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# Cell-Free Fungal Mycoherbicides for Sustainable Weed Management

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*Abstract*—**Weed infestations remain one of the major constraints to global agricultural productivity, causing significant yield losses and increasing dependence on synthetic herbicides. The rapid evolution of herbicide-resistant weed populations, environmental concerns associated with chemical herbicides, and increasing demand for sustainable crop production systems have intensified the search for alternative weed management strategies. Mycoherbicides, traditionally based on living fungal propagules, have emerged as promising biological weed control agents. However, limitations associated with living fungal formulations, including environmental sensitivity, short shelf life, inconsistent field performance, and regulatory challenges, have restricted their widespread commercialization.**

**Cell-free fungal broth-based mycoherbicides represent a new generation of bioherbicides that exploit fungal-derived bioactive compounds without requiring viable fungal propagules. These preparations offer several advantages, including improved formulation stability, enhanced biosafety, simplified regulatory requirements, easier standardization, and compatibility with modern formulation technologies.**

**This review critically examines the current status of cell-free fungal broth-based mycoherbicides, covering fungal strain selection, fermentation processes, metabolite production, characterization methodologies, formulation technologies, mechanisms of herbicidal action, field application strategies, commercialization prospects, and regulatory considerations. Future opportunities involving synthetic biology, metabolic engineering, artificial intelligence-assisted metabolite discovery, nanoformulations, and precision application systems are also discussed.**

**The integration of cell-free fungal bioherbicides into integrated weed management programs may provide environmentally sustainable solutions for reducing reliance on synthetic herbicides while supporting long-term agricultural productivity.**

*Index Terms*—**Cell-free fungal broth, mycoherbicide, fungal metabolites, phytotoxins, weed management, biocontrol, sustainable agriculture**

# I. INTRODUCTION

## 1.1 Global Impact of Weeds on Agricultural Productivity

Weeds are among the most destructive biological constraints limiting agricultural productivity worldwide. They compete aggressively with crop plants for essential resources including nutrients, water, light, and space, resulting in substantial reductions in crop growth and yield. Globally, weeds are responsible for greater economic losses in agriculture than insect pests and plant diseases, accounting for approximately 34% of total crop production losses under unmanaged conditions (Oerke 2006).

The increasing adoption of intensive agricultural practices and reliance on chemical herbicides have contributed to the evolution of herbicide-resistant weed populations, further complicating weed management strategies. To date, more than 270 weed species have evolved resistance to one or more herbicide modes of action, representing a major threat to global food security and agricultural sustainability (Heap 2025).

## 1.2 Limitations of Chemical Herbicides

Synthetic herbicides have remained the cornerstone of weed management for more than six decades due to their effectiveness, ease of application, and relatively low cost.

However, excessive and repeated use of herbicides has generated numerous agronomic, environmental, and regulatory concerns. One of the most significant challenges is the rapid evolution of herbicide resistance resulting from continuous selection pressure imposed by repeated application of herbicides with similar modes of action (Délye et al. 2013).

Environmental contamination arising from herbicide runoff and leaching has raised concerns regarding groundwater quality and ecosystem health. Increasing evidence also indicates that some herbicides may negatively affect soil microbial diversity and ecosystem functions, thereby compromising long-term soil fertility (Bünemann et al. 2006).

## 1.3 Biological Weed Management

Biological weed management has emerged as a sustainable and environmentally compatible alternative to conventional chemical control methods. Biological control involves the use of living organisms or their products to suppress weed populations below economically damaging levels (Charudattan 2001). Among biological control agents, fungal pathogens have attracted considerable attention because of their ability to infect and damage specific weed species while exhibiting minimal impacts on non-target plants (Singh & Pandey 2019).

Mycoherbicides are bioherbicidal products based on pathogenic fungi and their propagules. Despite their potential, conventional mycoherbicides often suffer from several limitations, including dependence on favorable environmental conditions, limited shelf life, inconsistent field performance, and challenges associated with large-scale production and storage of viable propagules (Auld and Morin 1995; Charudattan and Dinoor 2000).

