma lucidum

Optimization of the U6 promoter for *in vivo* sgRNA transcription and the gpd promoter for Cas9 expression has enabled the precise disruption of the *ura3* metabolic marker gene. Furthermore, editing the *ku70* gene has artificially suppressed the NHEJ pathway, forcing the cell to rely on HDR, thereby exponentially increasing the efficiency of targeted gene insertion for advanced metabolic engineering(Dong *et al*.2022).

Cordyceps militaris

A monumental leap in multiplex editing was achieved with the development of the marker-free CRISPR/Cas9-TRAMA system. By exploiting the multiplex RNA-processing function of endogenous tRNA elements, researchers simultaneously edited multiple synthetase genes responsible for *cordycepin* and *ergothioneine* production. Furthermore, the system successfully executed the deletion of massive 10-kb metabolic synthetic clusters. This targeted metabolic editing profoundly amplifies the yield of high-value pharmaceutical compounds and provides a blueprint for extracting rare secondary metabolites from medicinal mushrooms (Zhang *et al*.2023).

Next-Generation Cultivation Technologies and Precision Agriculture

The transition from environmentally exposed, seasonal substrates to highly controlled, indoor agro-industrial biomanufacturing requires the integration of sophisticated cyber-physical systems.

Smart Farming, IoT, and Edge Computing Architectures

The Internet of Things (IoT), functioning in tandem with Artificial Intelligence (AI), establishes dynamic, microclimate-controlled environments capable of optimizing biological yield while aggressively minimizing resource waste (Anonymous, 2026). Modern smart cultivation chambers employ dense sensor arrays to continuously and autonomously monitor critical physiological parameters: ambient temperature, relative humidity, substrate core moisture, CO_2 concentration, and precise lux levels (Omar *et al*. 2024).

Advanced predictive modeling, utilizing ensemble machine learning algorithms (such as Decision Trees, Random Forests, and Support Vector Machines), analyzes real-time sensor telemetry to forecast micro-weather states within the grow room. These algorithms then autonomously actuate climate control mechanisms—such as HVAC systems, ultrasonic humidifiers, and CO_2 exhaust vents—achieving predictive environmental control accuracies approaching 100% (Omar *et al.* 2024). A critical architectural advancement within these smart systems is the fundamental shift from traditional Device-Cloud protocols toward Edge Computing paradigms(Nguyen*et al*.2024). Modern greenhouse sensor networks and high-definition computer vision cameras generate massive datasets. Transmitting this raw Big Data directly to cloud servers incurs unacceptable latency, creates bandwidth bottlenecks, and drastically inflates commercial service costs. By implementing a multi-layered Sensor-AI-System



Fig. 1: Different kinds of mushroom cultivated in India . **A**- Oyster mushroom; **B**- Paddy Straw mushroom **C**- Button mushroom; **D**- Milky White mushroom ; **E**- Morchella; F- Split gill mushroom

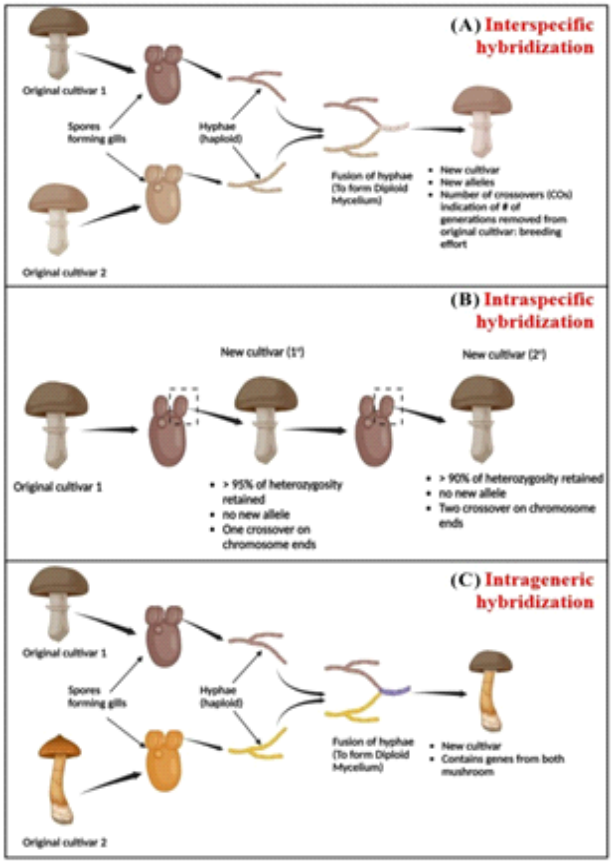


Fig.2: Inter-specific, intra-specific and intra-genic hybridization (Singh *et al*.2025)

(SAIS) that processes data locally at the "edge" utilizing lightweight AI for anomaly detection and temporal resampling, farms achieve low-cost data pre-processing. This enables instantaneous, localized actuation of environmental controls, which is absolutely crucial for maintaining the exceptionally narrow physiological tolerances required during the fungal pinning and fruiting phases (Nguyen *et al*.2024).

