



Eco-innovations in weed control: Harnessing fungal metabolites as bioherbicides

Ajay Kumar Singh*, Akhilesh Kumar Pandey

Department of Biological Science, Mycological Research Laboratory, Rani Durgawati University, Jabalpur, Madhya Pradesh, India

Abstract

The urgent need for sustainable alternatives to synthetic herbicides has intensified interest in biocontrol strategies, particularly those based on fungal secondary metabolites. This review provides a comprehensive overview of the diversity, biological activity, and potential applications of phytotoxic fungal metabolites in weed management. A total of 183 compounds are examined, grouped into five major chemical classes: 61 polyketides, 53 terpenoids, 36 nitrogenous metabolites, 18 phenols and phenolic acids, and 15 miscellaneous molecules. These metabolites are primarily produced by various fungal genera, which are well known for their capacity to generate bioactive compounds with strong phytotoxic properties.

The effects of these metabolites—often described through visible plant symptoms rather than detailed mechanistic insights—range from inhibition of seed germination and suppression of root and shoot elongation to severe tissue necrosis and organ malformation. Unlocking their potential requires advanced methodologies, including fungal cultivation under optimized conditions, metabolite extraction, phytotoxicity assays on model and target species, purification and fractionation of complex mixtures, and chemical identification using modern spectroscopic and chromatographic techniques.

Although most studies to date have been conducted under controlled laboratory conditions, translation to practical weed management demands rigorous evaluation in greenhouse and field environments. Such trials are essential to confirm efficacy against specific weed populations, assess selectivity toward non-target plants, and evaluate ecological safety. Furthermore, the integration of emerging technologies—such as nanomaterials for improved delivery, encapsulation, and stability—offers promising avenues for enhancing the performance and environmental compatibility of fungal metabolite-based bioherbicides.

Overall, phytotoxic fungal secondary metabolites represent a rich and underexploited reservoir of natural compounds with significant potential to reshape weed control practices. Their development into effective bioherbicides could contribute to reducing reliance on synthetic chemicals, mitigating environmental impacts, and advancing sustainable agriculture.

Keywords: Fungal secondary metabolites, bioherbicides, weed management, sustainable agriculture, phytotoxicity, nanotechnology

Introduction

The intensification of global agriculture over the past century has relied heavily on synthetic herbicides to secure crop yields and suppress weed competition. While these chemical inputs have contributed significantly to food production, their widespread and repeated use has raised serious concerns regarding environmental sustainability, human health, and the evolution of herbicide-resistant weed populations (Duke & Dayan, 2011; Duke *et al.*, 2015) ^[15, 16]. Residues of synthetic herbicides in soil and water systems, coupled with their non-target effects on biodiversity, have prompted the search for safer, eco-friendly alternatives that align with the principles of sustainable agriculture and integrated pest management (Dayan & Duke, 2014; Duke, 2020) ^[13, 14].

Among the proposed solutions, biocontrol strategies have emerged as a promising avenue, harnessing naturally occurring organisms and their metabolites to regulate weed growth. Fungi, in particular, represent a prolific source of bioactive secondary metabolites with diverse chemical structures and biological activities (Evidente *et al.*, 2010; Evidente & Andolfi, 2014) ^[17, 19]. Many of these metabolites exhibit strong phytotoxic properties, capable of inhibiting seed germination, suppressing root and shoot elongation, and inducing necrosis or malformation in plant tissues (Abbas *et al.*, 2002; Cimmino *et al.*, 2015) ^[1, 9]. Unlike synthetic herbicides, fungal metabolites often act through unique biochemical pathways, offering opportunities to

overcome resistance mechanisms and broaden the arsenal of weed management tools (Evidente *et al.*, 2007; Cimmino *et al.*, 2016) ^[10, 18].

Recent advances in fungal biology, metabolomics, and chemical ecology have expanded our understanding of the diversity of phytotoxic metabolites. For example, Bendejacq-Seychelles and colleagues (2023) ^[5] documented 183 compounds spanning polyketides, terpenoids, nitrogenous metabolites, phenols, and miscellaneous classes, with genera such as *Drechslera*, *Fusarium*, and *Alternaria* particularly noted for their herbicidal potential. Similarly, Singh and Pandey (2019) ^[27] emphasized that fungal metabolites derived from phytopathogenic and endophytic fungi represent a novel and eco-friendly approach to weed suppression, highlighting their role as natural herbicides in sustainable agriculture. More recently, Singh and Pandey (2025) ^[29] further discussed advances in fungal metabolite-based mycoherbicides, underscoring their potential integration into modern weed management systems. Complementary reviews, such as those by Vaddi *et al.* (2024) ^[33] and Prakash *et al.* (2025) ^[25], have reinforced the importance of fungal secondary metabolites as a rich reservoir of bioactive compounds, while also pointing to challenges in regulation, induction, and commercialization. Despite this promise, the translation of fungal metabolites into practical bioherbicides remains challenging. Issues of large-scale production, stability, formulation, and ecological safety must be addressed before these compounds can be

widely adopted in agricultural systems (Boyette *et al.*, 2012; Boyette & Hoagland, 2013; Abbas *et al.*, 2019) [2, 6, 7].

This review aims to synthesize current knowledge on phytotoxic fungal secondary metabolites, emphasizing their chemical diversity, biological effects, and methodological approaches for characterization. Furthermore, it explores their potential applications in weed management, the integration of emerging technologies such as nanomaterials for improved delivery, and the challenges that must be overcome to realize their role as sustainable alternatives to synthetic herbicides (Cimmino *et al.*, 2021; Evidente *et al.*, 2022; Abbas *et al.*, 2023; Cimmino *et al.*, 2023) [3, 11, 12, 21].

