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Mushroom-Derived Bioactive Metabolites as Eco-Friendly Antifungal Agents Against Major Crop Diseases

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Abstract

Fungal phytopathogens cause devastating yield losses ranging from 20% to 40% in global food crops, with conventional synthetic fungicides facing critical challenges including resistance emergence, environmental toxicity, and regulatory restrictions. Macrofungi (mushrooms) represent an underexploited reservoir of eco-friendly bioactive metabolites with potent antifungal properties. This review comprehensively examines mushroom-derived bioactive compounds as sustainable alternatives for crop disease management, encompassing their chemical diversity, mechanisms of action, key species profiles, production methodologies, and formulation strategies. Mushroom metabolites exhibit remarkable structural diversity, including polyketides (strobilurins), bioactive proteins (Lentin), polysaccharides (β-glucans), triterpenoids (ganoderic acids), and phenolic derivatives. These compounds function through direct antifungal pathways disrupting mitochondrial respiration, compromising cell membrane integrity, and inhibiting spore germination alongside indirect mechanisms that prime plant systemic acquired resistance through salicylic acid and jasmonic acid signaling cascades. Key species including *Strobilurus tenacellus*, *Lentinula edodes*, *Ganoderma lucidum*, and *Pleurotus ostreatus* demonstrate potent activity against major pathogens such as *Pyricularia oryzae*, *Botrytis cinerea*, *Fusarium* species, and *Colletotrichum* spp. Biotechnological production via submerged and solid-state fermentation, coupled with advanced nanoencapsulation strategies using biodegradable polymers (chitosan, polycaprolactone, zein), addresses critical stability and field application barriers. Despite promising efficacy, commercialization faces challenges in batch-to-batch standardization, fermentation economics, and regulatory modernization. Integrating mushroom-derived biofungicides within integrated pest management frameworks offers a sustainable pathway to reduce chemical inputs, mitigate resistance development, and enhance global food security while aligning with consumer demand for residue-free agricultural produce.

Keywords: Mushroom Bioactive Metabolites, Antifungal Agents, Phytopathogens, Sustainable Agriculture, Biofungicides, Nanoencapsulation, Systemic Acquired Resistance, Integrated Pest Management

1. Introduction

Fungal phytopathogens represent one of the most severe threats to global agricultural security, causing annual crop yield losses between 20% and 40% across staple food supply chains and cash crops. Under favorable environmental conditions and within intensive monoculture systems, localized disease outbreaks can compromise up to 60% of crop yields, driving severe post-harvest decay and the accumulation of hazardous mycotoxins (Gai & Wang, 2024). For decades, modern crop protection has relied on synthetic chemical fungicides, including azoles, strobilurins, dicarboximides, and benzimidazoles. While these chemical inputs initially drove substantial increases in agricultural productivity, their continuous and single-site intensive application has created significant ecological and evolutionary complications (Jørgensen & Heick, 2021).

The rapid emergence of fungicide resistance among high-priority fungal pathogens such as *Botrytis cinerea*, *Pyricularia oryzae*, *Fusarium oxysporum*, and *Colletotrichum* species has severely eroded the field efficacy of major agrochemical classes. Pathogenic fungi adapt to synthetic inputs through specific molecular mechanisms, including point mutations in target genes (such as the G143A substitution in cytochrome b conferring resistance to quinone outside inhibitors, or mutations in the $ERG11$ gene reducing azole binding affinity), metabolic bypass pathways via $ERG3$ mutations, and the upregulation of ATP-binding cassette (ABC) efflux pumps driven by genes like $CDR1$ and $CDR2$ (Bosland, & Barchenger, 2023). Furthermore, persistent chemical residues in soil and aquatic ecosystems pose toxicological risks to non-target organisms, disrupt beneficial plant microbiomes, and raise food safety concerns due to lingering dietary residues. Strict regulatory withdrawals of conventional active ingredients, particularly within the European Union, alongside expanding consumer demand for residue-free organic produce, have accelerated the search for sustainable, bio-based alternatives (Sondhia, 2014).