The Industrial Transition to Liquid Spawn Technology

Spawn formulation fundamentally dictates the speed, vigor, and ultimate success of substrate colonization. Historically, solid matrix spawn—utilizing sterilized cereal grains, sawdust, or wooden dowels—was the unquestioned industry standard (Gopinath *et al*. 2023). However, "liquid spawn" technology—where pure mycelial cultures are suspended and expanded within a continuously agitated, sterile liquid nutrient broth—is rapidly supplanting solid spawn in high-volume industrial applications.

The biomechanical advantage of liquid spawn lies in the sheer density and homogenous distribution

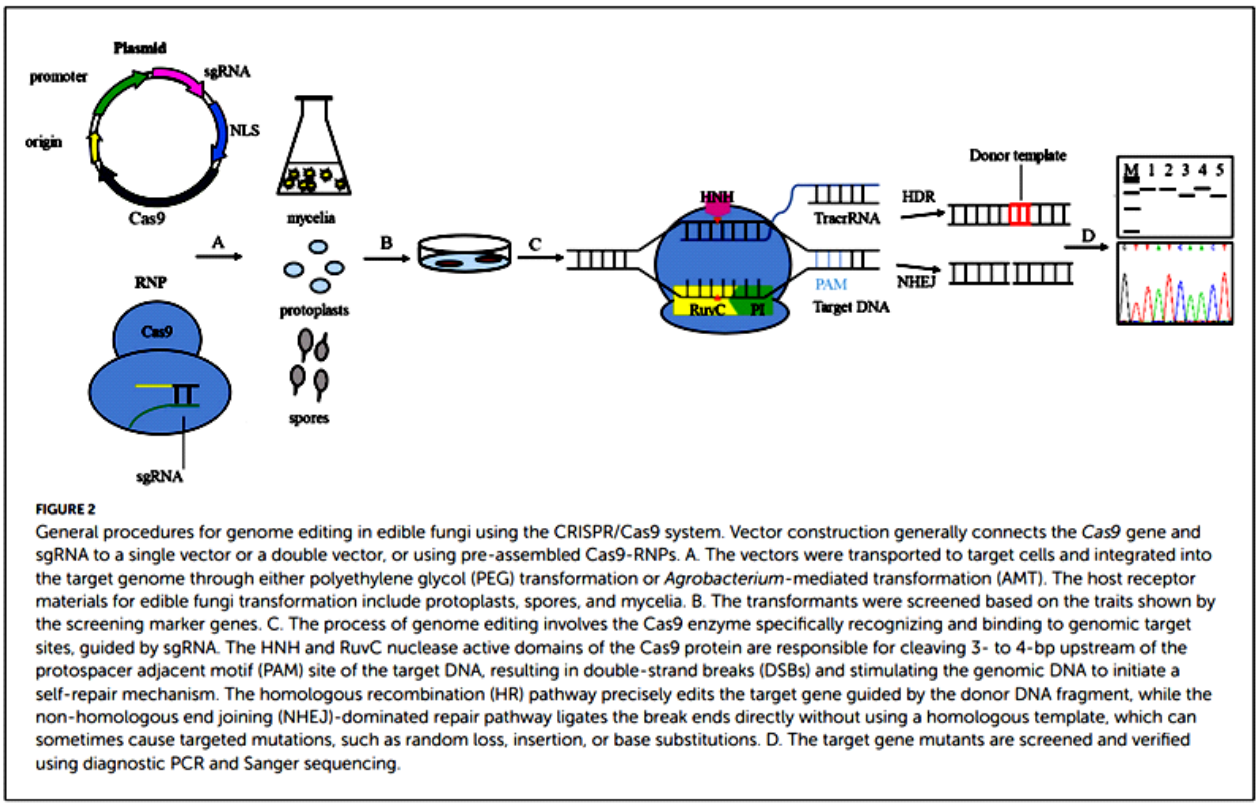


FIGURE 2
General procedures for genome editing in edible fungi using the CRISPR/Cas9 system. Vector construction generally connects the *Cas9* gene and sgRNA to a single vector or a double vector, or using pre-assembled Cas9-RNPs. A. The vectors were transported to target cells and integrated into the target genome through either polyethylene glycol (PEG) transformation or *Agrobacterium*-mediated transformation (AMT). The host receptor materials for edible fungi transformation include protoplasts, spores, and mycelia. B. The transformants were screened based on the traits shown by the screening marker genes. C. The process of genome editing involves the Cas9 enzyme specifically recognizing and binding to genomic target sites, guided by sgRNA. The HNH and RuvC nuclease active domains of the Cas9 protein are responsible for cleaving 3- to 4-bp upstream of the protospacer adjacent motif (PAM) site of the target DNA, resulting in double-strand breaks (DSBs) and stimulating the genomic DNA to initiate a self-repair mechanism. The homologous recombination (HR) pathway precisely edits the target gene guided by the donor DNA fragment, while the non-homologous end joining (NHEJ)-dominated repair pathway ligates the break ends directly without using a homologous template, which can sometimes cause targeted mutations, such as random loss, insertion, or base substitutions. D. The target gene mutants are screened and verified using diagnostic PCR and Sanger sequencing.

Fig. 3 : General procedure for genome editing in fungi using CRISPR/Cas9 System (Dong *et al.*2022)

Table 1. Global mushroom production trends, volume, and continent-wise CAGR distribution