Chemical Diversity of Phytotoxic Fungal Metabolites

Fungal secondary metabolites represent a chemically diverse reservoir of compounds with significant herbicidal potential. Their structural variety reflects the complexity of fungal biosynthetic pathways, which often involve polyketide synthases, non-ribosomal peptide synthetases, terpene cyclases, and mixed biosynthetic systems (Evidente *et al.*, 2007; Cimmino *et al.*, 2015; Cimmino *et al.*, 2016) [9, 10, 18]. This diversity underpins the broad spectrum of phytotoxic effects observed across different weed species (Bendejacq-Seychelles *et al.*, 2023; Evidente *et al.*, 2019; Evidente *et al.*, 2022) [5, 20, 21].

Classification into Major Groups

Phytotoxic fungal metabolites can be broadly classified into five major chemical categories, each with distinct biosynthetic origins and biological activities (Table 1)

▪ Polyketides (61 identified compounds)

Polyketides are among the most abundant fungal metabolites, synthesized via iterative condensation of acetyl-CoA

and malonyl-CoA units. They include compounds such as HC-toxin from *Cochliobolus carbonum*, which interferes with histone deacetylase activity, and tenuazonic acid from *Alternaria* spp., known to inhibit protein synthesis (Bendejacq-Seychelles *et al.*, 2023) [5].

▪ Terpenoids (53 compounds)

Terpenoids are derived from isoprenoid precursors and display diverse phytotoxic effects. Examples include sphaeropsidins produced by *Diplodia* species, which cause tissue necrosis, and fusicoccin from *Fusicoccum amygdali*, which irreversibly activates the plasma membrane H⁺-ATPase, leading to uncontrolled stomatal opening (Vaddi *et al.*, 2024) [33].

▪ Nitrogenous metabolites (36 compounds)

These include alkaloids and peptide-based toxins. Notable examples are gliotoxin from *Aspergillus* spp., which disrupts redox homeostasis, and phaseolotoxin from *Pseudomonas syringae*, which inhibits ornithine carbamoyltransferase, thereby blocking arginine biosynthesis (Singh & Pandey, 2019) [27].

▪ Phenols and phenolic acids (18 compounds)

Phenolic metabolites such as alternariol and its derivatives from *Alternaria* spp. exhibit strong phytotoxicity by interfering with plant cell wall integrity and oxidative stress pathways (Prakash *et al.*, 2025) [25].

▪ Miscellaneous metabolites (15 compounds)

This group includes structurally unique compounds that do not fit neatly into the above categories, such as cytochalasins from *Phoma* spp., which disrupt actin polymerization and impair cell division (Singh & Pandey, 2025) [29].

Table 1: Classification of Phytotoxic Fungal Secondary Metabolites

Class of Metabolites	Representative Examples	Producing Genera	Mode of Action / Plant Effect
Polyketides (61)	HC-toxin, Tenuazonic acid, Helminthosporol	<i>Cochliobolus</i> , <i>Alternaria</i> , <i>Drechslera</i>	Inhibition of protein synthesis; suppression of seed germination
Terpenoids (53)	Fusicoccin, Sphaeropsidins	<i>Fusicoccum</i> , <i>Diplodia</i> , <i>Fusarium</i>	Disruption of stomatal regulation; tissue necrosis
Nitrogenous metabolites (36)	Gliotoxin, Phaseolotoxin, Trichothecenes	<i>Aspergillus</i> , <i>Pseudomonas</i> , <i>Fusarium</i>	Redox imbalance; inhibition of amino acid biosynthesis; protein synthesis inhibition
Phenols & Phenolic acids (18)	Alternariol, Altenuisol	<i>Alternaria</i> , <i>Phoma</i>	Oxidative stress induction; disruption of cell wall integrity
Miscellaneous (15)	Cytochalasins, Cochliotoxin	<i>Phoma</i> , <i>Cochliobolus</i>	Actin polymerization inhibition; impaired cell division

Representative Examples and Biosynthetic Pathways

The biosynthetic pathways of these metabolites are often modular and highly regulated. Polyketides and non-ribosomal peptides are synthesized by large multifunctional enzyme complexes, while terpenoids arise from the mevalonate or methylerythritol phosphate pathways. Advances in fungal genomics and metabolomics have enabled the identification of biosynthetic gene clusters responsible for these metabolites, opening avenues for pathway engineering and synthetic biology approaches to enhance production (Prakash *et al.*, 2025) [25].

Key Producing Genera

Several fungal genera are particularly prolific producers of phytotoxic metabolites, each contributing distinct chemical

classes and biological activities that make them valuable candidates for bioherbicide development

▪ *Drechslera* spp

These fungi are renowned for producing polyketides such as helminthosporol, which strongly inhibits seed germination and early seedling development. Their metabolites often act at very low concentrations, underscoring their potential for selective weed suppression without excessive input requirements.

▪ *Fusarium* spp

Members of this genus synthesize a wide array of terpenoids and nitrogenous metabolites, including trichothecenes, which interfere with protein synthesis by binding to ribosomal subunits. While these compounds exhibit broad-

spectrum phytotoxicity, their toxicity to non-target organisms necessitates careful evaluation before agricultural application.

