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# Endophytic Mushroom-Associated Fungi for Enhancing Plant Immunity and Resistance to Phytopathogens

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**Abstract**

Endophytic mushroom-associated fungi represent an underexploited biological resource for sustainable crop protection, offering environmentally compatible alternatives to synthetic agrochemicals. While Ascomycota have historically dominated endophyte research, Basidiomycota particularly mushroom-forming Agaricomycetes possess sophisticated enzymatic systems, expansive secondary metabolic pathways, and complex immunomodulatory capabilities that remain largely untapped. This review comprehensively examines the taxonomic diversity, colonization dynamics, and dual mechanisms of pathogen suppression mediated by endophytic macrofungi. Direct antagonism operates through antimicrobial secondary metabolite synthesis (including phenolics, terpenoids, and alkaloids), lytic enzyme secretion (chitinases, β-1,3-glucanases, proteases), mycoparasitism, siderophore-mediated iron sequestration, and competitive niche displacement. Indirect defense mechanisms involve reprogramming host plant physiology via induced systemic resistance (ISR) and systemic acquired resistance (SAR), stabilization of reactive oxygen species (ROS) dynamics through enhanced antioxidant enzyme activity (SOD, CAT, POD), and reinforcement of structural barriers via lignin and callose deposition. Model endophytic systems, particularly *Serendipita indica* (formerly *Piriformospora indica*), demonstrate significant disease suppression across diverse crops including onion, chickpea, tomato, and cabbage, reducing pathogen severity by up to 69.5% in field trials. Emerging biotechnological innovations including nanoparticle biosynthesis, multi-omics integration, and activation of silent biosynthetic gene clusters through epigenetic modulation and microbial co-culturing promise to unlock the full metabolic potential of these fungi. This review synthesizes current knowledge on endophytic mushroom-associated fungi and proposes their integration into integrated pest and disease management frameworks, addressing the urgent need for sustainable agricultural practices that maintain crop productivity while reducing ecological damage from conventional agrochemicals.

**Keywords:** Endophytic fungi, Basidiomycota, biological control, induced systemic resistance, antimicrobial metabolites, *Serendipita indica*, sustainable agriculture

## 1. Introduction

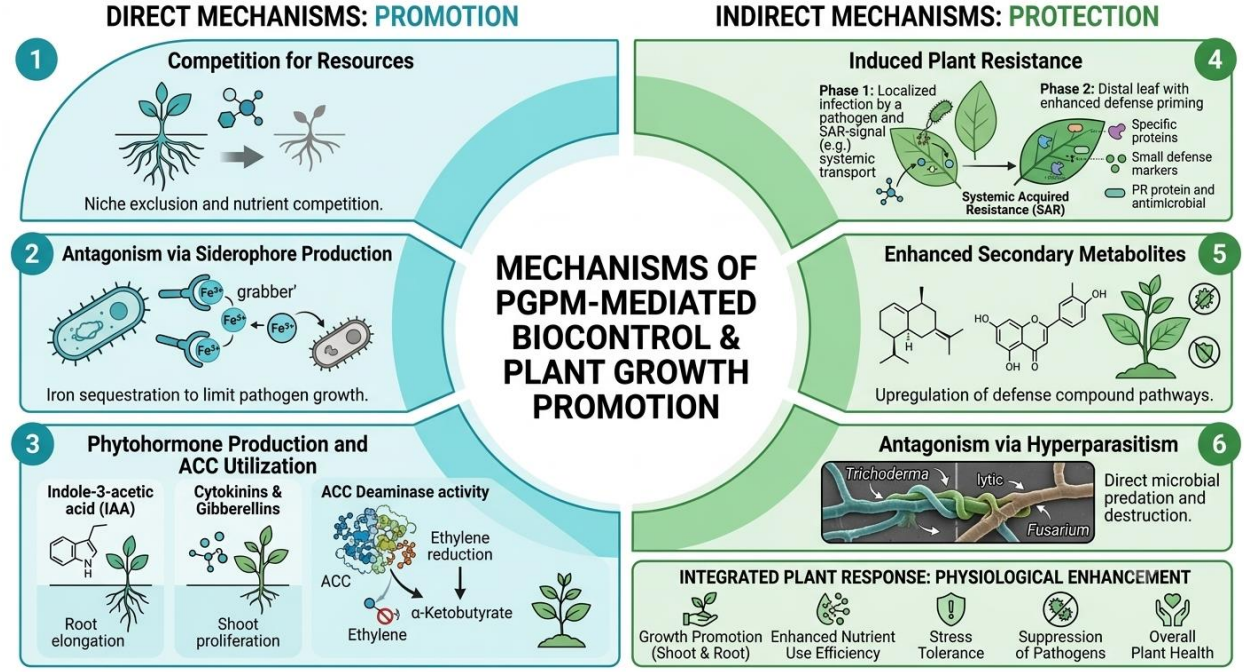
Modern agriculture faces an unprecedented global challenge: sustaining high crop yields to feed an expanding human population while simultaneously reducing reliance on synthetic agrochemicals. Decades of intensive chemical application have precipitated severe ecological consequences, including soil degradation, aquatic contamination, off-target environmental toxicity, bioaccumulation within human food networks, and the rapid emergence of fungicide-resistant phytopathogen biotypes (Fisher et al., 2018). The high evolutionary plasticity of fungal, bacterial, and viral plant pathogens enables them to continuously breach host resistance in monoculture cropping systems and overcome synthetic single-site chemical modes of action (Lucas et al., 2015). Consequently, agricultural research has increasingly focused on biological crop protection strategies that utilize naturally occurring, co-evolved plant-microbe symbioses (Compant et al., 2021).