Geographic Region	Global Production Share (%)	Production Volume (Million Tonnes, 2021)	Historical CAGR (2000-2021)
Asia	~95.0%	41.80	8.97%
Europe	~3.0%	1.30	1.29%
Americas	~1.0%	0.46	0.77%
Africa	<1.0%	0.01	6.31%

Table 2: Leading mushroom-producing states in India, market shares, and climatic alignment

State	National Production Share (%)	Dominant Cultivated Species	Climatic Adaptability
Bihar	11.30%	*Pleurotus* spp., *C. indica*, *A. bisporus*	Subtropical plains; winter window for Button
Odisha	10.96%	*Volvariella* spp., *Pleurotus* spp.	Warm coastal; ideal for Straw mushrooms
Maharashtra	10.34%	*A. bisporus*, *L. edodes*	Controlled indoor environments
Uttar Pradesh	7.10%	*C. indica*, *A. bisporus*	Subtropical plains; highly profitable Milky cultivation
Haryana	6.83%	Seasonal *A. bisporus*	Hot semi-arid; reliant on winter seasonality
Rajasthan	6.34%	*Pleurotus* spp.	Arid; requires active humidification
Punjab	6.27%	Seasonal *A. bisporus*, *Pleurotus* spp.	Hot semi-arid; winter crop rotation

Source: Pandya *et al.* 2024



Liquid spawn | Grain spawn | Saw dust spawn | Plug spawn

Fig. 4: Various types of spawn used for mushroom cultivation

Table 3 : Performance metrics and computational efficiency of deep learning models in mushroom toxicity classification

CNN Architecture	Overall Accuracy (%)	Precision (%)	F1 Score (%)	Computational Training Time
ResNet-50	99.50	99.33	99.66	5 min 50 s
GoogLeNet	99.50	99.35	99.97	2 min 20 s
EfficientNet-B0 (Hybrid with SVM)	99.50	-	-	-
AlexNet	99.00	98.61	99.30	1 min 42 s
Custom Region-CNN (R-CNN)	98.50	99.39	99.09	1 min 10 s

Source: Ketwongsa *et al*. 2022

Table 4 : Summary of advanced CRISPR-Cas9 genomic engineering applications in commercial fungi

Fungal Species	Targeted Gene Locus	Primary Cas9 Promoter	Transformation Delivery Method	Engineered Application
Agaricus bisporus	*ppo4*	gpd	Agrobacterium-mediated (AMT)	Shelf-life extension via browning inhibition
Ganoderma lucidum	*ura3*, *ku70*	Pgpd	Protoplast-mediated (PMT)	Metabolic pathway optimization; HDR enhancement
Cordyceps militaris	*Cns1B*, *Egt1*	cmcas9	PEG-mediated protoplast	Cordycepin a nd ergothioneine amplification
Shiraiabambusicola	*SbaPKS*, *ku80*	EF1α and Pgpd	Protoplast-mediated (PMT)	Hypocrellin biosynthesis enhancement
Flammulinafiliformis	*pyrG*, *Mads-8*	gpd	Protoplast-mediated (PMT)	Morphological and developmental control