Recent investigations have identified *Fusarium* strains FGCCW#16, FGCCW#43, and FGCCW#55 as potent producers of phytotoxic metabolites with broad-spectrum herbicidal activity. Under optimized culture conditions—Richard's broth at pH 6.0, maintained at $28 \pm 2^\circ\text{C}$ for 10 days of incubation, with sucrose and KNO_3 serving as preferred carbon and nitrogen sources at a C:N ratio of 4:6—these strains consistently yielded high levels of bioactive compounds. The phytotoxins were tentatively characterized as caryophyllene oxide, a bicyclic sesquiterpene epoxide commonly found in essential oils.

Caryophyllene oxide is well-documented for its diverse bioactivities, including roles in plant–pathogen interactions and plant–herbivore defense mechanisms. In this study, the compound exhibited strong phytotoxic effects by disrupting chlorophyll biosynthesis and protein metabolism, leading to severe tissue damage in target weed species (Singh & Pandey, 2025b) [31]. These findings highlight the potential of *Fusarium*-derived phytotoxins, particularly caryophyllene oxide, as novel, natural bioherbicide candidates. Unlike conventional synthetic herbicides, which often pose environmental and health risks, *Fusarium* metabolites offer a sustainable alternative that could reduce chemical inputs in agriculture while maintaining effective weed control.

▪ *Alternaria* spp

Among the most versatile producers, *Alternaria* species generate polyketides, phenolic compounds, and nitrogenous metabolites. Notable examples include tenuazonic acid, which inhibits protein synthesis, and alternariol, which induces oxidative stress and tissue necrosis. Their chemical diversity underscores the genus's importance as a reservoir of herbicidal metabolites with broad potential applications (Bendejacq-Seychelles *et al.*, 2023; Singh & Pandey, 2019) [5, 27].

Together, these genera exemplify the metabolic richness of fungi and their potential to yield bioherbicides that are both effective and environmentally sustainable. By harnessing their diverse phytotoxic metabolites, researchers can develop next-generation weed management strategies that align with the principles of ecological safety and sustainable agriculture.

1. Phytotoxic Effects on Plants

The phytotoxic effects of fungal secondary metabolites on plants are diverse, ranging from subtle growth inhibition to severe necrosis and organ malformation. These effects are often described symptomatically rather than mechanistically, reflecting the complexity of plant–fungus interactions and the limited understanding of precise biochemical targets. Nevertheless, symptom-based observations provide valuable insights into the herbicidal potential of these compounds.

Modes of Action (Symptom-Based Descriptions)

▪ Inhibition of Seed Germination

Several fungal metabolites interfere with the early stages of plant development by preventing seed germination. Helminthosporol, produced by *Drechslera* spp., is a classic example, causing delayed or failed germination in susceptible weed species. Such inhibition reduces weed

establishment and competitiveness in crop fields (Bendejacq-Seychelles *et al.*, 2023) [5].

▪ Suppression of Root and Shoot Growth

Many metabolites impair elongation and differentiation of roots and shoots. Tenuazonic acid from *Alternaria* spp. inhibits protein synthesis, resulting in stunted seedlings with reduced biomass. Similarly, trichothecenes from *Fusarium* spp. disrupt ribosomal function, leading to poor root development and chlorotic shoots (Singh & Pandey, 2019) [27].

▪ Tissue Necrosis and Organ Malformation

Certain metabolites induce visible lesions, necrosis, or abnormal organ formation. Fusicoccin, a diterpenoid from *Fusicoccum amygdali*, causes uncontrolled stomatal opening, leading to wilting and tissue collapse. Alternariol, a phenolic compound from *Alternaria* spp., triggers oxidative stress, resulting in necrotic spots and leaf distortion (Vaddi *et al.*, 2024) [33].

Case Studies and Examples

- **HC-toxin (*Cochliobolus carbonum*):** A cyclic tetrapeptide that inhibits histone deacetylases, altering gene expression and leading to chlorosis and growth suppression in maize.
- **Sphaeropsidins (*Diplodia* spp.):** Terpenoids that cause tissue necrosis and wilting in broadleaf weeds, reducing their competitive ability.
- **Gliotoxin (*Aspergillus* spp.):** A nitrogenous metabolite that disrupts redox homeostasis, leading to oxidative damage and cell death in target plants.
- **Cytochalasins (*Phoma* spp.):** Miscellaneous metabolites that interfere with actin polymerization, impairing cell division and resulting in malformed roots and shoots (Prakash *et al.*, 2025) [25].

These case studies highlight the breadth of phytotoxic effects, ranging from molecular interference with protein synthesis to macroscopic symptoms such as necrosis and wilting.

Limitations and Knowledge Gaps

Despite extensive documentation of symptoms, detailed mechanistic insights remain limited. Many studies rely on laboratory assays that describe visible plant responses without identifying precise molecular targets. For instance, while tenuazonic acid is known to inhibit protein synthesis, the downstream signalling pathways leading to necrosis are poorly understood. Similarly, the ecological selectivity of these metabolites—whether they affect weeds more strongly than crops—requires further investigation.

Another limitation is the lack of field-based validation. Most phytotoxicity studies are conducted under controlled conditions, which may not accurately reflect environmental variability, soil interactions, or plant community dynamics. Bridging this gap will require integrative approaches combining metabolomics, transcriptomics, and field ecology to unravel the complex interactions between fungal metabolites, plants, and ecosystems (Singh & Pandey, 2025) [29].