### 1.4 Emergence of Cell-Free Fungal Metabolite-Based Mycoherbicides

Recent advances in fungal biotechnology, fermentation science, and natural product chemistry have stimulated interest in cell-free fungal metabolite-based mycoherbicides as a next-generation weed management strategy (Singh & Pandey 2024; Singh & Pandey 2025). These preparations typically contain extracellular phytotoxins, secondary metabolites, hydrolytic enzymes, organic acids, peptides, and other bioactive molecules capable of inducing phytotoxic effects in susceptible weed species (Hoagland 2001; Evidente et al. 2006).

Cell-free mycoherbicides offer several advantages over conventional fungal bioherbicides. Because they do not contain viable fungal propagules, they exhibit improved storage stability, reduced environmental risks, simplified quality control, and greater formulation flexibility. Consequently, cell-free fungal metabolite-based products are increasingly recognized as promising components of integrated weed management programs aimed at reducing dependence on synthetic herbicides while promoting sustainable agricultural production systems. Table 1 Representative studies reporting the use of fungal culture filtrates, cell-free broths, phytotoxic metabolites, and fungal secondary metabolites for biological control of economically important terrestrial and aquatic weeds. The table highlights the diversity of fungal taxa, target weeds, active compounds, and reported herbicidal effects documented in the literature.

Table 1. Representative Studies on Cell-Free Fungal Broths and Metabolite-Based Mycoherbicides Against Major Weeds

| Year | Fungal Species / Source | Target Weed | Cell-Free Metabolite / Broth Component | Reported Efficacy* | Reference |
|---|---|---|---|---|---|
| 1977 | *Alternaria alternata* | Various broadleaf weeds | Tentoxin | Chlorosis, inhibition of chloroplast development | Liebermann et al. (1977) |
| 1996 | *Fusarium oxysporum* | Various weeds | Fusaric acid | Growth inhibition and wilting symptoms | Bacon et al. (1996) |
| 2003 | *Alternaria alternata* | Eichhornia crassipes | Phytotoxic metabolites in culture filtrate | Severe leaf necrosis and plant decline | Babu et al. (2003) |
| 2004 | *Alternaria alternata* | Eichhornia crassipes | Cell-free toxin-containing filtrates | Up to 79% tissue necrosis under optimized conditions | El-Morsy (2004) |
| 2005 | *Alternaria eichhorniae* | Eichhornia crassipes | Phytotoxic metabolites | Complete weed suppression | Shabana (2005) |

| | | | | | |
|---|---|---|---|---|---|
| | | | and extracellular products | under favorable conditions | |
| 2006 | *Curvularia lunata* | Grassy weeds | Curvularin | Root growth inhibition and biomass reduction | Evidente et al. (2006) |
| 2009 | *Phoma* spp. | Broadleaf weeds | Cytochalasins and secondary metabolites | Significant seedling growth suppression | Vurro et al. (2009) |
| 2011 | *Alternaria alternata* | Parthenium hysterophorus | Culture filtrate phytotoxins | Reduced germination and severe foliar injury | Javaid and Ali (2011) |
| 2014 | *Phoma dimorpha* | Multiple weed species | Membrane-filtered phytotoxic metabolites | Strong phytotoxicity under greenhouse conditions | Chaves et al. (2014) |
| 2024 | Various fungi (*Alternaria*, *Colletotrichum*, *Curvularia*, *Fusarium*, *Phoma*) | *Amaranthus*, *Bidens*, *Conyza*, *Brachiaria*, *Parthenium* | Cell-free fermentation filtrates | Significant inhibition of germination, growth, and biomass accumulation | Ocán-Torres et al. (2024) |
| 2024 | *Alternaria alternata* | Parthenium hysterophorus | Cell-free metabolites | Severe damage to seeds and leaves; strong bioherbicidal potential | Singh & Pandey (2024) |

*Efficacy values reported in different studies were measured using different parameters (weed mortality, chlorosis, necrosis, biomass reduction, disease severity, or germination inhibition), therefore direct numerical comparison should be interpreted cautiously.

## II. CONCEPT AND PRINCIPLES OF CELL-FREE FUNGAL METABOLITE-BASED MYCOHERBICIDES

### 2.1 Definition

Cell-free fungal metabolite-based mycoherbicides are bioherbicidal preparations derived from the extracellular products of phytopathogenic fungi without the presence of viable fungal propagules (Fig 1). These preparations are typically obtained by cultivating selected fungal isolates under controlled fermentation conditions followed by removal of fungal biomass through filtration, centrifugation, or membrane separation techniques (Hoagland 2001; Evidente et al. 2006).