Source: Zhang *et al.*2023

of active mycelial fragments. Upon injection into a sterilized substrate block, the liquid percolates evenly, providing thousands of simultaneous inoculation points. This extreme uniformity radically accelerates the colonization rate (the spawn run duration), which drastically reduces the temporal window in which competitive microbial contaminants (such as *Trichoderma*molds) can establish a foothold(Dong *etal*. 2026) (Fig.4). In commercial facilities, the adoption of liquid spawn has compressed the production cycle of Yellow Enoki mushrooms from 60 days down to a mere 7 days, facilitating the automated inoculation of up to 10,000 bags

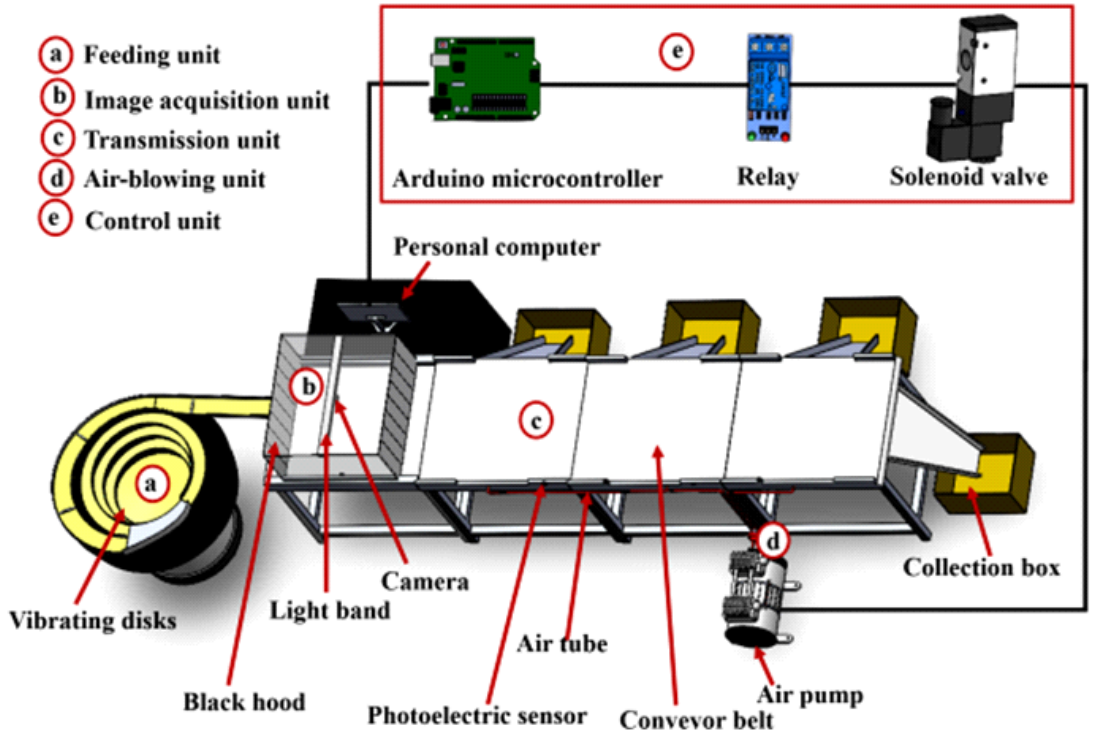


Fig. 5: The designed automatic mushroom-grading system(Lv *etal.* 2025**)**

Table 5 : Biological Efficiency metrics of secondary mushroom cultivation utilizing recycled SMS

Primary Mushroom Species	Secondary Cultivated Species	Supplementation Strategy	Resulting Biological Efficiency
Mixed SMS	*Pleurotus pulmonarius*	Wheat bran, soybean flour, $CaCO_3$	208%
Mixed SMS	*Pleurotus ostreatus*	Wheat bran, soybean flour, $CaCO_3$	185%
P. nameko, *H. erinaceus*	*Pleurotus ostreatus*	Wheat straw, bran (3%), gypsum (1%)	66–73%
Mixed SMS	*Auricularia polytricha*	Rice bran (9.5%), $CaCO_3$ (0.5%)	41–59%

Source: Martín *et al.*2023

per worker per day, thereby maximizing capital turnover (Anonymous,2018).The economic implications are staggering. In rigorous scientific trials optimizing *Tremella fuciformis* cultivation, the application of liquid spawn—specifically co-cultured with the essential symbiotic fungus *Annulohypoxylonstygium*—increased total fruiting body yield by 11.1% over solid spawn controls, while simultaneously eliminating the batch-to-batch variation that plagues solid spawn production (Lin *et al*.2025).