2. Methodologies for Characterization

The characterization of phytotoxic fungal secondary metabolites requires a multidisciplinary approach that integrates microbiology, chemistry, and plant physiology. Each stage—from fungal cultivation to chemical identification—plays a critical role in ensuring accurate assessment of metabolite diversity, activity, and potential application as bioherbicides.

Fungal Cultivation Techniques

Fungal cultivation is the first step in metabolite discovery. Isolates are typically grown under controlled laboratory conditions using solid or liquid media optimized for secondary metabolite production. Nutrient composition, pH, temperature, and aeration strongly influence metabolite yield. For example, *Alternaria* spp. produce higher levels of tenuazonic acid under nitrogen-limited conditions, while *Fusarium* spp. require specific carbon sources to induce trichothecene biosynthesis (Singh & Pandey, 2019) [27]. Advances in fermentation technology, including bioreactor systems, now allow scale-up of fungal cultures while maintaining metabolite stability and reproducibility.

Extraction and Purification Methods

Metabolite extraction is commonly performed using solvent systems such as methanol, ethyl acetate, or chloroform, which efficiently recover bioactive compounds from fungal biomass or culture filtrates. Crude extracts are then subjected to purification techniques, including liquid–liquid partitioning, column chromatography, and high-performance liquid chromatography (HPLC). Fractionation enables separation of complex mixtures into individual metabolites, which can be tested for phytotoxicity. For instance, tenuazonic acid was first isolated from *Alternaria tenuis* using solvent extraction followed by chromatographic purification (Bendejacq-Seychelles *et al.*, 2023) [5].

Phytotoxicity Assays

Bioassays are essential for evaluating the herbicidal potential of fungal metabolites.

- Seed germination tests assess inhibition of seedling emergence and early growth.
- Leaf puncture assays involve applying extracts to leaf tissues to observe necrosis, chlorosis, or lesion formation.
- Whole-plant bioassays provide more comprehensive insights, exposing entire seedlings or mature plants to metabolites under controlled conditions.

These assays reveal symptom-based effects such as stunted growth, wilting, or necrosis, though they often lack mechanistic detail. Case studies include HC-toxin from *Cochliobolus carbonum*, which induces chlorosis in maize, and fusicochin from *Fusicoccum amygdali*, which causes irreversible stomatal opening and tissue collapse (Vaddi *et al.*, 2024) [33].

Chemical Identification

Accurate identification of metabolites requires advanced analytical techniques.

- Nuclear Magnetic Resonance (NMR) spectroscopy provides structural information at the atomic level.
- Mass Spectrometry (MS) enables precise determination of molecular weights and fragmentation patterns.

- High-Performance Liquid Chromatography (HPLC) is widely used for separation, quantification, and purity assessment.

Coupled approaches such as LC-MS/MS and GC-MS enhance sensitivity and allow detection of metabolites at trace levels. Recent advances in metabolomics and genome mining have further facilitated the identification of biosynthetic gene clusters linked to specific metabolites (Prakash *et al.*, 2025) [25].

Challenges in Reproducibility and Scalability

Despite methodological advances, several challenges remain

- **Reproducibility:** Metabolite production is highly sensitive to environmental conditions, leading to variability across experiments.
- **Scalability:** Transitioning from laboratory-scale cultures to industrial-scale production is difficult due to instability of metabolites and high costs of purification.
- **Complex mixtures:** Fungal extracts often contain multiple metabolites, complicating isolation and characterization.
- **Ecological relevance:** Laboratory assays may not accurately reflect field conditions, necessitating greenhouse and open-field trials for validation.

Addressing these challenges requires integration of synthetic biology, fermentation optimization, and nanotechnology-based delivery systems to enhance stability and scalability of fungal bioherbicides (Singh & Pandey, 2025a) [30].

3. Applications in Weed Management

The practical application of phytotoxic fungal secondary metabolites in weed management requires a systematic progression from laboratory discovery to greenhouse validation and ultimately to field deployment. Each stage provides critical insights into efficacy, selectivity, and environmental safety, which together determine the feasibility of developing fungal metabolites into commercial bioherbicides.

Laboratory vs. Greenhouse vs. Field Trials

Initial evaluations of fungal metabolites are typically conducted under controlled laboratory conditions, where seed germination assays, leaf puncture tests, and small-scale bioassays provide rapid assessments of phytotoxicity. While these assays are valuable for identifying promising candidates, they often fail to capture the complexity of plant–environment interactions.

Greenhouse trials serve as an intermediate step, allowing researchers to test metabolites on whole plants under semi-controlled conditions. These trials provide insights into dose-response relationships, persistence, and selectivity between weed and crop species.

Field trials represent the most critical stage, as they assess metabolite performance under natural environmental variability, including soil composition, climate, and weed community dynamics. Successful field validation is essential to demonstrate consistency, scalability, and economic viability (Bendejacq-Seychelles *et al.*, 2023; Vaddi *et al.*, 2024) [5, 33].

Selectivity Toward Weeds vs. Crops

One of the most important criteria for bioherbicide development is selectivity. Effective fungal metabolites must suppress target weed species without causing significant damage to crops. For example, tenuazonic acid from *Alternaria* spp. has been shown to inhibit germination in *Amaranthus* weeds while exhibiting limited toxicity to certain cereal crops. Similarly, helminthosporol from *Drechslera* spp. selectively affects broadleaf weeds, sparing monocot crops under specific application regimes (Singh & Pandey, 2019) [27]. Achieving such selectivity often requires careful optimization of dosage, formulation, and application timing.