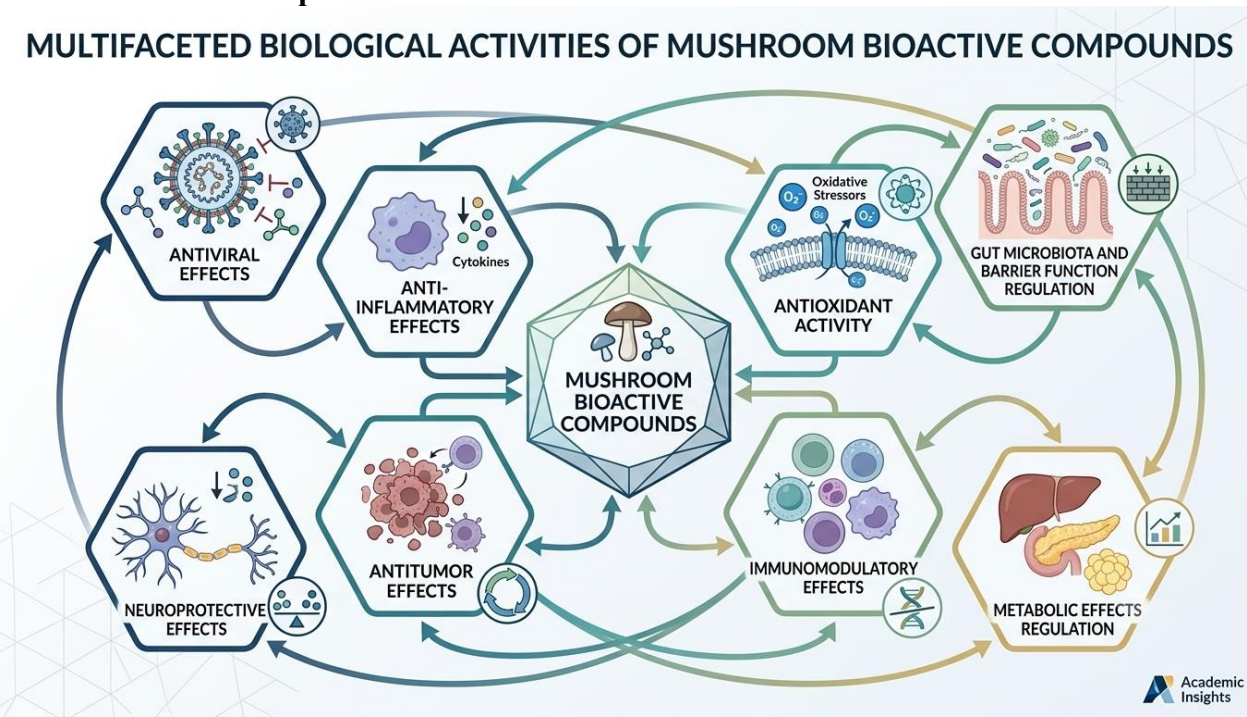
Macrofungi (mushrooms) belonging to the Basidiomycota and Ascomycota phyla represent an underutilized reservoir of bioactive molecules with strong antifungal properties. Operating as saprophytic decomposers, mycorrhizal symbionts, or parasites, macrofungi have evolved specialized metabolic machinery that synthesizes complex chemical defenses to compete in competitive microbial niches. Mushroom-derived metabolites offer diverse modes of action ranging from direct disruption of fungal mitochondrial respiration and cell wall synthesis to the indirect priming of plant systemic acquired resistance (SAR) (dos Santos et al., 2026).

Driven by integrated pest management (IPM) directives and organic farming trends, the market for biological crop protection is undergoing rapid expansion. Global market evaluations project the microbial biofungicide sector to grow from USD 1040 million to USD 4330 million over the next decade, representing an overall expansion of 415%. Concurrently, the overall biofungicide market is forecast to expand at a compound annual growth rate (CAGR) of 13.5%, rising from USD 2.5 billion to over USD 5.5 billion (Baker et al., 2020). This review examines mushroom-derived bioactive metabolites, evaluating their structural diversity, mechanisms of antifungal action, key species profiles, upstream production, downstream extraction, and nano-formulation strategies required to translate these natural products into commercial biofungicides (Sánchez, 2017).

2. Chemical Diversity and Classification of Mushroom Bioactive Metabolites

Macrofungi synthesize a vast array of chemical structures during their developmental phases, fruiting body formation, and vegetative mycelial expansion. These molecules are broadly categorized into high-molecular-weight primary metabolites and low-molecular-weight secondary metabolites (Niego et al., 2021).

Figure 1. Overview of the multifaceted biological activities and systemic effects of mushroom-derived bioactive compounds.



2.1 Primary Metabolites

Primary bioactive metabolites are integral to cellular architecture, growth, and energetic processes, yet many possess pronounced antimicrobial and immunomodulatory activity. Polysaccharides and glycoproteins represent the primary structural components of macrofungal cell walls, dominated by β-D-glucans featuring a β-(1\$\rightarrow3) $-$ $linkedglucosebackbonewithvariable\$\beta\$-$(1\rightarrow\$6) side branching. Prominent examples include lentinan isolated from *Lentinula edodes*, schizophyllan from *Schizophyllum commune*, and water-soluble heteropolysaccharides from *Ganoderma lucidum*. These biopolymers interact directly with host plant cellular receptors to act as pathogen-associated molecular patterns (PAMPs), initiating downstream defense signaling (Koti et al., 2025).