Among the diverse microflora inhabiting agroecosystems, endophytic fungi have emerged as vital drivers of plant fitness and multi-trophic interactions. Defined as non-pathogenic fungi that reside within the living internal tissues of host plants including roots, stems, leaves, and reproductive organs without inducing immediate overt disease symptoms, endophytes operate across a dynamic mutualism-parasitism continuum (Schulz & Boyle, 2005). While microfungal endophytes belonging to the phylum Ascomycota have historically dominated scientific literature, macrofungi and mushroom-associated fungi belonging to the Basidiomycota (and select basal fungal lineages) represent an underutilized functional reservoir (Jia et al., 2016).

Macrofungi possess sophisticated enzymatic systems, complex cellular structures, and expansive secondary metabolic pathways capable of synthesizing novel antimicrobial, nematicidal, and immunomodulatory compounds. When establishing endophytic or mycorrhizal-like associations within plant hosts, mushroom-associated fungi exert dual protection. Directly, they suppress invading pathogens through hyperparasitism, competitive nutrient sequestration, cell wall-degrading enzyme secretion, and antimicrobial metabolite synthesis (Lugtenberg et al., 2017). Indirectly, they reconfigure host plant physiology by reprogramming transcriptional defense networks, activating Induced Systemic Resistance (ISR) and Systemic Acquired Resistance (SAR), stabilizing reactive oxygen species (ROS) dynamics, and accelerating structural barrier deposition (Pieterse et al., 2014).

Understanding the mechanisms through which endophytic mushroom-associated fungi fortify host plants against biotic threats offers a transformative framework for sustainable agriculture. This review provides a comprehensive analysis of the taxonomic diversity, colonization dynamics, direct and indirect defense mechanisms, model biological systems, and technological frontiers associated with macrofungal endophytes in crop protection (Harman et al., 2021).

**Figure 1: Overview of Direct Plant Growth Promotion and Indirect Protection Mechanisms Mediated by Plant Growth-Promoting Microorganisms (PGPM)**




## 2. Diversity, Isolation Dynamics, and Colonization Strategies

### 2.1 Taxonomic Diversity and the Ascomycota-Basidiomycota Disparity

Global surveys of endophyte diversity reveal a pronounced taxonomic skew: Ascomycetous taxa typically comprise 80% to 95% of total isolated endophytes across diverse angiosperm, gymnosperm, and non-vascular hosts, whereas Basidiomycetes consistently account for less than 10%. This underrepresentation does not necessarily reflect true ecological scarcity, but rather stems from isolation artifacts, culture media biases, and physiological growth characteristics inherent to macrofungi (McDonald & Stukenbrock, 2016).

Standard isolation protocols rely heavily on nutrient-rich media such as Potato Dextrose Agar and short incubation windows of five to ten days, which selectively favor fast-growing, highly sporulating Ascomycota. In contrast, endophytic Basidiomycetes frequently exhibit slow hyphal extension rates, strict nutrient requirements, and prolonged vegetative phases without producing diagnostic conidia or fruitbodies in vitro (Pimentel & Burgess, 2014). Consequently, non-sporulating basidiomycetous mycelia were historically misclassified as mycelia sterilia or discarded. The deployment of specialized, selective media such as malt extract agar amended with benomyl, chloramphenicol, and streptomycin combined with extended incubation periods and high-throughput internal transcribed spacer (ITS) rRNA gene sequencing, has revealed that endophytic Basidiomycetes are ubiquitous across forest, agricultural, and aquatic ecosystems (Savary et al., 2019).

Characteristically, basidiomycetous endophytes can be recognized microscopically by the presence of hyphal clamp connections, which facilitate nuclear distribution during dikaryotic growth. Taxonomically, these endophytes span diverse orders within the Agaricomycetes, including Sebacinales (e.g., Serendipita indica), Polyporales (e.g., Trametes versicolor, Ganoderma spp., Irpex lacteus), Hymenochaetales, and Agaricales (e.g., Schizophyllum commune) (FAO, 2022).