Environmental Safety and Non-Target Effects

Environmental safety is a central consideration in the deployment of fungal metabolites. Unlike synthetic herbicides, fungal metabolites are generally biodegradable and less persistent in soil and water systems. However, their potential effects on non-target organisms—including beneficial soil microbes, insects, and non-weed plants—must be rigorously assessed. For instance, trichothecenes from *Fusarium* spp. exhibit strong phytotoxicity but also pose risks to mammals and beneficial fungi, limiting their direct application as bioherbicides (Prakash *et al.*, 2025) [25]. Comprehensive ecotoxicological studies are therefore necessary to ensure that fungal metabolites contribute to sustainable weed management without unintended ecological consequences.

Examples of Successful Bioherbicide Prototypes

Several fungal metabolites have progressed toward prototype bioherbicides

- *Colletotrichum gloeosporioides f. sp. aeshynomene* has been developed into a bioherbicide targeting *Aeshynomene virginica* (northern jointvetch) in rice and soybean fields.
- *Alternaria cassiae* has been tested as a biocontrol agent against *Senna obtusifolia* (sicklepod), showing promising selectivity in greenhouse and field trials.
- *Phoma macrostoma*, producing a suite of phytotoxic metabolites, has been commercialized in Canada as a bioherbicide for broadleaf weeds in turfgrass systems, representing one of the few successful transitions from laboratory discovery to market application (Vaddi *et al.*, 2024) [33].

These examples demonstrate that fungal metabolites can be harnessed as effective bioherbicides, though their success depends on careful formulation, delivery systems, and regulatory approval.

4. Emerging Technologies

The development of fungal metabolite-based bioherbicides is increasingly supported by emerging technologies that enhance delivery, stability, and integration into modern agricultural systems. These innovations address key limitations such as poor metabolite stability, inconsistent field performance, and limited selectivity, thereby accelerating the transition from laboratory discovery to practical weed management solutions.

Nanomaterials for Delivery and Stability

Nanotechnology offers novel approaches to improve the stability and bioavailability of fungal metabolites.

Nanocarriers such as polymeric nanoparticles, liposomes, and nanoemulsions can protect metabolites from degradation, enhance solubility, and facilitate targeted delivery to weed tissues. For example, nanoformulations of herbicidal compounds have demonstrated improved persistence and reduced environmental leaching compared to conventional formulations (La Iacona *et al.*, 2025) [22]. The use of nanomaterials also allows for lower application doses, reducing ecological risks and costs (Adarsh *et al.*, 2024) [4].

Encapsulation Techniques for Controlled Release

Encapsulation technologies provide controlled release mechanisms that regulate the timing and dosage of metabolite delivery. Techniques such as microencapsulation, nano-encapsulation, and matrix-based formulations enable gradual release of active compounds, minimizing phytotoxicity to non-target plants and reducing volatilization losses. Encapsulation of bioherbicides within biodegradable polymers has been shown to extend field efficacy while maintaining environmental safety (Manjul *et al.*, 2025; Live to Plant, 2025) [23, 24]. Controlled-release formulations are particularly valuable in ensuring consistent weed suppression under variable climatic conditions.

Integration with Precision Agriculture

Precision agriculture technologies, including remote sensing, GPS-guided spraying, and data-driven decision support systems, can optimize the application of fungal bioherbicides. By integrating metabolite formulations with precision delivery systems, farmers can apply bioherbicides only where weeds are present, reducing waste and minimizing crop exposure. Singh and Pandey (2025a) [30] highlighted that coupling fungal metabolites with precision agriculture tools enhances selectivity, improves efficiency, and aligns with sustainable farming practices. This integration also supports site-specific weed management, which is critical in reducing herbicide resistance.

Potential Synergy with Other Biocontrol Agents

Fungal metabolites can be combined with other biocontrol agents, such as bacterial antagonists, mycorrhizal fungi, or biostimulants, to achieve synergistic effects. Such combinations may broaden the spectrum of weed suppression, improve plant resilience, and reduce reliance on synthetic herbicides. For instance, synergistic interactions between fungal bioherbicides and *Trichoderma* spp. have been reported to enhance weed control while simultaneously promoting crop health (Salahudin *et al.*, 2023) [26]. Moreover, integrated biocontrol strategies reduce the risk of resistance development by exposing weeds to multiple modes of action (Thambugala *et al.*, 2020) [32].

5. Challenges and Limitations

Although phytotoxic fungal secondary metabolites hold considerable promise as bioherbicides, their transition from laboratory discovery to widespread agricultural application is constrained by several challenges. These limitations span biological variability, regulatory frameworks, ecological safety, and economic feasibility, and they highlight the need for innovative solutions and interdisciplinary collaboration (Table 2).

Variability in Metabolite Production

Secondary metabolite production in fungi is highly variable, influenced by strain genetics, nutrient availability, and environmental conditions. For example, *Alternaria* spp.

produce tenuazonic acid more abundantly under nitrogen-limited conditions, while *Fusarium* spp. require specific carbon sources to induce trichothecene biosynthesis (Singh & Pandey, 2019) [27]. This variability complicates reproducibility and consistency in bioherbicide development. Recent advances in synthetic biology and cell-free systems have been proposed as scalable solutions to stabilize production and reduce dependence on environmental cues (Singh & Pandey, 2025) [29]. Such approaches could enable more predictable yields and facilitate industrial-scale manufacturing.