In addition to structural carbohydrates, macrofungi produce bioactive proteins, lectins, ribonuclease-like enzymes, and ribosome-inactivating proteins that directly suppress fungal hyphal growth. A representative example is Lentin, a 27.5 kDa antifungal protein isolated from *Lentinula edodes* fruiting bodies. Lentin exhibits sequence similarity to endoglucanases and demonstrates direct inhibitory activity against diverse phytopathogenic fungi by compromising hyphal membrane integrity (Sánchez, 2017).

2.2 Secondary Metabolites

Secondary metabolites are low-molecular-weight compounds (< 1000 Da) synthesized via specialized biosynthetic gene clusters (BGCs) that are not strictly essential for basic vegetative survival, but provide competitive ecological advantages. Polyketides assembled by polyketide synthases (PKS) include some of the most successful agrochemical frameworks. The premier example is the strobilurin family (e.g., strobilurin A, B, D, E, F, G, H) isolated from *Strobilurus tenacellus* and related basidiomycetes. Strobilurins feature a central E-β-methoxyacrylate or related toxophore responsible for disrupting electron transport in fungal mitochondria (Marinelli & Marcone, 2011).

Terpenoids and steroids, synthesized via the mevalonate or non-mevalonate pathways, encompass sesquiterpenoids, diterpenoids, and oxygenated triterpenoids. Notable members include ganoderic acids (lanostane-type triterpenoids) from *Ganoderma lucidum*, rufuslactone from *Lactarius rufus*, and

various bioactive ergosterol derivatives. Nitrogen-containing secondary metabolites derived from amino acids encompass diketopiperazines, indole derivatives, cytochalasins, and oxindoles. Compounds such as 3-hydroxy-3-methyloxindole from *Pleurotus ostreatus* disrupt fungal cellular signaling, membrane fluidity, and cell division (Dewick, 2002). Furthermore, macrofungi synthesize simple phenolics, hydroxycinnamic acids (e.g., caffeic acid, ferulic acid, chlorogenic acid), catechins, and phthalide derivatives. Compounds such as 4,6-dimethoxyphthalide and 5,7-dimethoxyphthalide exert direct toxicity against fungal spore germination and hyphal differentiation (Vazquez-Armenta et al., 2022).

Table 1. Key Bioactive Metabolite Classes from Macrofungi and Their Structural Features.

Chemical Class	Representative Mushroom Species	Key Bioactive Metabolites	Target Pathogens	Primary Mechanism of Action
Polyketides	*Strobilurus tenacellus*, *Oudemansiella mucida*	Strobilurin A, B, D; Oudemansin A	*Pyricularia oryzae*, *Puccinia recondita*, *Erysiphe graminis*	Reversible binding to the Q_o site of the mitochondrial cytochrome bc_1 complex, arresting ATP production.
Proteins & Peptides	*Lentinula edodes*	Lentin (27.5 kDa protein)	*Botrytis cinerea*, *Physalospora piricola*, *Mycosphaerella arachidicola*	Cell wall hydrolytic action, cell membrane perforation, and disruption of apical hyphal extension.
Polysaccharides	*Lentinula edodes*, *Schizophyllum commune*, *Ganoderma lucidum*	Lentinan, Schizophyllan, β-(1\rightarrow3)(1\rightarrow6)-glucans	*Colletotrichum* spp., *Botrytis cinerea*, *Fusarium* spp.	Priming host plant innate immunity via Systemic Acquired Resistance (SAR); activation of PAL, POD, and PPO enzymes.
Triterpenoids	*Ganoderma lucidum*	Ganoderic acids (A, B, C, D)	*Colletotrichum capsici*, *Magnaporthe oryzae*, *Fusarium* spp.	Disruption of fungal cell membrane integrity, ergosterol interaction, and induction of ROS-

				mediated oxidative stress.
Phthalides & Oxindoles	*Pleurotus ostreatus*	4,6-Dimethoxyphthalide, 3-Hydroxy-3-methyloxindole	*Pyricularia oryzae*, *Alternaria alternata*, *Sclerotinia sclerotiorum*	Inhibition of conidial germination, germ tube elongation, and appressorium differentiation.

3. Mechanisms of Antifungal Action

The efficacy of mushroom-derived biofungicides stems from a dual-action framework combining direct suppression of fungal development with indirect amplification of host plant defenses (Stajic et al., 2017).