### 2.2 Host Colonization and Environmental Transmission

Endophytic colonization by mushroom-associated fungi occurs primarily through horizontal transmission, where fungal propagules such as basidiospores, hyphal fragments, or chlamydospores present in the phyllosphere, soil, or rhizosphere breach host plant barriers. Vertical transmission via

seeds is rare among Basidiomycetes under natural field conditions, emphasizing the critical role of environmental microclimates, soil properties, and host genotypic filtering in shaping endophytic community structure (Hardoim et al., 2015).

Entry into host tissues follows a multi-step recognition and enzymatic cascade. Fungal spores or chlamydospores germinate on plant surfaces, producing germ tubes and appressoria-like structures that penetrate host tissues through natural openings such as stomata and lenticels, micro-wounds, or epidermoid intercellular junctions. To navigate the rigid structural barrier of the plant cell wall, endophytes secrete regulated, non-lethal quantities of cell wall-degrading enzymes (CWDEs), including endoglucanases, cellulases, xylanases, and pectinases (Rodriguez et al., 2009).

Once inside, the host plant encapsulates the hyphae within host-derived membrane compartments, preventing direct cytoplasm-to-cytoplasm contact and suppressing hyper-inflammatory plant defense responses. Hyphae colonize the intercellular apoplast or form intracellular coils within root epidermal and cortical cells, particularly concentrated in the zone of cellular differentiation. Crucially, during this biotrophic phase, the endophyte suppresses host apoptotic defense pathways through targeted effectors, maintaining tissue integrity and establishing long-term metabolic dialogue (Hyde et al., 2019).

## 3. Direct Mechanisms of Pathogen Antagonism

Endophytic mushroom-associated fungi possess a robust armory of direct antagonistic mechanisms that inhibit phytopathogen proliferation prior to host tissue invasion. These direct modes of action encompass secondary metabolite synthesis, lytic enzyme production, mycoparasitism, and competitive niche displacement (Stone et al., 2000).

### 3.1 Antimicrobial Secondary Metabolites and Volatile Organic Compounds

Macrofungal endophytes are prolific producers of specialized secondary metabolites belonging to diverse chemical classes, including sesquiterpenes, diterpenes, polyketides, complex alkaloids, phenolics, and bioactive steroids. These molecules exhibit high target specificity and lower non-target toxicity compared to synthetic agrochemicals (Rahi & Malik, 2016).

For instance, endophytic strains of Schizophyllum commune isolated from medicinal host plants yield ethyl acetate extracts rich in bioactive phenolics and terpenoids. These extracts exert potent, bactericidal effects against critical bacterial pathogens, disrupting cell wall integrity and destroying established sessile bacterial biofilms. Similarly, endophytic wood-decaying mushrooms such as Daldinia concentrica and Irpex lacteus synthesize mycoactive alkaloids, flavonoids, and saponins. These compounds permeate phytopathogenic fungal membranes, inducing electrolyte leakage, metal ion depletion, and cell lysis ((Lindequist et al., 2005).

In addition to non-volatile secondary metabolites, endophytic macrofungi generate complex mixtures of Volatile Organic Compounds (VOCs), including low-molecular-weight alcohols, esters, ketones, and aromatic hydrocarbons. These gaseous metabolites function as bio-fumigants, suppressing spore germination, hyphal elongation, and mycotoxin synthesis in postharvest phytopathogens such as Botrytis cinerea, Aspergillus flavus, and Fusarium species without requiring physical contact (Köhl et al., 2019).

### 3.2 Lytic Enzyme Secretion and Mycoparasitism

The fungal cell wall is composed of a rigid cross-linked matrix of chitin ($\beta$-1,4-N-acetylglucosamine polymers), $\beta$-1,3- and $\beta$-1,6-glucans, mannans, and structural glycoproteins. Pathogenic bacteria, conversely, rely on peptidoglycan meshworks. Endophytic Basidiomycetes secrete hydrolytic enzymes capable of digesting these structural polymers, serving as a primary defense against invading phytopathogens (Noman et al., 2020).

Hydrolytic enzyme profiles typically include:

- Chitinases (EC 3.2.1.14), which hydrolyze internal $\beta$-1,4-glycosidic bonds of chitin into oligomeric chitooligosaccharides, degrading structural rigidity in phytopathogenic fungi.
- $\beta$-1,3-Glucanases (EC 3.2.1.39), which cleave the primary glucan scaffold of fungal cell walls, acting synergistically with chitinases.
- Proteases and peptidases, which digest structural cell wall proteins and virulence factors, disabling pathogen-host interaction platforms (Compant et al., 2021).