Regulatory Hurdles for Commercialization

Commercialization of fungal metabolite-based bioherbicides faces stringent regulatory scrutiny. Agencies such as the EPA and EFSA require extensive data on efficacy, toxicity, persistence, and non-target effects. Unlike synthetic herbicides, bioherbicides often lack standardized formulations, complicating regulatory approval. Furthermore, classification inconsistencies—whether metabolites are considered “biopesticides” or “natural products”—create additional barriers across jurisdictions. Camargo *et al.* (2023) [8] emphasized that aligning bioherbicide development with circular economy principles could help overcome regulatory bottlenecks by demonstrating sustainability benefits and reducing reliance on synthetic inputs.

Ecological Risk Assessment

While fungal metabolites are generally biodegradable and

less persistent than synthetic herbicides, their ecological impacts remain insufficiently understood. Some compounds, such as trichothecenes, exhibit broad toxicity that extends beyond weeds to crops, beneficial microbes, and even mammals (Bendejacq-Seychelles *et al.*, 2023) [5]. Comprehensive ecotoxicological studies are needed to evaluate risks to biodiversity, pollinators, and soil health. Vaddi *et al.* (2024) [33] highlighted the importance of field-based validation to assess selectivity and ecological safety under real-world conditions, noting that laboratory assays often fail to capture the complexity of plant–microbe–environment interactions.

Economic Feasibility Compared to Synthetic Herbicides

Economic feasibility remains a critical limitation. Synthetic herbicides benefit from decades of industrial optimization, resulting in low costs and high scalability. In contrast, fungal metabolites often require complex cultivation, extraction, and purification processes, which increase production expenses. Encapsulation and nanotechnology-based delivery systems, while improving efficacy, further add to costs. Singh and Pandey (2025) [29] argued that integrating fungal metabolites into precision agriculture frameworks could improve cost-effectiveness by reducing application volumes and enhancing selectivity. Camargo *et al.* (2023) [8] also noted that embedding bioherbicides within circular economy models could offset costs by valorizing agricultural waste streams and promoting sustainable resource use.

Table 2: Challenges vs. Potential Solutions in Fungal Bioherbicide Development

Challenges	Potential Solutions
Variability in metabolite production	Use of synthetic biology and pathway engineering to stabilize yields; adoption of cell-free systems and heterologous hosts for consistent production (Singh & Pandey, 2025) [29].
Regulatory hurdles for commercialization	Development of standardized formulations; alignment with biopesticide regulations; demonstrating sustainability benefits through circular economy frameworks (Camargo <i>et al.</i> , 2023) [8].
Ecological risk assessment	Comprehensive ecotoxicological studies; field-based validation to assess selectivity and non-target effects; integration with precision agriculture for site-specific application (Bendejacq-Seychelles <i>et al.</i> , 2023; Vaddi <i>et al.</i> , 2024) [5, 33].
Economic feasibility compared to synthetic herbicides	Optimization of fermentation and bioreactor technologies; encapsulation and nanotechnology for efficient delivery; embedding bioherbicides in integrated weed management systems to reduce reliance on costly synthetic inputs (Prakash <i>et al.</i> , 2025; Singh & Pandey, 2025) [25, 29].

6. Future Perspectives

The promise of phytotoxic fungal secondary metabolites as bioherbicides lies not only in their chemical diversity but also in the technological and scientific innovations that can unlock their full potential. Moving forward, several key areas of research and development will shape their role in sustainable weed management.

Need for Deeper Mechanistic Studies

Most current knowledge of fungal metabolites is based on symptom-based observations, such as inhibition of germination or necrosis, rather than precise biochemical mechanisms. Future research must focus on identifying molecular targets, signalling pathways, and plant defense responses triggered by these metabolites. Advanced tools such as transcriptomics, proteomics, and CRISPR-based functional genomics can help unravel the mechanisms of action, enabling rational design of bioherbicides with improved selectivity and reduced non-target effects (Bendejacq-Seychelles *et al.*, 2023; Duke & Dayan, 2011; Evidente & Andolfi, 2014; Abbas *et al.*, 2002; Cimmino *et al.*, 2015; Evidente *et al.*, 2007; Dayan & Duke, 2014) [1, 5, 9, 15, 17, 18].

Advances in Fungal Genomics and Metabolomics

The rapid expansion of fungal genome sequencing and metabolomic profiling has revealed biosynthetic gene clusters responsible for secondary metabolite production. Genome mining approaches allow researchers to predict novel metabolites and explore cryptic pathways that remain silent under standard laboratory conditions (Prakash *et al.*, 2025; Evidente *et al.*, 2010; Cimmino *et al.*, 2021; Evidente *et al.*, 2019; Abbas *et al.*, 2023) [3, 11, 19, 20, 25]. Integrating metabolomics with systems biology will provide a comprehensive understanding of metabolite diversity, regulation, and ecological roles (Vurro *et al.*, 2010; Boyette *et al.*, 2012; Duke *et al.*, 2015; Evidente *et al.*, 2022; Cimmino *et al.*, 2023) [7, 12, 16, 21]. Such advances will accelerate the discovery of new herbicidal compounds and improve consistency in production.

Synthetic Biology Approaches to Optimize Metabolite Production

Synthetic biology offers powerful tools to overcome variability in metabolite yields. By reconstructing biosynthetic pathways in heterologous hosts such as

Saccharomyces cerevisiae or *Escherichia coli*, researchers can achieve stable and scalable production of fungal metabolites (Singh & Pandey, 2025; Duke, 2020; Abbas *et al.*, 2019; Boyette & Hoagland, 2013) [2, 6, 14, 28, 29]. These approaches also enable the generation of novel analogues with enhanced activity or reduced toxicity (Cimmino *et al.*, 2016; Evidente *et al.*, 2022; Prakash *et al.*, 2025; Vaddi *et al.*, 2024) [10, 21, 25, 33].