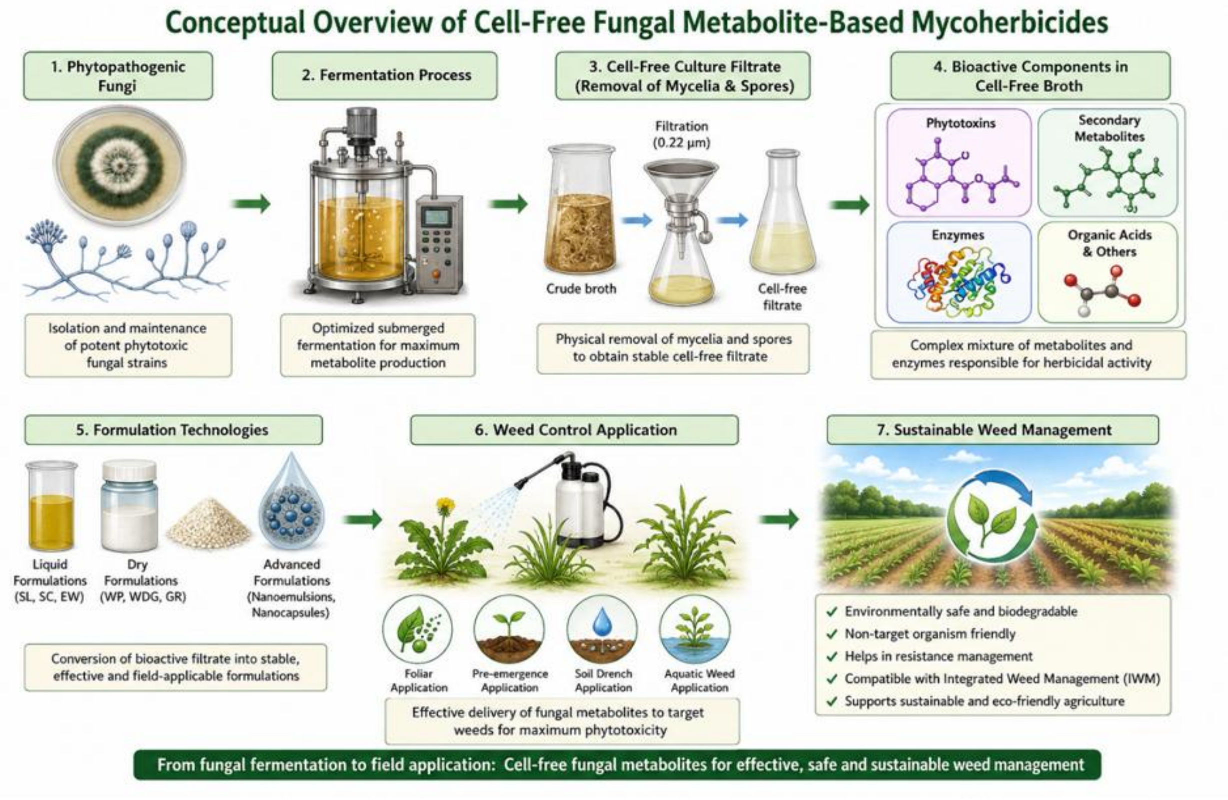

Fig 1. Cell-free fungal metabolite-based mycoherbicides

## 2.2 Components of Cell-Free Fungal Broths

### 2.2.1 Phytotoxic Metabolites

Phytotoxic metabolites constitute the primary active ingredients of most cell-free mycoherbicidal preparations. These compounds are typically low-molecular-weight secondary metabolites capable of interfering with essential plant physiological processes (Singh & Pandey 2020; Singh & Pandey 2024).

Alternariol is a dibenzopyrone derivative produced by *Alternaria* species that induces oxidative stress, chlorosis, and growth inhibition in susceptible plants (Tsuge et al. 2013).

Tentoxin is a cyclic tetrapeptide produced by Alternaria alternata that disrupts chloroplast development and photosynthetic activity, resulting in chlorosis and reduced plant growth (Liebermann et al. 1977).

Fusaric acid is synthesized by several Fusarium species and interferes with ion transport, cellular metabolism, and vascular function, leading to wilting and tissue necrosis (Bacon et al. 1996).