In hyperparasitic interactions, exemplified by endophytic Trichoderma and polypore strains, the endophyte detects chemical signals such as oligomers released by constitutive CWDE activity emanating from a host phytopathogen. The endophyte grows tropically toward the pathogen, coils around its hyphae, forms specialized appressoria-like structures, and localizedly secretes concentrated chitinases and proteases. This process causes localized wall dissolution, cytoplasm penetration, and cellular collapse of pathogens such as Sclerotinia sclerotiorum, Rhizoctonia solani, and Fusarium oxysporum (Alfiky & Weisskopf, 2021).

### 3.3 Nutrient Competition and Siderophore Secretion

Iron is an essential micronutrient for microbial growth, metabolic enzyme function, and virulence factor synthesis. However, the bioavailability of ferric iron ($Fe^{3+}$) in soil and apoplastic environments is extremely low due to the formation of insoluble hydroxides. Endophytic Basidiomycetes synthesize and secrete high-affinity, low-molecular-weight iron-chelating agents termed siderophores, primarily belonging to the hydroxamate and carboxylate structural classes (Wei et al., 2025).

By secreting siderophores into the rhizosphere and apoplastic spaces, macrofungal endophytes efficiently sequester $Fe^{3+}$, forming stable complexes that are selectively reabsorbed by the endophyte via dedicated transmembrane transport systems. This action depletes soluble iron pools in the immediate microenvironment, starving competing phytopathogens such as Fusarium, Pythium, and Pseudomonas species, thereby preventing spore germination and host colonization. Furthermore, endophytes aggressively occupy spatial niches within host apoplasts, preventing pathogen attachment and physical establishment (Vijay et al., 2023).

## 4. Indirect Mechanisms: Host Immune Modulation and Systemic Resistance

Beyond direct physical and chemical suppression of pathogens, endophytic mushroom-associated fungi reconfigure the host plant's internal physiological state. By acting as biological immunomodulators, these endophytes prime host defense networks, leading to faster and more robust immune activation upon subsequent pathogen challenge (David & Slot, 2022).

### 4.1 Signaling Networks: ISR and SAR Crosstalk

Plant immune networks rely on major phytohormonal defense pathways to combat biotic stress: the Salicylic Acid (SA) pathway, predominantly effective against biotrophic and hemibiotrophic pathogens, and the Jasmonic Acid (JA) and Ethylene (ET) pathways, effective against necrotrophic pathogens and herbivorous insects (Roychowdhury et al., 2024).

Endophytic colonization triggers two distinct forms of systemic plant immunity:

- Induced Systemic Resistance (ISR): Typically activated by root-colonizing mutualistic endophytes, ISR is mediated by JA and ET signaling cascades. ISR does not induce an immediate, metabolically costly defense response; instead, it primes the host plant. Upon subsequent attack by necrotrophic pathogens such as Botrytis cinerea or pests, ISR-primed plants exhibit accelerated transcript expression of defense genes and rapid accumulation of phytoalexins (Fang et al., 2025).
- Systemic Acquired Resistance (SAR): Induced by localized tissue perception of fungal microbe-associated molecular patterns (MAMPs), such as chitin fragments or $\beta$-glucans. SAR relies on SA accumulation, triggering systemic translocation of signal molecules like methyl

salicylate and activating Pathogenesis-Related (PR) genes (PR-1, PR-2 [glucanase], PR-5 [thaumatin-like]) throughout the plant architecture (Lugtenberg et al., 2017).

Endophytic Basidiomycetes fine-tune the crosstalk between SA and JA/ET signaling, preventing mutual inhibition of these pathways and allowing the host plant to mount customized, multi-layered resistance tailored to the lifestyle of the invading pathogen (Piotr & Mariola, 2026).