Nanotechnology in Mushroom Bio-fortification and Cultivation

The application of nanoscale fertilizers (NFs) and metal oxide nanoparticles fundamentally alters nutrient delivery dynamics. Owing to their exceptionally high surface-area-to-volume ratio, nanoparticles can bypass traditional cellular barriers (such as the Casparian strip in higher plants) and facilitate highly efficient cellular uptake via apoplastic or symplastic pathways (Gulaiya *et al*. 2025).

In a landmark investigation targeting nutritional

enrichment, lignocellulosic substrates were biofortified with Zinc Oxide Nanoparticles (ZnONPs) at specific concentrations of 20 and 40 ppm during the spawning stage of *Pleurotus djamor* and *P. florida*. The introduction of ZnONPs not only accelerated mycelial radial growth and increased overall biological efficiency but also actively upregulated critical anti-oxidative enzymatic pathways. This resulted in elevated intracellular levels of phenols and flavonoids, effectively biofortifying the resulting fruiting bodies with highly bioavailable micro-nutrients (Leema *et al.* 2025).

Conversely, mushrooms themselves are being utilized in the "green synthesis" of nanotechnologies. "Myco-NPs"—nanoparticles synthesized utilizing the rich matrix of mushroom polysaccharides, flavones, and reductases as both reducing and stabilizing agents (e.g., silver nanoparticles derived from *Agaricus bisporus* and *Ganoderma* spp.)—function as highly potent, eco-friendly antimicrobial agents, offering sustainable alternatives to synthetic agrochemicals(Torós *et al,* 2025).

Autonomous Harvesting and Computer Vision Systems

To resolve the global labour shortages, rising agricultural wage inflation, and the delicate biomechanics of fruiting bodies, the industry is deploying advanced robotic harvesting systems. These fully automated platforms integrate multi-axis robotic arms equipped with soft-grip pneumatic end-effectors, synchronized with state-of-the-art computer vision arrays (Anonymous, June, 2026). Utilizing RGB-D (color and depth) cameras, such as the Microsoft Azure DK 3D, the vision system captures the complex spatial geometry of the growing beds. Deep learning instance segmentation models—specifically architectures like YOLOv8-seg, Detectron2, and Mask R-CNN—analyze the imagery to identify mature caps amidst dense visual clutter and overlapping fruiting bodies (Rowland *et al.* 2025).

The algorithms compute precise spatial coordinates and identify optimal stem cut-points. YOLOv8-seg, for instance, has demonstrated a mean average precision (mAP) of 67.9, allowing the robotic manipulator to approach and sever the mushroom with calculated force feedback (Rowland *et al*. 2025). This precision ensures uniform force application, effectively eliminating mechanical bruising. Consequently, automated harvesting translates to a direct 15-25% improvement in marketable yield for highly sensitive, premium varieties like Shiitake and Oyster mushrooms.

IoT Sensor Networks and Environmental Micro-Management

Modern smart mushroom farms deploy highly decentralized wireless sensor networks (WSNs) utilizing robust microcontrollers such as the ESP32, Arduino Uno, or Raspberry Pi. These computing nodes are integrated with precision environmental sensors, notably the DHT-11 or AM2302 for temperature and humidity tracking, and MQ135 gas sensors for precise CO2 and air quality monitoring.High-Speed Automated Grading and Sorting (Chong *et al*.2023).

Computer Vision Applications in Post-Harvest Quality Assessment

Post-harvest, computer vision is further deployed to eliminate the subjectivity, inconsistency, and inefficiency of manual quality grading. High-speed, non-destructive optical sorting systems utilize lightweight convolutional neural networks (such as modified YOLOv5) to evaluate mushrooms based on rigorous morphological criteria, including color uniformity, spatial shape, stem cut angle, and cap veil opening (Shui *et al*.2026).