Other notable phytotoxic compounds include cercosporin, ascochitine, curvularin, and cytochalasins, each exhibiting distinct herbicidal mechanisms and target specificities.

### 2.2.2 Extracellular Enzymes

In addition to phytotoxins, many phytopathogenic fungi secrete extracellular hydrolytic enzymes that contribute significantly to herbicidal activity. Cellulases, pectinases, and xylanases facilitate degradation of plant cell walls. Proteolytic enzymes and lipases further contribute to cellular damage and disruption (Walton 1994; Agrios 2005).

The combined action of extracellular enzymes and phytotoxic metabolites often produces synergistic herbicidal effects that exceed the activity of individual compounds alone.

### 2.2.3 Secondary Metabolites

Secondary metabolites constitute a chemically diverse group of fungal natural products. Major classes include polyketides, terpenoids, alkaloids, and peptides, each contributing to the overall herbicidal potential of cell-free fungal broths through multiple mechanisms of action (Keller et al. 2005; Schulz et al. 2002).

## III. DEVELOPMENT OF CELL-FREE MYCOHERBICIDES

### 3.1 Selection of Fungal Isolates

The selection of suitable fungal isolates represents the most critical step in the development of cell-free mycoherbicides. Ideal fungal candidates should produce potent phytotoxic metabolites with consistent herbicidal activity against target weed species while exhibiting minimal adverse effects on economically important crops and non-target organisms (Vurro et al. 2009).

Important Fungal Genera for Cell-Free Mycoherbicide Development

Alternaria spp. produce diverse phytotoxins including alternariol, alternariol monomethyl ether, and tentoxin, inducing chlorosis, necrosis, and photosynthetic inhibition.

Colletotrichum spp. produce polyketides and diketopiperazines with significant potential against broadleaf and grassy weeds.

Fusarium spp. synthesize fusaric acid, beauvericin, and enniatins that interfere with cellular metabolism and membrane integrity.

Curvularia spp. produce curvularin and related macrolides exhibiting strong phytotoxic effects on seed germination and seedling growth.

Exserohilum spp., Phoma spp., and Drechslera spp. have also attracted attention due to their production of highly active phytotoxins.

### 3.2 Fermentation Technologies for Metabolite Production

### 3.2.1 Submerged Fermentation (SmF)

Submerged fermentation is the most widely utilized method for industrial production of fungal metabolites, offering high scalability, precise environmental control, efficient mixing, automation capability, and simplified downstream processing. These features make SmF particularly suitable for large-scale production of extracellular phytotoxins and enzymes (Pandey et al. 2000).

### 3.2.2 Solid-State Fermentation (SSF)

Solid-state fermentation involves fungal cultivation on moist solid substrates, utilizing agricultural residues such as wheat bran, rice bran, and sugarcane bagasse. This approach offers lower production costs, utilization of inexpensive wastes, reduced wastewater generation, and often higher metabolite concentrations compared with submerged fermentation (Mitchell et al. 2006).

### 3.2.3 Process Optimization

Carbon and Nitrogen Sources: Carbon sources and organic nitrogen sources significantly influence metabolite production. High carbon-to-nitrogen ratios generally favor secondary metabolite production.

pH and Temperature: Most phytopathogenic fungi exhibit optimal metabolite production at pH 4.5-6.5 and temperatures of 25-30°C.

Aeration and Agitation: Adequate aeration promotes efficient respiration and metabolite accumulation, while optimal agitation speeds balance oxygen transfer and fungal physiological stability.

## IV. CHARACTERIZATION OF CELL-FREE FUNGAL BROTHS

### 4.1 Chemical Characterization

### 4.1.1 Chromatographic Methods

High-Performance Liquid Chromatography (HPLC) is widely used for quantification of fungal phytotoxins and serves as a standard quality control tool (Singh & Pandey 2024; Strobel et al. 1991).

Liquid Chromatography–Tandem Mass Spectrometry (LC–MS/MS) enables simultaneous identification and quantification of multiple metabolites, becoming one of the most powerful tools for studying fungal bioactive compounds (Maffi et al. 2018).