### 4.2 Redox Homeostasis and Antioxidant Defense Systems

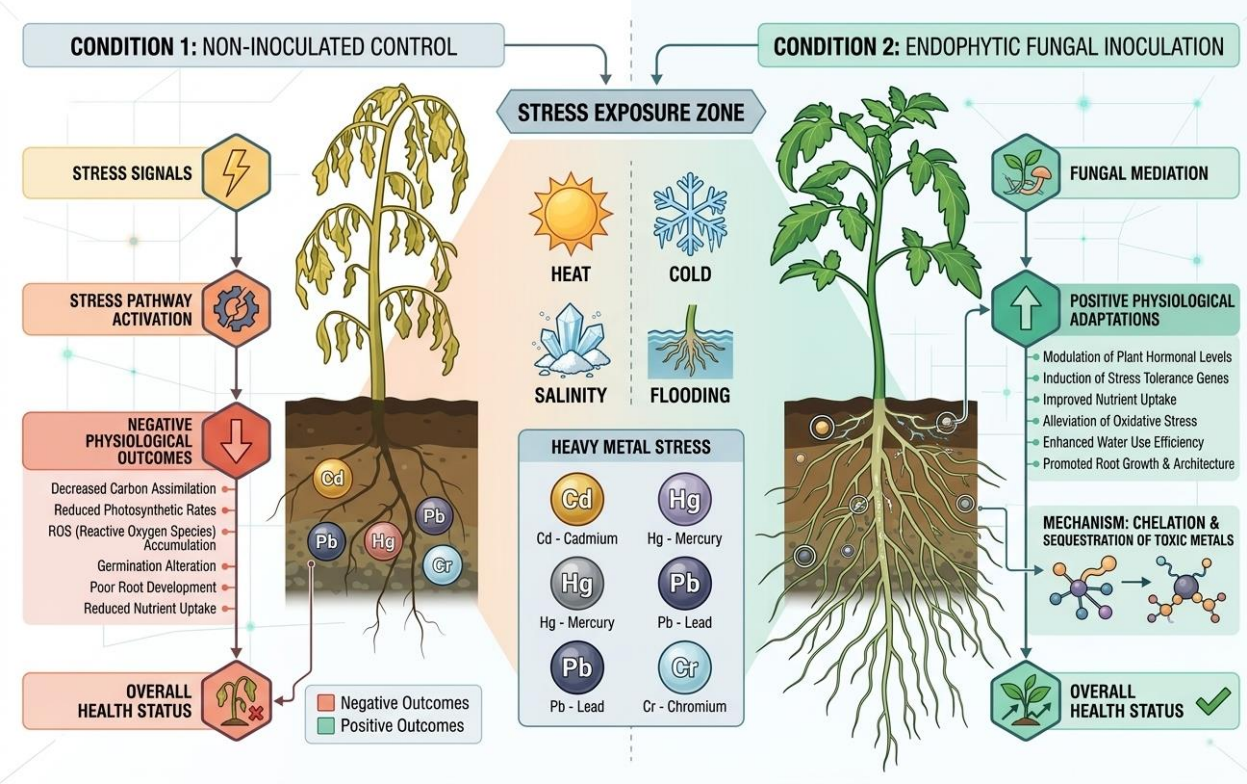
Pathogen invasion triggers an early plant immune event known as the oxidative burst, characterized by rapid accumulation of reactive oxygen species (ROS) such as hydrogen peroxide ($H_2O_2$), superoxide anions ($O_2^{\cdot-}$), and hydroxyl radicals ($OH^{\cdot}$) in host apoplasts. While ROS serve as essential defense signals and direct antimicrobial agents, sustained high ROS levels cause severe host cell damage, membrane lipid peroxidation, enzyme inactivation, and tissue necrosis (Haghpanah et al., 2025).

Colonization by endophytic mushroom fungi modulates host redox homeostasis. Upon pathogen attack, endophytes enhance the activity of key enzymatic antioxidants:

- Superoxide Dismutase (SOD), which catalyzes the dismutation of toxic $O_2^{\cdot-}$ into $H_2O_2$ and $O_2$.
- Catalase (CAT) and Peroxidase (POD), which rapidly break down $H_2O_2$ into water and oxygen, preventing toxic radical formation.
- Phenylalanine Ammonia-Lyase (PAL), the key enzyme in the phenylpropanoid pathway that converts L-phenylalanine to trans-cinnamic acid, driving the biosynthesis of phenolics, flavonoids, and lignin (Low & Merida, 1996).

This enzymatic regulation attenuates lipid peroxidation, as quantified by reduced malondialdehyde (MDA) accumulation. Consequently, host cell membrane integrity is preserved during severe pathogen challenge, preventing necrotrophic pathogens from causing widespread lesion development (Bolwell & Daudi, 2009).

**Figure 2: Comparative Physiological Outcomes of Non-Inoculated Control Plants Versus Endophytic Fungal-Inoculated Plants Under Environmental and Heavy Metal Stresses**



### 4.3 Histological and Structural Cell Wall Reinforcements

To physically restrict pathogen penetration and vascular movement, endophytes stimulate structural modifications within host tissues. Endophyte-inoculated plants exhibit elevated rates of cell wall lignification, suberin deposition, and callose apposition at sites of attempted pathogen entry (Grabka et al., 2022).

For instance, interaction with root endophytes elevates phenylpropanoid pathway transcript levels, accelerating the accumulation of insoluble phenolic compounds that cross-link structural cell wall polymers. This structural reinforcement creates a mechanical barrier resistant to enzymatic degradation by phytopathogenic CWDEs, effectively trapping pathogens within localized papillae and halting systemic spread through host vascular systems (Lòpez-Fernàndez et al., 2016).