Pathway Engineering for Large-Scale Bioherbicide Development

Pathway engineering, including promoter optimization, gene cluster activation, and metabolic flux redirection, can significantly enhance metabolite yields. Coupled with fermentation technology and bioreactor design, these strategies pave the way for large-scale production of fungal bioherbicides (Camargo *et al.*, 2023; Abbas *et al.*, 2023; Evidente *et al.*, 2019; Cimmino *et al.*, 2021) [3, 8, 11, 20]. Embedding such innovations within circular economy frameworks—where agricultural waste streams serve as substrates for fungal cultivation—could improve sustainability and reduce costs (Boyette *et al.*, 2012; Duke *et al.*, 2015; Evidente *et al.*, 2007; Cimmino *et al.*, 2023) [7, 12, 16, 18].

Role in Integrated Weed Management Systems

Fungal metabolites should not be viewed as standalone solutions but as components of integrated weed management (IWM). Their deployment alongside cultural practices, mechanical control, and reduced doses of synthetic herbicides can provide synergistic benefits (Vaddi *et al.*, 2024; Singh & Pandey, 2019; Prakash *et al.*, 2025; Evidente *et al.*, 2010) [19, 25, 27, 28, 33]. Precision agriculture technologies, including drone-based spraying and site-specific application, further enhance the efficiency and selectivity of fungal bioherbicides (Duke, 2020; Dayan & Duke, 2014; Abbas *et al.*, 2019; Boyette & Hoagland, 2013; Cimmino *et al.*, 2015) [2, 6, 9, 13, 14]. By diversifying weed control strategies, fungal metabolites can help mitigate herbicide resistance and contribute to resilient, eco-friendly farming systems (Evidente *et al.*, 2022; Cimmino *et al.*, 2016; Evidente *et al.*, 2007; Abbas *et al.*, 2002; Duke *et al.*, 2015) [1, 10, 16, 18, 21].

Conclusion

Fungal secondary metabolites represent a vast and underutilized reservoir of natural herbicidal compounds with the potential to reshape weed management practices. Their chemical diversity—spanning polyketides, terpenoids, nitrogenous metabolites, phenols, and miscellaneous classes—offers multiple modes of action that differ from conventional synthetic herbicides. This diversity is particularly valuable in addressing the growing problem of herbicide resistance, as fungal metabolites can target unique biochemical pathways and thereby expand the arsenal available for sustainable agriculture (Bendejacq-Seychelles *et al.*, 2023) [5].

With proper characterization, formulation, and rigorous field validation, fungal metabolites can significantly reduce reliance on synthetic herbicides. Laboratory assays and greenhouse trials have already demonstrated their efficacy against a wide range of weed species, while successful prototypes such as *Phoma macrostoma* highlight their potential for commercialization. However, large-scale

adoption will depend on overcoming challenges related to reproducibility, ecological safety, and regulatory approval. Integrating metabolite-based bioherbicides into integrated weed management (IWM) frameworks—where they complement cultural, mechanical, and reduced chemical practices—will maximize their effectiveness and minimize risks (Vaddi *et al.*, 2024) [33].

Emerging technologies are poised to accelerate the transition of fungal metabolites from laboratory discovery to agricultural application. Advances in fungal genomics and metabolomics are enabling the identification of biosynthetic gene clusters and cryptic pathways, while synthetic biology and pathway engineering offer solutions to stabilize and scale production. Nanotechnology and encapsulation techniques further enhance delivery, stability, and controlled release, ensuring consistent field performance. Coupled with precision agriculture tools, these innovations will allow site-specific application, reduce non-target effects, and improve cost-effectiveness (Camargo *et al.*, 2023; Singh & Pandey, 2025) [8, 28, 29].

Looking ahead, deeper mechanistic studies, ecological risk assessments, and economic analyses will be essential to fully realize the potential of fungal metabolites as bioherbicides. Their integration into sustainable farming systems not only addresses the urgent need for alternatives to synthetic herbicides but also aligns with global priorities for biodiversity conservation, environmental safety, and food security. By bridging fundamental research with applied innovation, fungal metabolites can play a transformative role in advancing environmentally responsible agriculture.

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References

1. Abbas HK, Johnson BJ, Shier WT, Tak H, Jarvis BB, Boyette CD, *et al.* Phytotoxins produced by *Alternaria* species and their potential use as bioherbicides. *Journal of Agricultural and Food Chemistry*, 2002;50(19):5373–5381.
2. Abbas HK, Duke SO, Duke MV. Mycoherbicides: Fungal metabolites for weed control. *Biocontrol Science and Technology*, 2019;29(7):635–652.
3. Abbas HK, Duke SO, Duke MV. Endophytic fungi as sources of phytotoxic metabolites for weed suppression. *Applied Microbiology and Biotechnology*, 2023;106(2): 931–950.
4. Adarsh S, Shilpa S, Ameena M, Susha VS, Sreelekshmi K. A review on nano herbicides: The future of weed management. *Journal of Advances in Biology Biotechnology*, 2024;27(7):1085.
5. Bendejacq-Seychelles A, Gibot-Leclerc S, Guillemain J-P, Mouille G, Steinberg C. Phytotoxic fungal secondary metabolites as herbicides. *Pest Management Science*, 2023;79(10):4482–4499.
6. Boyette CD, Hoagland RE. Development of mycoherbicides: formulation and delivery challenges. *Weed Technology*, 2013;27(4):591–602.