In a highly optimized implementation for *Agaricus bisporus*, individual mushrooms are singulated onto a conveyor belt via a 60 Hz vibrating disk—a frequency determined to maximize throughput without inducing physical damage 51. As the mushrooms traverse the conveyor at 130.17 mm/s, an overhead industrial camera captures continuous image frames. The PC-based YOLOv5 model instantly classifies the mushroom's grade and transmits the data to an Arduino microcontroller. Synchronized with photoelectric sensors, the microcontroller

actuates specific solenoid valves, utilizing targeted blasts of compressed air to cleanly blow the mushroom into its designated quality bin 49. This frictionless, air-blown system achieves an astounding grading speed of 1066.67 mushrooms per minute while maintaining an accuracy rate of 97.87%, representing a monumental leap over human capacity (Shui *et.al.* 2026) (Fig.5).

Methods for increasing biological efficiency

The biological efficiency (BE) of paddy straw mushrooms (*Volvariella volvacea*) in India varies in-between 8–10% in traditional methods which can be improved to over 18%, by using cagemethod of cultivation (12.10% to 15.2%); four -layered bed(17.6%). Cultivation in a thatched shed (16.40% BE) or climate-controlled rooms provides a higher, more stable yield compared to outdoor, uncovered beds.Pre-soaking of paddy straw @ 2% calcium carbonate solution for 8 hours can increase BE, up to 14.52%.Application of Indole Acetic Acid (IAA) at 200 ppm, or growth regulators like Gibberellic Acid (GA3) at 50 ppm, has been shown to increase BE to 13.14% (Biswas and Layak, 2014)

Oyster mushrooms in India can achieve biological efficiency (BE) exceeding 100% when cultivated on optimized substrates. A 1:1 mixture of paddy and wheat straw, supplemented with 5–10% rice or wheat bran, provides a favorable carbon-to-nitrogen ratio for enhanced growth and yield. The use of a 4–5% spawn rate, maintenance of 70–80% relative humidity, and an optimal substrate quantity of 3 kg (wet weight) further improve productivity by balancing spawn run speed and yield. Effective substrate pasteurization through hot-water treatment for 2 hours or formalin–bavistin treatment is essential for reducing contamination and ensuring successful cultivation.Key methods involve optimal substrate preparation (pasteurization/fermentation), substratesupplementation, and proper environmental control, boosting yields up to 125.75% (Biswas and Biswas, 2015).The Hb4 strain is a highly productive, genetically improved hybrid of the white button mushroom (*Agaricus bisporus*) developed by agricultural researchers in India. It consistently yields higher (26.94 % BE) and produces firmer, more uniform mushrooms compared to traditional parental lines (Sharma *et al.* 2016).

Domestication of Novel High-Value Species

The Morchella Paradigm

While *Agaricus*, *Pleurotus*, and *Lentinula* dominate aggregate global volumes, the strategic domestication of ultra-high-value, previously uncultivable wild species represents the absolute frontier of modern mycology. The Black Morel (*Morchella* spp.) is a highly prized, astronomically expensive culinary mushroom whose complex, poorly understood soil ecology and obligate symbiotic relationships previously precluded artificial cultivation(Liu *et al.,*2023) (Fig.1 E).Recent ecological insights and methodological innovations have finally facilitated the transition of *Morchella* from a strictly wild-foraged commodity to a cultivated crop, typically managed under semi-controlled conditions such as high tunnels or specialized shade greenhouses. Cultivators prepare well-draining outdoor beds comprising a mixture of soil, compost, peat moss, and hardwood chips, carefully buffered to a pH range of 6.0 to 7.5(Liu *et al*.2023). Recent ecological insights and methodological innovations have finally facilitated the transition of ^{2}. Grain spawn is inoculated directly into the soil when ambient temperatures rest between 50-70°F. Application of sterilized, compressed nutrient blocks directly onto the surface of the colonized soil. Following the complete assimilation of the Exogenous Nutrient Bag Innovation ENBs, the depleted bags are physically removed. The sclerotia-laden bed must then undergo an obligatory period of cold stratification. This precise transition from cold conditioning to spring warming triggers the sclerotia to expend their stored energy, initiating the explosive growth of the Morel fruiting bodies(Anonymous,2026 b).