**Table 1: Functional Diversity and Biocontrol Profiles of Representative Endophytic Mushroom-Associated Fungi**

| Endophytic Fungal Species | Taxonomic Class | Isolated Host / Applied Crop | Target Phytopathogen / Pest | Dominant Biocontrol & Defense Mechanisms |
|---|---|---|---|---|
| Serendipita indica (Piriformospora indica) | Agaricomycetes (Sebacinales) | Allium cepa, Cicer arietinum, Brassica oleracea | Stemphylium vesicarium, Fusarium oxysporum, Xanthomonas campestris | ISR induction, SOD/CAT/PAL elevation, ROS mitigation, reduced MDA lipid peroxidation |
| Hendersonia sp. (GanoEF Strain) | Sordariomycetes (Mushroom-Assoc.) | Elaeis guineensis (Oil Palm) | Ganoderma boninense | Elevated PAL, POD, chitinase & glucanase activity, lignin deposition, root tissue barrier formation |
| Schizophyllum commune (Strain Sch1) | Agaricomycetes (Agaricales) | Aloe vera, Agricultural Crops | Staphylococcus aureus, Klebsiella pneumoniae, Fungal Pathogens | Secretion of bioactive phenolics & terpenoids, bactericidal action, biofilm matrix dispersion |
| Trichoderma sp. (Endophytic Isolate) | Sordariomycetes (Mushroom-Assoc.) | Ricinus communis, Horticultural Crops | Botrytis cinerea, Locusta migratoria | Chitinase & protease secretion, acetylcholinesterase (AChE) inhibition, direct mycoparasitism |
| Daldinia concentrica & Irpex lacteus | Sordariomycetes / Agaricomycetes | Hardwoods, Crop Plants | Broad Fungal Phytopathogens | Production of mycoactive alkaloids, flavonoids, & saponins causing pathogen membrane disintegration |
| Trametes versicolor & Ganoderma lucidum | Agaricomycetes (Polyporales) | Model Crop Systems | Foliar & Root Pathogens, Weeds | Secretion of glucanases, chitinases, antibacterial polysaccharides, phytostimulation |

Table 2 provides a comparative overview distinguishing direct physical/chemical antagonism from indirect host-mediated immune responses.

**Table 2: Direct vs. Indirect Defense Mechanisms Mediated by Endophytic Mushroom Fungi**

| Defense Category | Molecular / Cellular Elicitor | Primary Target / Biological Process | Impact on Pathogen / Resistance |
|---|---|---|---|
| **Direct: Antibiosis** | Secondary Metabolites (Alkaloids, Terpenoids, VOCs) | Pathogen plasma membrane integrity, respiratory chain enzymes | Cell lysis, electrolyte lea inhibition of spore germi and mycotoxin synthesis |
| **Direct: Enzymatic Lysis** | Chitinases, $\beta$-1,3-Glucanases, Proteases | Pathogen structural cell wall polymers (Chitin, Peptidoglycan) | Dissolution of fungal h walls, degradation of bac cell envelopes |
| **Direct: Iron Sequestration** | Hydroxamate & Carboxylate Siderophores | Soluble Ferric Iron ($Fe^{3+}$) pools in apoplast/rhizosphere | Depletion of bioavailable restricting pathogen growt virulence expression |
| **Indirect: Defense Priming** | MAMPs (Chitin, $\beta$-Glucans), JA/ET/SA Signaling | Host defense genes (PR-1, PR-2, PR-5, LOX, PAL) | Accelerated, heigh expression of host def upon pathogen cha (ISR/SAR) |
| **Indirect: Redox Balance** | SOD, CAT, POD Enzymes, Phenolics | Host Reactive Oxygen Species ($H_2O_2$, $O_2^{\cdot-}$) dynamics | ROS scavenging, mitigati lipid peroxidation (M membrane stabilization |
| **Indirect: Wall Reinforcement** | PAL activation, Phenylpropanoid metabolites | Host apoplastic cell walls, extracellular matrix | Deposition of lignin, su and callose, creating ph barriers to invasion |

## 5. Model Endophytic Systems and Agronomic Applications

To illustrate the translational efficacy of endophytic mushroom-associated fungi, key model systems demonstrate how these organisms transition from laboratory assays to field applications (Rai & Prasad, 2023).

### 5.1 Case Study: Serendipita indica as a Paradigm Model

**Serendipita indica** (order Sebacinales, Basidiomycota), formerly *Piriformospora indica*, represents the most extensively researched root-endophytic macrofungus. Originally isolated from the Thar Desert in India, *S. indica* mimics the functional attributes of arbuscular mycorrhizal fungi (AMF) by promoting nutrient uptake and stress tolerance across an broad host range of monocotyledonous and dicotyledonous crops (Varma et al., 1999). Unlike obligate AMF, however, *S. indica* can be cultivated axenically on synthetic media, producing large quantities of resilient pear-shaped chlamydospores suitable for commercial formulation (Qiang et al., 2012).