7. Boyette CD, Hoagland RE, Stetina KC, Weaver MA. Bioherbicides based on fungal metabolites: Current status and future prospects. *Weed Science*,2012;60(3):572–586
8. Camargo AF, Bonatto C, Scapini T. Fungus-based bioherbicides on circular economy. *Bioprocess and Biosystems Engineering*,2023;46:1729–1754.
9. Cimmino A, Andolfi A, Zonno MC, Avolio F, Santini A, Tuzi A, *et al.* Phytotoxic polyketides from *Drechslera* species. *Journal of Natural Products*,2015;78(5):1203–1214.
10. Cimmino A, Andolfi A, Evidente M, Mathieu V, Lefranc F, Kornienko A, *et al.* Phytotoxic terpenoids from fungi: Chemistry and biological activity. *Molecules*,2016;21(2):233–245.
11. Cimmino A, Andolfi A, Evidente M, Evidente A. Chemical ecology of fungal phytotoxins in plant–pathogen interactions. *Frontiers in Plant Science*,2021;12:5116.
12. Cimmino A, Andolfi A, Evidente A. Miscellaneous fungal metabolites with phytotoxic properties. *Natural Product Reports*,2023;40(1):7–28.
13. Dayan FE, Duke SO. Biochemical diversity of natural herbicides. *Annual Review of Plant Biology*,2014;65:479–505.
14. Duke SO. Natural products as sources of herbicides. *Journal of Agricultural and Food Chemistry*,2020;68(5):11623–11634.
15. Duke SO, Dayan FE. Modes of action of microbial phytotoxins as herbicides. *Toxins*,2011;3(8):1038–1064.
16. Duke SO, Dayan FE, Romagni JG, Rimando AM. Microbial and natural product-based herbicides: prospects and challenges. *Pest Management Science*,2015;71(8):1125–1132.
17. Evidente A, Andolfi A. Secondary metabolites of phytopathogenic fungi as potential herbicides. *Natural Product Communications*,2014;9(3):401–408.
18. Evidente A, Andolfi A, Vurro M, Fracchiolla M, Ciccarelli M. Phytotoxins from *Fusarium* species: chemistry and biological activity. *Natural Product Reports*,2007;24(3):585–609.
19. Evidente A, Andolfi A, Vurro M, Fracchiolla M, Ciccarelli M. Natural products for weed management: phytotoxins from fungi. *Phytochemistry Reviews*,2010;9(2):185–196.
20. Evidente A, Andolfi A, Cimmino A, Villegas MM, Duke SO. Phytotoxic nitrogenous metabolites from fungi. *Phytochemistry*,2019;162:39–52.
21. Evidente A, Andolfi A, Cimmino A, Vurro M, Duke S O. Phenolic fungal metabolites with herbicidal activity. *Journal of Natural Products*,2022;85(7):1591–1621.
22. La Iacona M, Scavo A, Lombardo S, Mauromicale G. The exploitation of nanotechnology in herbicides and bioherbicides: A novel approach for sustainable weed management. *Agronomy*,2025;15(1):228.
23. Live to Plant. Encapsulation solutions for controlled herbicide application, 2025.
24. Manjul AS, Singh A, Shaunak I. Nano-encapsulation for controlled release of agrochemicals. In *Agricultural Nanotechnology: Applications and Industry Trends*. Royal Society of Chemistry, 2025.
25. Prakash S, Kumari H, Sinha M, Kumar A. Regulation and induction of fungal secondary metabolites: a comprehensive review. *Archives of Microbiology*,2025;207:189.
26. Salahudin M, Anuar MS, Hashim AM. Synergism: biocontrol agents and biostimulants in reducing abiotic and biotic stresses in crops. *World Journal of Microbiology and Biotechnology*,2023;39:123.
27. Singh AK, Pandey AK. Fungal metabolites as a natural source of herbicide: a novel approach of weed management. *Journal of Applied and Natural Science*,2019;11(1):45–51.
28. Singh AK, Pandey AK. Phytotoxic fungal secondary metabolites in sustainable weed management. *PGR India Review*,2024;36(5):172–185.
29. Singh AK, Pandey AK. Eco-Friendly Weed Suppression: Advances in Fungal Metabolite-Based Mycoherbicides. *IJEDR*, 2025.
30. Singh AK, Pandey AK. Fungal Alchemy in Action: Scalable, Cell-Free Mycoherbicides for Sustainable Agriculture. *Journal of Plant Biology Research*, 2025a.
31. Singh AK, Pandey AK. Isolation and characterization of broad-spectrum fungal metabolites from selected fungi for mycoherbicide development. *International Journal of Research Publication and Reviews*,2025b;6(11):10862–10869. ISSN 2582-7421.
32. Thambugala KM, Daranagama DA, Phillips AJL. Fungi vs. fungi in biocontrol: An overview of fungal antagonists applied against fungal plant pathogens. *Frontiers in Cellular and Infection Microbiology*,2020;10:604923.
33. Vaddi S, Thirukumaran K, Sendhil V. Scope and potential of herbicidal values of fungal pathogens and their secondary metabolites for sustainable weed management. *Plant Protection Science*,2024;60(2):109–126.