3.1 Direct Fungicidal and Fungistatic Pathways

Direct antifungal mechanisms involve physical or biochemical interactions between mushroom metabolites and target fungal cell structures. The classic paradigm of respiration inhibition is exemplified by strobilurins, which bind site-specifically to the quinone outside (Q_o) site (also termed the ubihydroquinone oxidation center) of the cytochrome bc_1 complex (Complex III) within the inner mitochondrial membrane (Jha et al., 2023). By binding to cytochrome b, these molecules block electron transfer to the Rieske iron-sulfur protein and cytochrome c_1. This electron blockade halts proton translocation across the inner membrane, preventing the generation of the proton motive force required for ATP synthase. Deprived of cellular energy, fungal spore germination, germ tube elongation, and active nutrient transport immediately cease, ultimately driving cell death (Trumpower, 1981).

Direct structural damage is also mediated through cell wall degradation and cell membrane disruption. Bioactive terpenoids and lipophilic sterols intercalate into the fungal plasma membrane, destabilizing the phospholipid bilayer, causing leakage of intracellular potassium ions and phosphate, and depleting ergosterol content. Concurrently, bioactive proteins like Lentin hydrolyze structural cell wall polymers, inducing apical hyphal tip swelling, vacuolization, protoplast collapse, and cellular lysis (Lou et al., 2025). Low-molecular-weight phenolics, phthalides, and oxindoles penetrate conidial walls to arrest early morphogenesis. By suppressing conidial germination and appressorium differentiation (the specialized structures required for cuticular penetration), these metabolites prevent pathogenic fungi from breaching host plant epidermal layers (Thines et al., 2004).

3.2 Indirect Mechanisms: Host Defense Priming and Systemic Resistance

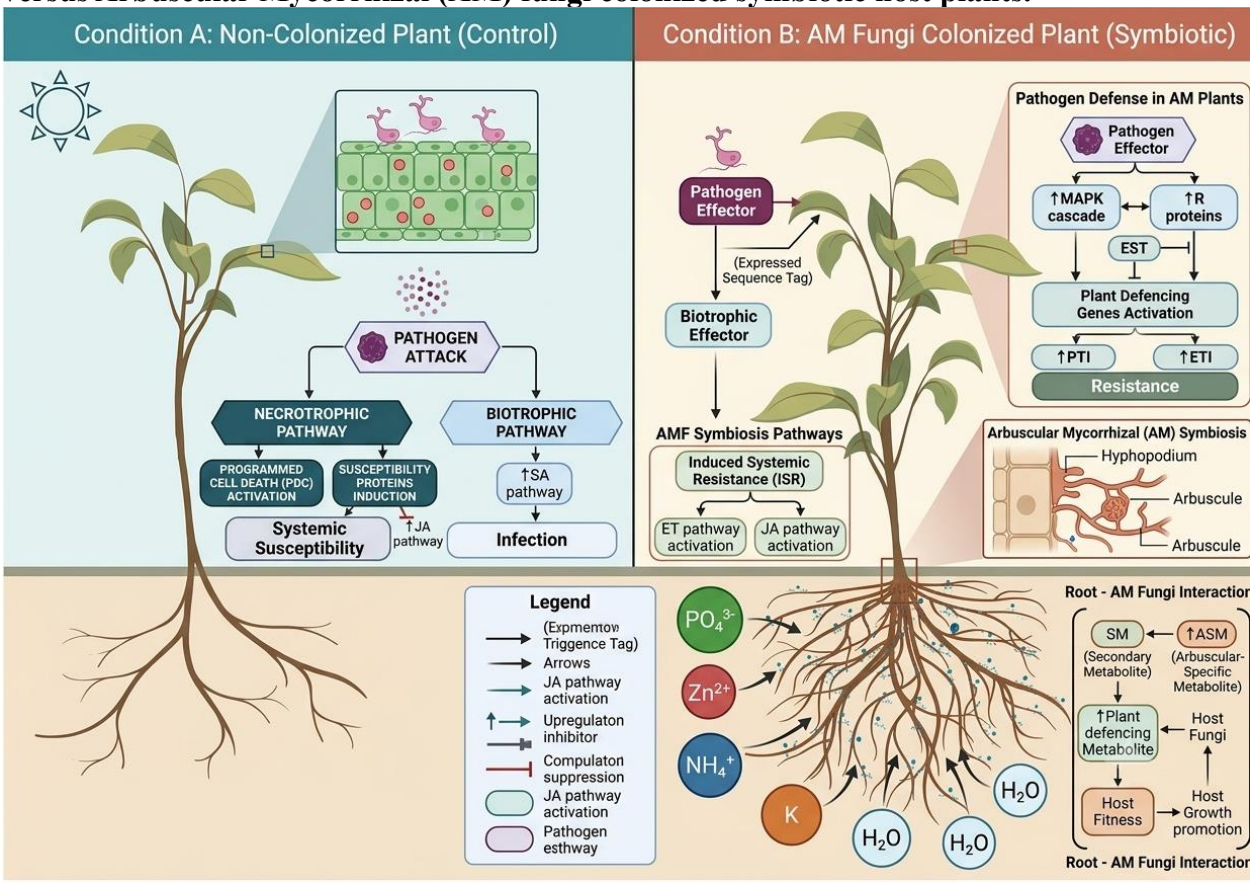
Beyond direct toxicity, macrofungal biopolymers function as potent elicitors of plant immunity, providing systemic, broad-spectrum protection. Exogenous application of mushroom β-glucans (such as lentinan and schizophyllan) to plant foliage or root systems triggers host pattern recognition receptors (PRRs). This interaction initiates a systemic signaling cascade mediated by salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) pathways. The plant enters a primed state, enabling rapid, heightened defense mobilization upon subsequent pathogen challenge (Chavanke & Dalvi, 2025).