The disease mitigation cascade orchestrated by *S. indica* operates through a synchronized physiological pathway. Upon encountering a pathogen challenge such as *Stemphylium vesicarium* or *Fusarium oxysporum*, root colonization by *S. indica* establishes an extensive intercellular and intracellular hyphal network within host root tissues. This physical establishment directly suppresses pathogens through competitive space and nutrient exclusion. Simultaneously, the fungus triggers host physiological modulations, stimulating endogenous indole-3-acetic acid (IAA) synthesis and accelerating macronutrient absorption (nitrogen, phosphorus, potassium) (Lahrmann et al., 2013).

These primary metabolic shifts feed directly into host antioxidant and defense pathways, causing a marked upregulation of superoxide dismutase, catalase, peroxidase, and phenylalanine ammonia-lyase activity (Peskan-Berghöfer et al., 2004). The resulting stabilization of redox homeostasis suppresses destructive hydrogen peroxide accumulation and attenuates malondialdehyde lipid peroxidation levels. On a transcriptional level, this metabolic state primes Induced Systemic Resistance, accelerating structural lignin deposition and upregulating key defense transcripts (*AcLOX1, AcLOX2, AcPAL1,*

*AcCHI, AcWRKY1,* and *AcWRKY70*). Conclusively, this integrated cascade leads to a broad systemic reduction in disease severity, lesion development, and overall pathogen burden (Waller et al., 2005).
In onion (*Allium cepa*), inoculation with *S. indica* significantly reduces leaf blight severity caused by *Stemphylium vesicarium* under both controlled greenhouse conditions and multi-year field trials (Jogaiah et al., 2018). Similarly, in chickpea (*Cicer arietinum*) and tomato (*Solanum lycopersicum*), *S. indica* colonization suppresses vascular wilt caused by *Fusarium oxysporum*. In cabbage (*Brassica oleracea*), substrate or seed inoculations reduce black rot disease caused by *Xanthomonas campestris* pv. *campestris* by up to 27.9%, driven by indirect physiological priming rather than direct antibiosis (Oelmüller et al., 2009).

### 5.2 Macrofungal Endophytes in Crop Protection

Beyond S. indica, macrofungal endophytes are increasingly integrated into integrated pest and disease management (IPDM) systems across high-value agricultural commodities (Sherameti et al., 2005).

**Oil Palm Basal Stem Rot Control**

Basal Stem Rot (BSR), caused by the hemibiotrophic polypore Ganoderma boninense, is a critical threat to global palm oil production, incurring substantial economic losses. The application of Hendersonia sp., a specialized endophytic fungus isolated from native oil palm roots, has demonstrated efficacy against G. boninense. Formulated into commercial biofertilizers such as GanoEF, Hendersonia colonizes root cortical tissues, increasing host lignin content and inducing defensive enzyme cascades including peroxidase, PAL, chitinase, and $\beta$-1,3-glucanase. In nursery and field trials, treated palms exhibited up to a 69.5% reduction in disease incidence and a significant decrease in seedling mortality (Jogawat et al., 2020).

**Phytopathogenic Mycotoxin Reduction**

Endophytic Agaricomycetes including Bjerkandera adusta and Trametes cubensis function as biological control agents against mycotoxigenic fungi in cereal crops. When co-cultured with Aspergillus flavus and Aspergillus niger on corn substrate, these endophytes reduce carcinogenic Aflatoxin B1 ($AFB_1$) and Ochratoxin A (OTA) concentrations to undetectable levels (0 ng/g) through competitive nutrient uptake and enzymatic degradation of the toxin molecules 9 Vahabi et al., 2015).

**Dual Biocontrol and Entomopathogenic Activity**

Endophytic isolates of Trichoderma recovered from mushroom tissues exhibit combined antifungal and insecticidal properties. When colonizing castor leaf tissues (Ricinus communis), Trichoderma synthesizes extracellular chitinases, proteases, and acetylcholinesterase (AChE)-inhibiting alkaloids. These secondary metabolites disrupt the cell wall integrity of phytopathogenic Botrytis cinerea while simultaneously impairing digestive peritrophic membranes and neuromuscular signaling in herbivorous pests such as Locusta migratoria (Kumar et al., 2009).

## 6. Biotechnological Innovations and Future Horizons

The development of sustainable crop protection products derived from endophytic macrofungi is accelerated by advances in nanotechnology, multi-omics, and metabolic engineering (Narayan et al., 2017).