Liquid Chromatography–Quadrupole Time-of-Flight Mass Spectrometry (LC–QTOF–MS) provides accurate mass measurements and high-resolution molecular characterization, valuable for discovery of novel phytotoxic metabolites (Wolfender et al. 2019).

### 4.1.2 Spectroscopic Techniques

FTIR Spectroscopy facilitates rapid screening of chemical classes including phenolics, ketones, alcohols, and peptides (Stuart 2004).

Nuclear Magnetic Resonance (NMR) Spectroscopy remains the gold standard for structural elucidation of natural products and is extensively employed for determining structures of fungal phytotoxins (Dewick 2009).

Raman Spectroscopy offers a rapid and non-destructive method for molecular characterization with complementary information to FTIR.

### 4.2 Biological Characterization

Seed Germination Assays provide rapid screening of phytotoxic activity by measuring effects on germination percentage and seedling vigor.

Root Growth Inhibition Assays assess effects on root elongation and biomass accumulation, serving as reliable indicators of metabolite potency.

Chlorophyll Reduction Studies provide information regarding effects on photosynthetic machinery through quantification of chlorophyll content and fluorescence parameters.

Electrolyte Leakage Tests measure membrane integrity and reflect membrane disruption and lipid peroxidation.

## V. FORMULATION TECHNOLOGIES

### 5.1 Challenges in Formulation Development

Despite promising herbicidal potential, fungal metabolites face formulation challenges including metabolite instability, UV degradation, oxidative breakdown, and rapid environmental degradation. Advanced formulation strategies are required to protect bioactive compounds and optimize their herbicidal efficacy under field conditions (Singh & Pandey 2025; Boyette et al. 2015; Bailey 2014).

### 5.2 Liquid Formulations

Soluble Liquid Concentrates (SL) consist of bioactive fungal metabolites dissolved in aqueous systems, providing rapid biological activity and ease of dilution.

Suspension Concentrates (SC) contain finely dispersed metabolites in liquid carrier systems with stabilizing agents, offering enhanced storage stability.

Oil-in-Water (O/W) Emulsions distribute oil droplets containing bioactive compounds, improving leaf adhesion and protection of lipophilic metabolites.

### 5.3. Dry Formulations

Wettable Powders (WP) adsorb metabolites onto inert carriers, offering excellent storage stability and ease of manufacturing. Water-Dispersible Granules (WDG) are dust-free formulations that rapidly disintegrate when mixed with water, providing improved handling safety. Microgranules contain encapsulated or adsorbed metabolites, useful for soil applications and controlled release delivery.

### 5.4. Encapsulation and Nanoformulations

Alginate Encapsulation protects metabolites within biodegradable gel matrices, enabling controlled release (John et al. 2011). Chitosan Microcapsules provide antimicrobial activity, biodegradability, and enhanced surface adhesion (Campos et al. 2014). Liposomes are phospholipid vesicles enhancing cellular penetration and improving metabolite stability (Kah and Hofmann 2014). Nanoemulsions are thermodynamically stable dispersions improving foliar coverage and penetration. Polymeric Nanoparticles improve metabolite stability and facilitate targeted delivery significantly enhancing field performance (Fraceto et al. 2016).

## VI. MECHANISMS OF HERBICIDAL ACTION

The herbicidal activity of cell-free fungal metabolite-based mycoherbicides arises from complex biochemical and physiological disruptions (Fig 2). Unlike synthetic herbicides targeting single pathways, fungal metabolites affect multiple cellular processes simultaneously, resulting in multifaceted phytotoxic effects that reduce resistance development likelihood (Singh & Pandey 2022; Dayan and Duke 2014; Berestetskiy 2008).