This primed state manifests through elevated transcription of defense-related genes and increased activity of key protective enzymes. Phenylalanine ammonia-lyase (PAL), the rate-limiting enzyme in phenylpropanoid synthesis, is upregulated to accelerate the accumulation of endogenous phytoalexins

and lignin precursors. Peroxidase (POD) and polyphenol oxidase (PPO) activities increase concurrently, polymerizing phenolic substrates into structural lignin to reinforce plant cell walls against enzymatic degradation by necrotrophic invaders (Mauch-Mani et al., 2017). Furthermore, superoxide dismutase (SOD) and catalase (CAT) activities are modulated to manage reactive oxygen species (ROS) dynamics, balancing defense signaling while protecting host tissues from self-directed oxidative damage (Anwar et al., 2024).

Macrofungal extracts can also neutralize fungal virulence factors through the suppression of mycotoxigenesis without halting vegetative growth. For instance, non-lethal concentrations of *Lentinula edodes* extracts downregulate mycotoxin biosynthetic gene clusters in *Fusarium verticillioides*, reducing fumonisin B_1 (FB_1) production by up to 75%. Neutralizing these essential necrotrophic virulence factors prevents systemic tissue necrosis, rendering the pathogen avirulent and easily controlled by the host plant's endogenous immunity (Savoie et al., 2019).

Figure 2. Comparative schematic of plant pathogen interactions in non-colonized (control) versus Arbuscular Mycorrhizal (AM) fungi colonized symbiotic host plants.



4. Key Mushroom Species and Their Bioactive Profiles Against Major Crop Diseases

Different macrofungal species possess distinct metabolic signatures that can be targeted against specific economic crop pathogens.

4.1 Strobilurus tenacellus and Respiration Inhibitors

The discovery of natural strobilurins from the wood-rotting basidiomycete *Strobilurus tenacellus* (alongside related species such as *Oudemansiella mucida*, *Xerula* spp., and *Mycena tintinnabulum*) represents a major milestone in agricultural chemistry. Natural strobilurins (strobilurin A, B, C, D, E,

F, G, H) exhibit potent antifungal activity across all major plant pathogenic fungal classes, including Ascomycetes, Basidiomycetes, and Oomycetes (Sidorova & Voronina, 2019).

However, un-modified natural strobilurins are unsuitable for direct agricultural field application due to severe photolability, rapidly decomposing under ambient solar UV radiation. Industrial chemistry overcomes this limitation by utilizing the natural molecule as a structural template: replacing the photolabile central polyene bridge with stable aromatic rings while preserving the E-β-methoxyacrylate pharmacophore (Bartlett et al., 2002). This rational design yielded commercial synthetic strobilurin fungicides such as azoxystrobin, pyraclostrobin, trifloxystrobin, kresoxim-methyl, and picoxystrobin which remain cornerstone treatments against rice blast (*Pyricularia oryzae*), cereal rusts (*Puccinia* spp.), late blight (*Phytophthora infestans*), and powdery mildews (Earley et al., 2012).

4.2 Lentinula edodes (Shiitake)

Lentinula edodes produces both high-molecular-weight immunomodulators and low-molecular-weight antifungal agents. The purified antifungal protein Lentin (27.5 kDa) demonstrates strong growth inhibition against high-impact crop pathogens, including *Botrytis cinerea* (causing gray mold in horticultural crops), *Physalospora piricola*, and *Mycosphaerella arachidicola* (Gaitán-Hernández et al., 2019)

Organic extracts (chloroform and ethyl acetate fractions) from *L. edodes* mycelium and spent mushroom substrate exhibit minimum inhibitory concentrations (MIC) ranging from 1.95 to 3.9 mg/mL against aggressive *Fusarium* species (*F. oxysporum*, *F. verticillioides*, *F. graminearum*). In addition to suppressing radial mycelial growth, these fractions alter fungal growth kinetics and downregulate mycotoxin synthesis, decreasing FB_1 accumulation by 75%. Simultaneously, the structural polysaccharide lentinan functions as a foliar biostimulant, inducing systemic resistance across tomato, cucumber, and tobacco crops (Dharani et al, 2026).