### 6.1 Nanotechnology and Biosynthesis of Metallic Nanoparticles

Fungi possess high heavy-metal tolerance, rapid biomass accumulation, and high levels of secreted extracellular enzymes, making them excellent platforms for the biological synthesis of metallic nanoparticles (NPs). Endophytic macrofungi reduce precursor metal salts into stable, monodisperse nanoparticles through enzyme-mediated electron transport cascades. In this bioprocess, extracellular fungal reductases and electron donors transfer electrons to cationic metal precursor ions ($M^+$), driving intracellular or extracellular reduction to zero-valent metallic nuclei ($M^0$), which subsequently aggregate under controlled protein capping into functional nanoparticles (Baltruschat et al., 2008).

$$M^{+} + \text{Extracellular Reductase / Electron Donor} \longrightarrow M^{0} \text{ (Nanoparticle Nucleation)}$$

Synthesized nanoparticles include:

- Silver Nanoparticles (AgNPs), which display broad-spectrum bactericidal and antifungal action by generating localized ROS, penetrating cell walls, and binding to genomic DNA.
- Copper (Cu), Iron (Fe), and Zinc (Zn) Nanoparticles, which serve a dual purpose as micro-nutritional bio-fortificants and targeted antimicrobial agents that disrupt phytopathogen cell membranes at significantly lower concentrations than conventional chemical copper or sulfur sprays.

The integration of endophyte-derived capped nanoparticles provides enhanced catalytic stability, lower environmental leaching, and targeted delivery within host plant vascular systems (Ghorbani et al., 2018).

### 6.2 Omics Integration and Activation of Silent Biosynthetic Gene Clusters

Genome sequencing of Basidiomycetes reveals that many secondary metabolic pathways are controlled by silent or cryptically expressed Biosynthetic Gene Clusters (BGCs) that remain unexpressed under standard axenic laboratory conditions. Fungal endophytes often downregulate secondary metabolite production when removed from the complex chemical environment of host plant tissues (Harman et al., 2021).

To unlock this hidden biosynthetic potential, modern biotechnology employs multi-omics approaches (metagenomics, transcriptomics, metabolomics) alongside targeted metabolic elicitation strategies. The process begins with genomic mining of the endophytic macrofungus to identify dormant polyketide synthase (PKS), non-ribosomal peptide synthetase (NRPS), and terpenoid synthase gene clusters. Researchers then apply distinct activation mechanisms:

- One Strain-Many Compounds (OSMAC) strategies, which systematically alter physical and nutritional parameters such as carbon/nitrogen sources, pH, temperature, and aeration to induce transcript expression (Schulz & Boyle, 2005).
- Epigenetic modifications, using small-molecule chemical elicitors like histone deacetylase (HDAC) inhibitors or DNA methyltransferase inhibitors to alter chromatin structure, opening silenced genomic loci for active transcription.
- Microbial co-culturing, which simulates competitive natural microbiomes by introducing live phytopathogens or host plant cell extracts to trigger defensive metabolic pathways.
- Heterologous expression, transferring identified BGCs into fast-growing model microbial hosts (Piotr & Mariola, 2026).

Through these convergent strategies, silent gene cascades are transcriptionally activated, yielding novel secondary metabolites with broad antimicrobial efficacy against resistant crop pathogens (Bolwell & Daudi, 2009).

## 7. Conclusions

Endophytic mushroom-associated fungi constitute a functionally diverse and ecologically significant group of plant symbionts with substantial potential for sustainable crop protection. Unlike conventional biological control agents that rely predominantly on direct pathogen antagonism, macrofungal endophytes operate through a sophisticated dual strategy: direct suppression of phytopathogens via antimicrobial metabolites, lytic enzymes, mycoparasitism, and nutrient competition, coupled with indirect host-mediated immune modulation through ISR and SAR priming, ROS homeostasis regulation, and structural cell wall reinforcement. Model organisms such as *Serendipita indica* illustrate the translational feasibility of these mechanisms, demonstrating consistent disease suppression across multiple cropping systems under both controlled and field conditions. Despite these promising attributes, significant knowledge gaps persist regarding the taxonomic diversity of endophytic Basidiomycetes, the molecular basis of host-endophyte signaling, and the ecological

factors governing colonization success in agricultural environments. Future research priorities should focus on large-scale metagenomic surveys to catalog endophytic macrofungal diversity across major cropping systems, functional characterization of secondary metabolites and effector proteins, and development of robust formulation and delivery technologies for commercial application. Integration of multi-omics approaches with advanced biotechnological tools including nanoparticle synthesis and epigenetic elicitation of silent biosynthetic gene clusters will accelerate the discovery of novel bioactive compounds and enhance the efficacy of endophyte-based biocontrol products. Realizing the full potential of endophytic mushroom-associated fungi requires interdisciplinary collaboration among mycologists, plant pathologists, biotechnologists, and agricultural practitioners to develop cost-effective, scalable solutions that address the global challenge of reducing agrochemical dependency while ensuring food security.

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