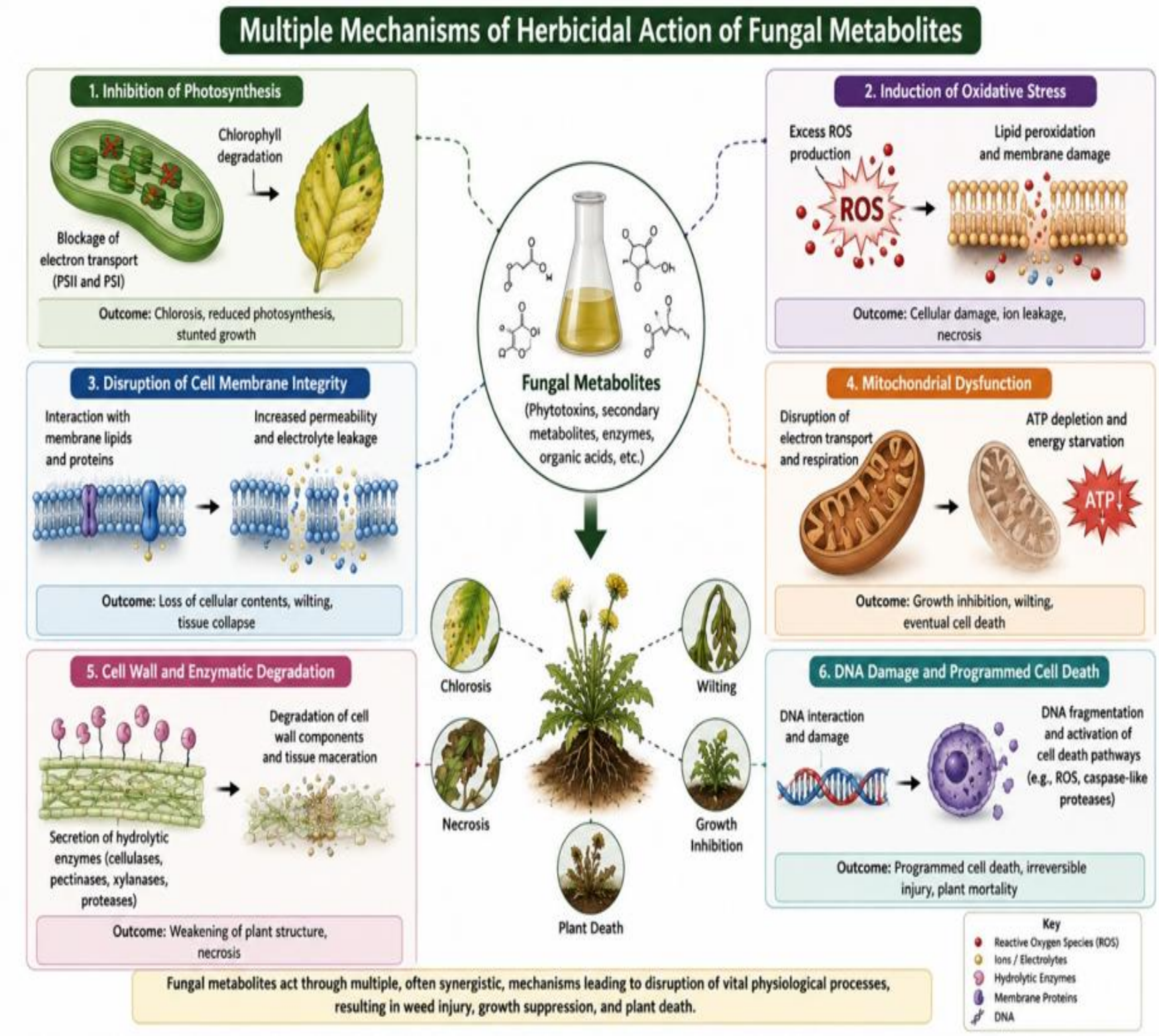

Fig.2. Mechanism of herbicidal action of fungal metabolites

### 6.1 Photosynthesis Disruption

Chlorophyll Degradation: Several metabolites induce chlorosis through chlorophyll degradation or inhibited biosynthesis, reducing the plant & capacity to capture light energy.

Photosystem II Inhibition: Metabolites interfere with PSII function, disrupting electron flow and reducing ATP and NADPH production (Liebermann et al. 1977).
Reduced Photosynthetic Efficiency: Cumulative effects decline photosynthetic performance, contributing to growth inhibition, necrosis, and plant death.

### 6.2 Oxidative Stress and ROS Generation

ROS Production: Metabolites stimulate excessive production of superoxide radicals, hydrogen peroxide, and hydroxyl radicals, overwhelming antioxidant defenses (Apel and Hirt 2004).
Cellular Damage: The oxidative burst causes chlorosis, lipid peroxidation, protein oxidation, DNA damage, and cell death (Daub and Ehrenshaft 2000; Gill and Tuteja 2010).