4.3 Ganoderma lucidum (Reishi)

Ganoderma lucidum synthesizes over 400 identified bioactive constituents, dominated by lanostane-type oxygenated triterpenoids (ganoderic acids) and complex soluble polysaccharides. Solvent extracts derived from cell-free culture filtrates of *G. lucidum* display marked antagonism against hemibiotrophic pathogens responsible for anthracnose diseases (Raza et al., 2024).

In vitro bioassays confirm that *G. lucidum* secondary metabolites significantly reduce spore germination and hyphal growth in *Colletotrichum capsici* (chilli fruit rot) and *Colletotrichum gloeosporioides* (anthracnose across tropical fruits). The underlying bioactivity combines direct disruption of fungal plasma membrane ergosterol by ganoderic acids with host plant SAR activation mediated by soluble polysaccharides (da Cruz et al., 2022).

4.4 Pleurotus ostreatus (Oyster Mushroom)

Pleurotus ostreatus produces a distinct arsenal of antifungal metabolites. Bioassay-guided fractionation of *P. ostreatus* culture filtrates has identified potent low-molecular-weight active compounds, including pleurotin, phthalide derivatives (4,6-dimethoxyphthalide and 5,7-dimethoxyphthalide), and 3-hydroxy-3-methyloxindole (Annang et al., 2018).

The dimethoxyphthalide derivatives exhibit nanomolar to micromolar IC_{50} values against conidial germination and germ tube elongation in *Pyricularia oryzae*. Furthermore, *Pleurotus* species express robust extracellular lignin-degrading enzymes particularly laccases and peroxidases capable of degrading phenolic virulence factors secreted by necrotrophic pathogens such as *Sclerotinia sclerotiorum*, *Alternaria alternata*, and *Gaeumannomyces graminis* var. *tritici* (the causal agent of wheat take-all disease) (Cabutaje et al., 2023).

4.5 Schizophyllum commune and Other Macrofungi

Schizophyllum commune secretes schizophyllan, a neutral β-1,3-glucan with β-1,6 side branches. When applied as a protective foliar spray, schizophyllan forms a biopolymeric film across leaf surfaces that reinforces host cell walls and physically blocks appressorium formation by *Botrytis* and *Colletotrichum* specie (Garcia et al., 2026).

Other macrofungi yield specialized bioactive candidates: *Lactarius rufus* secretes rufuslactone (a sesquiterpenoid) active against *Alternaria brassicae* and *Fusarium graminearum*; *Albatrellus dispansus* yields grifolin, which depolarizes fungal plasma membranes; and *Bovistella utriformis* produces polyphenolic fractions with broad-spectrum antifungal activity against plant pathogenic fungi (Clericuzio et al., 2008).

Table 2. Overview of Selected Mushroom Species, Primary Bioactive Metabolites, Target Pathogens, and Key Antifungal Mechanisms.

Mushroom Species	Bioactive Metabolite / Fraction	Target Pathogen	Disease / Crop System	Reported Activity / Field Impact
Strobilurus tenacellus	Strobilurin A & B	*Pyricularia oryzae*, *Phytophthora infestans*	Rice blast, Solanaceous late blight	Respiratory inhibition at Complex III; served as lead template for synthetic QoI fungicides.
Lentinula edodes	Lentin (27.5 kDa protein)	*Botrytis cinerea*, *Physalospora piricola*	Gray mold, Pome fruit rot	Mycelial growth inhibition, hyphal vacuolization, and cellular breakdown.
Lentinula edodes	Chloroform extract / Phenolics	*Fusarium verticillioides*	Maize ear rot	MIC 1.95–3.9 mg/mL; 75% reduction in FB_1 mycotoxin production.
Ganoderma lucidum	Ganoderic acids & Polysaccharides	*Colletotrichum capsici*, *C. gloeosporioides*	Chilli fruit rot, Tropical fruit anthracnose	Suppresses spore germination; induces host SAR and defense enzymes (PAL, PPO).
Pleurotus ostreatus	4,6-Dimethoxyphthalide, Oxindoles	*Pyricularia oryzae*, *Alternaria alternata*	Rice blast, Tomato early blight	Low IC_{50} values against conidial germination and germ tube elongation.
Schizophyllum commune	Schizophyllan	*Botrytis cinerea*, *Colletotrichum* spp.	Multi-crop foliar protection	Biostimulant; reinforces cell walls and blocks fungal infection structures.