Split Gill Mushroom (Schizophyllum commune)

Split Gill Mushroom (*Schizophyllum commune*) cultivation in India is a promising, climate-resilient enterprise that grows well at 25–35°C on lignocellulosic substrates such as paddy straw

and sawdust (Fig.1F). Cultivation involves substrate soaking, sterilization or pasteurization, spawning, and maintenance of 80–90% relative humidity for successful fruiting and harvest. The mushroom is particularly popular in Northeast India and is valued for its high protein content and the presence of the bioactive â-glucan schizophyllan, which possesses prebiotic and medicinal properties. These attributes make it an important functional food with growing commercial and nutritional significance(Singh *et al*. 2021).

Post-Harvest Physiology, Value Addition, and Shelf-Life Extension

The very biological characteristics that make fresh mushrooms culinarily desirable—high moisture content, lack of a protective epidermal cuticle, and exceptionally high post-harvest respiration rates—render them highly perishable due to microbial spoilage, and pervasive enzymatic browning (melanosis) driven by polyphenol oxidase activity(Liufang *et al*. 2024).

Advanced Post-Harvest Preservation Modalities

To maintain intracellular turgor, suppress respiration, and chemically inhibit oxidative decay, the industry is moving beyond standard refrigeration to deploy advanced physiochemical interventions:

A.***Ultrasound-Enhanced Freezing (UIF):***

UIF utilizes high-intensity ultrasonic waves (300-400 W) to physically agitate the water molecules during the freezing process. This acoustic cavitation accelerates the freezing rate and forces theformation of microscopic, highly uniform intra-cellular ice crystals. The UC300 treatment effectively preserves the mushroom's microstructural integrity, significantly reducing post-thaw weight loss, maintaining textural metrics like gumminess and chewiness, and recording the lowest Total Color Difference (E) and Browning Index (BI) values(Priya *et al.* 2025).

B.***Ergothioneine (EGT) Application and Nano-Coatings*:**

Ergothioneine is a unique, highly stable thiol-containing amino acid synthesized by specific fungi. When applied exogenously at a concentration of 0.12 mmol L^{-1}, EGT acts as a potent antioxidant, effectively penetrating the mushroom tissue to inhibit polyphenol oxidase activity and dramatically delay melanosis(Priya *et al.* 2025). Furthermore, the application of advanced biopolymer coatings—utilizing chitosan-dextran films, nano-gelatin layers, or chitosan infused with essential oils—creates a semi-permeable barrier on the mushroom cap. This artificial cuticle restricts oxygen ingress, slows the physiological respiration rate, and can extend refrigerated shelf-life up to an impressive 28 days.

C. *Modified Atmosphere Packaging (MAP*):

Modified Atmosphere Packaging (MAP) alters the gaseous environment within the package to reduce respiration and metabolic activity in mushrooms. The use of multi-layer PET/LLDPE barrier films to maintain an atmosphere of approximately 15% O, , 5% CO, , and 80% N, effectively delays oxidative browning and inhibits aerobic microbial growth, thereby extending shelf life beyond 10 days under refrigerated storage at 4°C (Priya *et al.* 2025).

D. ***Strategic Value Addition and Functional Foods*:**

To overcome the perishability of fresh mushrooms, surplus or lower-grade produce is increasingly

processed into value-added functional foods and nutraceutical products that capitalize on their bioactive compounds. Mushroom powders derived from mycelial biomass and fruiting bodies, particularly from *Pleurotus* species, are incorporated into processed meat products as functional binders, improving texture, reducing fat content, and enhancing dietary fibre levels. In the dairy industry, extracted â-glucans serve as effective prebiotics in products such as yogurt and soft cheeses, improving both gut health benefits and rheological properties. Additionally, extracts from medicinal mushrooms such as *Ganoderma lucidum* are incorporated into bakery products and herbal beverages, providing immune-modulatory and other health-promoting

benefits to consumers(Sangeeta *et.al.* 2025).

Spent Mushroom Substrate (SMS) and Spent Mushroom Compost (SMC) : Valuable Resources in the Circular Bioeconomy