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### 6.3 Membrane Damage and Cellular Leakage

Lipid Peroxidation: ROS attack unsaturated fatty acids, compromising membrane fluidity and permeability.
Electrolyte Leakage: Membrane disruption results in leakage of ions and cellular solutes, indicating severe impairment (Hoagland 2001).
Cellular Collapse: Progressive degradation leads to turgor loss, cellular dehydration, and tissue necrosis.

### 6.4 Cell Wall Degradation

Extracellular enzymes hydrolyze cellulose, hemicellulose, and pectin, degrading plant cell wall architecture. Cellulases weaken cell wall integrity, xylanases degrade hemicelluloses, and pectinases cause cell separation and tissue maceration (Walton 1994; Agrios 2005).

### 6.5 Mitochondrial Dysfunction and Programmed Cell Death

ATP Depletion: Disruption of oxidative phosphorylation reduces ATP production, limiting energy for essential cellular processes (Tiwari et al. 2002).
Programmed Cell Death: Fungal metabolites trigger PCD pathways through DNA fragmentation, caspase-like protease activation, and autophagy induction (Lam et al.
2001; Liu and Bassham 2012).

Multi-target mechanisms distinguish cell-free mycoherbicides from conventional herbicides and provide foundations for sustainable weed management. Table 2 represents Commercially registered and experimental fungal-derived mycoherbicides, including both propagule-based products and emerging cell-free metabolite formulations. The table highlights target weeds, formulation types, commercialization status, and current development stages. Commercial products such as DeVine®, Collego®, BioMal®, Chontrol™, and Sarritor® represent important milestones in biological weed control, whereas cell-free fungal metabolite formulations constitute the next generation of bioherbicidal technologies.

Table 2. Commercial and Experimental Mycoherbicides Derived from Fungi: Product Status, Target Weeds, Formulation Type, and Commercialization Stage

| Product Name | Active Fungus / Agent | Target Weed(s) | Formulation Type | Commercialization Stage | Country/ Region | Status | Key Reference |
|---|---|---|---|---|---|---|---|
| DeVine® | *Phytophthora palmivora* | Strangler vine (*Morrenia odorata*) | Liquid inoculum | Fully commercialized | USA | First registered mycoherbicide (1981) | Charudattan (2001); Cordeau et al. (2016) |
| Collego® | *Colletotrichum gloeosporioides* f. sp. *aeschynomene* | Northern jointvetch (*Aeschynomene virginica*) | Spore suspension | Commercialized | USA | Registered and marketed | Charudattan (2001); Cordeau et al. (2016) |
| BioMal® | *Colletotrichum gloeosporioides* f. sp. *malvae* | Round-leaved mallow (*Malva pusilla*) | Wettable powder | Commercialized (limited) | Canada | Commercial product | Bailey (2014); Cordeau et al. (2016) |
| Woad Warrior® | *Puccinia thlaspeos* | Dyer's woad (*Isatis tinctoria*) | Rust inoculum | Commercialized | USA | Niche market | Cordeau et al. (2016) |
| Chontrol™ | *Chondrostereum purpureum* | Woody weeds and hardwood sprouts | Paste formulation | Commercialized | Canada/Europe | Forestry application | Cordeau et al. (2016); |
| Smoulder® | *Chondrostereum purpureum* | Deciduous woody weeds | Stump treatment paste | Commercialized | Europe | Forestry bioherbicide | Cordeau et al. (2016) |
| Sarritor® | *Sclerotinia minor* | Dandelion (*Taraxacum officinale*) | Granular formulation | Commercialized | Canada | Turf weed management | Cordeau et al. (2016) |
| Alternaria-based | *Alternaria alternata* | *Parthenium* | Cell-free broth / | Experimental | India, Egypt | Greenhouse and | Javaid and Ali |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| cell-free filtrates | | *hysterophorus*, aquatic weeds | metabolite formulation | | | field evaluation | (2011); Shabana (2005) |
| Fusarium metabolite formulations | *Fusarium oxysporum* | Broadleaf weeds | Cell-free metabolite concentrates | Experimental | Global | Laboratory and greenhouse stage | Bacon et al. (1996); Berestetskiy (2008) |
| Curvularin-based bioherbicides | *Curvularia lunata* | Grasses and broadleaf weeds | Purified metabolite formulation | Experimental | Global | Pre-commercial research | Evidente et al. (2006) |
| Phoma metabolite formulations | *Phoma* spp. | Various annual weeds | Cell-free filtrates | Experimental | Europe | Greenhouse validation | Vurro et al. (2009) |
| Exserohilum metabolite formulations | *Exserohilum* spp. | *Echinochloa crus-galli* and grasses | Fermentation broth concentrates | Experimental | USA, Europe | Research phase | Hoagland (2001) |
| Cercosporin-based formulations | *Cercospora* spp. | Broadleaf weeds and aquatic weeds | Purified phytotoxin formulation | Experimental | Global | Laboratory development | Daub and Ehrenshaft (2000) |
| Nano-encapsulated fungal metabolite formulations | Various fungi | Multiple herbicide-resistant weeds | Nanoemulsion / nanocapsule | Emerging technology | Global | Proof-of-concept | Kah et al. (2018); Fraceto et al. (2016) |