5. Biotechnological Production and Upstream Extraction Approaches

Transitioning mushroom bioactive metabolites from laboratory discovery to commercial biofungicide manufacturing requires optimized upstream fermentation and downstream extraction pipelines (Manta et al., 2025).

5.1 Upstream Production: Submerged Fermentation vs. Solid-State Fermentation

Macrofungal biomass and bioactive secondary metabolites are produced using two principal culture systems. Submerged Fermentation (SmF) involves culturing mushroom mycelium in liquid nutrient media within industrial bioreactors. SmF is the preferred industrial route for producing uniform extracellular metabolites, proteins, and soluble polysaccharides (Zhong & Xiao, 2009). Liquid culture permits precise regulation of dissolved oxygen, temperature, pH, agitation shear stress, and carbon-to-nitrogen (C/N) ratios. Adjusting nutrient parameters (e.g., choosing glucose versus complex starch carbon sources or inorganic ammonium salts) can direct fungal metabolism toward target secondary metabolite pathways (Mehrotra et al., 2007).

Solid-State Fermentation (SSF) utilizes solid agricultural residues (e.g., wheat straw, sugarcane bagasse, sawdust) at low free-moisture levels, mimicking the wood-decay microenvironment of basidiomycetes. SSF is highly cost-effective and suitable for generating high mycelial density, fruiting body biomass, and extracellular enzymes like laccases. However, SSF faces engineering limitations in process control, heat dissipation, batch-to-batch consistency, and downstream extraction efficiency compared to liquid bioreactors (Sadh et al., 2018).

Table 3. Comparative Evaluation of Extraction Methodologies for Mushroom Bioactives.

Extraction Method	Processing Mechanism	Main Advantages	Key Limitations	Typical Bioactive Targets
Ultrasound-Assisted (UAE)	Acoustic cavitation ruptures fungal cell walls.	High yield, low processing time, operates at mild temperatures.	Equipment scaling costs; localized temperature rises require cooling.	Thermolabile proteins (Lentin), soluble β-glucans, phenolics.
Microwave-Assisted (MAE)	Volumetric heating via dipole rotation and ionic conduction.	Rapid extraction kinetics, minimal solvent usage.	Potential thermal degradation of heat-sensitive compounds.	Polyphenols, flavonoids, stable small-molecule secondary metabolites.
Supercritical Fluid ($SFE\text{-}CO_2$)	Supercritical CO_2 acts as a tunable lipophilic solvent.	Solvent-free extracts, non-thermal, highly selective, eco-friendly.	High initial capital investment; requires specialized high-pressure equipment.	Triterpenoids (ganoderic acids), sterols, essential fatty acids.
High-Pressure (HPP)	Isostatic non-thermal pressure disruption.	Preserves tertiary protein structures and volatile profiles.	Batch operation limitations, high capital equipment costs.	Heat-sensitive enzymes, antifungal peptides, volatile fractions.

6. Formulation Engineering and Nanotechnology Integration

Despite strong in vitro antifungal activity, translating crude mushroom extracts into commercial crop protection products requires overcoming significant environmental stability barriers.

6.1 Stability and Environmental Bottlenecks

Natural mushroom metabolites face several degradation pathways when applied in field settings. Unmodified polyketides (such as natural strobilurins) undergo rapid photolysis under solar UV radiation, losing biological activity within hours. Low-molecular-weight phenolics and terpenoids degrade rapidly when exposed to atmospheric oxygen, high temperatures, and broad pH fluctuations (Rajarathnam et al., 1989). Furthermore, many bioactive secondary metabolites (e.g., ganoderic acids, phthalides) are hydrophobic, resulting in poor dispersion in aqueous spray tanks and limited cuticular penetration on target plant leaves. Unformulated aqueous extracts also lack rain fastness, washing off foliar surfaces during rain events and requiring frequent re-applications (Huang et al., 2021).

6.2 Nanoencapsulation Strategies

Nanotechnology offers solutions to these physical and environmental stability barriers by enclosing bioactive metabolites within functional nanocarriers (10--300 nm). Natural and synthetic biodegradable polymers such as Polycaprolactone (PCL), Chitosan, Zein, Sodium Alginate, and Starch nanocrystals are widely used as encapsulation wall materials. PCL nanocapsules form a protective polymeric shell around an inner core oil matrix containing lipophilic bioactives. This core-shell architecture shields photolabile compounds from UV radiation while regulating active release (Sharma et al., 2017).

Chitosan-based nanocarriers are particularly advantageous because chitosan possesses intrinsic antifungal activity and acts as a plant immune elicitor. Chitosan nanoparticles cross-linked with tripolyphosphate (TPP) encapsulate mushroom extracts via ionic gelation. The positively charged amino groups on chitosan bind electrostatically to negatively charged plant leaf cuticles, improving foliar adhesion and rain fastness (Poznanski et al., 2023).