The industrial cultivation of mushrooms generates staggering volumes of lignocellulosic waste. For every single kilogram of fresh mushrooms harvested, the process generates between 3 to 5 kilograms of Spent Mushroom Substrate (Luoa andChen, 2024).In 2018, the global mushroom industry generated approximately 64 million tonnes of spent mushroom substrate (SMS), with production projected to exceed 100 million tonnes by 2026. SMS of oyster mushroom has been demonstrated as biofertilzer for growth improvement of *Capsicum annum* (Roy *etal*. 2015). Traditionally regarded as an environmental waste, SMS was often disposed of through landfilling, open dumping, or incineration, leading to groundwater contamination and the emission of greenhouse gases such as methane (CH_4) and carbon dioxide (CO_2). However, SMS is now increasingly recognized as a valuable resource within the agricultural circular bioeconomy due to its potential for reuse in crop production, soil improvement, animal feed, bioenergy generation, and other value-added applications (Table 5). The ability of spent mushroom compost (SMC) of *Agaricus bisporus* to reduce root rot disease of mandarin caused by *Fusarium oxysporum*as well as enhancing plant growth could be attributed to the alteration of physical structure of soil and enhancement of plant friendly microbes by the compost (Barman *et al*. 2022). Induced resistance in mandarin plants against*F. oxysporum* following SMC treatment was further confirmed by immuno localization of defense enzymes both in root and lead tissues. The spent mushroom compost left after final crop harvest of button mushroom is a matter of concern for all of us to utilize for agricultural productivity as well as it can also ameliorate the problem of soild waste disposal in the mushroom industry.

Chemical Composition and Structural Degradation

SMS is not merely “dirt”; it is a complex, biochemically altered matrix. It comprises the remnants of the initial lignocellulosic raw materials (straw, sawdust, cottonseed hulls), substantial quantities of residual fungal mycelium, extracellular enzymes secreted during the growth phase, and non-cell-wall carbohydrates and proteins (Luoaand Chen,2024).During the primary cultivation cycle, the aggressive enzymatic action of white-rot fungi (such as *Pleurotus* and *Lentinula*) severely degrades the substrate’s structural integrity, resulting in massive mass losses: 26–46% of initial cellulose, 57–77% of hemicelluloses, and an astounding 61–75% of the highly recalcitrant lignin[59]. This pre-digested, lignin-stripped state renders SMS highly amenable to secondary biological and thermochemical processing.

Valorization Pathways and Biological Recycling

The most immediate and economically viable application of SMS is its re-utilization in successive cycles of mushroom cultivation. Because the complex lignin barriers have been enzymatically dismantled.SMS can be sterilized, re-supplemented with concentrated nitrogen sources (such as wheat bran or soybean flour), and utilized as a highly efficient base substrate. Cultivation trials leveraging recycled SMS have yielded phenomenal results; cultivating *Pleurotus pulmonarius* on optimally supplemented plain SMS achieved a record-breaking Biological Efficiency (BE) of 208%, while *Pleurotus ostreatus* achieved 185% BE (Martín *et al.* 2023).

Beyond secondary cultivation, SMS serves as a critical feedstock for environmental remediation and bio-energy production. Through advanced thermochemical processes such as hydro-thermal carbonization, SMS is efficiently converted into bioethanol, biogas, and high-porosity hydrochars(Sunil and Shamshina, 2026). These hydrochars function as exceptionally potent commercial biosorbents; when applied to highly toxic heavy-metal mine tailings, SMS-derived materials successfully sequestered and reduced the bioavailability of lead (Pb), zinc (Zn), copper (Cu), and cadmium (Cd), facilitating the phytoremediation of the site[58]. Furthermore, the rich organic carbon profile and abundance of residual bioactive compounds allow raw, composted SMS to function as a premium agricultural bio-fertilizer, directly improving soil

microbiomes, increasing crop yields, and mitigating plant-disease vectors without the application of synthetic agrochemicals(Martín *et al.* 2023).

CONCLUSION

The global mushroom industry has evolved into a technology-driven bioeconomy sector, powered by advances in molecular diagnostics, artificial intelligence, CRISPR-based breeding, IoT-enabled precision cultivation, and automation. These innovations are enhancing productivity, quality, and sustainability while transforming mushroom farming into a predictable and efficient enterprise. For emerging economies such as India, the adoption of these technologies offers immense opportunities for nutritional security, rural livelihood enhancement, and export growth. Coupled with advanced post-harvest management and the circular utilization of spent mushroom substrate, the mushroom industry represents a sustainable, zero-waste model for future agriculture and bio-based economic development.

ACKNOWLEDGEMENT

The author sincerely thanks the Indian Mycological Society, Kolkata, for selecting him for the prestigious Prof. S. B. Chattopadhyay Memorial Lecture Award. The author also gratefully acknowledges the opportunity to publish this review article in the Journal of Mycopathological Research and appreciates the support and encouragement received from the Society and the Journal.

DECLARATION

Conflict of Interest. Author declares no conflict of interest**.**

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