Nano-encapsulated biopesticides exhibit controlled Fickian or anomalous diffusion release kinetics. Rather than an initial burst release followed by rapid degradation, nanocarriers maintain active metabolite concentrations above the minimum inhibitory concentration (MIC) over extended periods. Studies on nano-encapsulated active ingredients demonstrate that nano-formulations can achieve equivalent or superior field disease control against targets like *Fusarium* and *Alternaria* at up to a ten-fold reduction in active ingredient dosage compared to unencapsulated forms (Punelas–Villanueva et al., 2025).

In addition to carrier encapsulation, cell-free culture supernatants from basidiomycetes rich in extracellular reductases (e.g., laccases, nitrate reductases) can reduce silver (Ag^{+}) or copper (Cu^{2+}) ions into biogenic metallic nanoparticles. These mycogenic silver (AgNPs) and copper oxide (CuONPs) nanoparticles exhibit strong, broad-spectrum antifungal action against *Rhizoctonia solani*, *Sclerotium rolfsii*, and *Fusarium oxysporum* (Roman et al., 2024).

7. Technical Bottlenecks, Regulatory Frameworks, and Commercial Outlook

Translating mushroom-derived bioactive metabolites into commercial biofungicides requires addressing several key industrial hurdles. A primary challenge is batch-to-batch chemical standardization. Unlike synthetic chemical inputs that contain single active ingredients, crude mushroom extracts are complex chemical mixtures influenced by substrate composition, fermentation temperature, light exposure, and developmental stage. Ensuring reproducible chemical profiles and standardized bioactive concentrations across commercial batches requires validated chromatographic (HPLC, LC-MS/MS) fingerprinting protocols (Abdelkhalek et al., 2025).

Upstream fermentation economics present another constraint. High-density submerged fermentation requires capital-intensive bioreactors, energetic aeration, and precise sterile processing. Downstream purification of single high-purity active compounds can be cost-prohibitive for broad-acre agricultural applications. Consequently, commercialization strategies are shifting toward standardized,

concentrated cell-free supernatants or crude extract fractions formulated with protective nanocarriers (Ramdas et al., 2026).

Regulatory registration pathways also require modernization. Historically, biopesticide registration frameworks were adapted from synthetic chemical paradigms, requiring expensive toxicological, ecotoxicological, and environmental fate testing. Modernizing regulatory systems to establish bio-specific risk assessment criteria recognizing the lower environmental persistence and favorable mammalian safety profiles of natural biopolymers is essential to reduce registration timelines and costs (Deeksha et al., 2025).

Finally, macrofungal biofungicides play a vital role in integrated resistance management. While single-site synthetic inputs (such as early strobilurins) selected for target-site resistance mutations like G143A, deploying multi-component mushroom extracts or multi-action formulations provides multi-site protection. Combining direct fungicidal action with systemic host immune priming lowers selective pressure on single fungal targets, preserving long-term product efficacy under field conditions (Ons et al, 2020).

8. Conclusion

Mushroom-derived bioactive metabolites represent a promising, eco-friendly alternative to conventional synthetic fungicides for managing major crop diseases. The structural diversity of macrofungal compounds encompassing polyketides, proteins, polysaccharides, terpenoids, and phenolics provides multiple antifungal mechanisms that combine direct pathogen suppression with host defense priming. Key species such as *Strobilurus tenacellus*, *Lentinula edodes*, *Ganoderma lucidum*, and *Pleurotus ostreatus* have demonstrated potent activity against economically devastating pathogens including *Pyricularia oryzae*, *Botrytis cinerea*, *Fusarium* spp., and *Colletotrichum* spp. The integration of advanced biotechnological production through submerged fermentation and solid-state fermentation, coupled with nanoencapsulation strategies using biodegradable polymers (chitosan, polycaprolactone, zein), addresses critical stability, bioavailability, and field application barriers that have historically limited natural product deployment. Despite these advances, successful commercialization requires overcoming challenges in batch-to-batch chemical standardization, fermentation economics, and regulatory framework modernization. The dual-action nature of mushroom metabolites combining direct fungicidal activity with systemic resistance induction positions them strategically within integrated pest management programs to reduce chemical input dependence, mitigate fungicide resistance development, and promote sustainable agricultural practices. Future research should prioritize optimization of cost-effective production systems, development of standardized formulation protocols, and comprehensive field validation across diverse cropping systems. As global markets for biological crop protection expand rapidly, mushroom-derived biofungicides offer a viable pathway to enhance food security while addressing environmental and consumer safety concerns associated with synthetic chemical inputs.

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