## VII. AGRICULTURAL APPLICATIONS

Cell-free fungal metabolite-based mycoherbicides offer significant potential for managing diverse weed species under varying environmental conditions. Their broad spectrum of activity and multiple modes of action provide opportunities for sustainable weed management in agricultural, horticultural, forestry, and aquatic ecosystems (Charudattan 2001; Hoagland 2001).

### 7.1 Broadleaf Weed Control

*Parthenium hysterophorus*: Fungal metabolites from *Alternaria, Fusarium*, and *Phoma* species suppress seed germination and induce severe foliar necrosis (Evans 1997; Javaid and Ali 2011).
*Xanthium strumarium*: *Colletotrichum, Alternaria*, and *Curvularia* metabolites show inhibitory effects on seedling emergence and photosynthetic activity (Singh and Pandey, 2019a).
*Amaranthus* Species: Fungal phytotoxins provide alternative strategies for managing herbicide-resistant populations (Dayan and Duke 2014).

### 7.2 Grass Weed Management

*Echinochloa crus-galli*: *Exserohilum, Curvularia*, and *Alternaria* metabolites inhibit seed germination and seedling establishment (Vurro et al. 2009).
*Cynodon dactylon*: Cell-free filtrates reduce vegetative growth and suppress regrowth from underground propagules (Hoagland 2001).
*Phalaris minor*: Fungal metabolites show inhibitory effects on germination and seedling growth (Chauhan and Mahajan 2014).

### 7.3 Aquatic Weed Control

*Eichhornia crassipes: Alternaria eichhorniae, Cercospora piaropi*, and *Fusarium* metabolites induce chlorosis and necrosis, providing environmentally compatible control (Shabana 2005).
*Pistia stratiotes* and *Salvinia molesta*: Fungal metabolites impair photosynthesis and reduce biomass accumulation (Charudattan 2001; Room et al. 1981).

### 7.4 Integration into Integrated Weed Management (IWM)

Cell-free fungal metabolites can be effectively integrated into IWM programs through herbicide rotation, mechanical combinations, cover crop systems, precision applications, and site-specific strategies. Their multiple mechanisms of action help delay resistance evolution while improving overall efficacy (Norsworthy et al. 2012).

## VIII. FIELD EVALUATION AND EFFICACY ASSESSMENT

Rigorous evaluation under greenhouse and field conditions determines efficacy, selectivity, environmental compatibility, and economic viability. Field trials remain essential for assessing real-world performance under variable environmental conditions (Charudattan 2001; Boyette et al. 2015).

### 8.1 Experimental Design

Randomized Complete Block Design (RCBD) effectively controls field heterogeneity and remains the most widely employed design, typically involving three to six replications per treatment (Gomez and Gomez 1984).

Split-Plot Design is useful for evaluating multiple factors simultaneously, investigating interaction effects between variables (Steel et al. 1997).

### 8.2 Evaluation Parameters

Weed Mortality: Most direct herbicidal indicator, assessed at regular intervals using standardized scales (Charudattan 2001).

Biomass Reduction: Measures herbicidal efficacy through fresh and dry weight reduction (Vurro et al. 2009).

Chlorophyll Content: SPAD meter readings and spectrophotometric analysis assess physiological damage (Dayan and Duke 2014).

Crop Safety and Selectivity: Essential for determining practical herbicide performance (Boyette et al. 2015).

Yield Response: Ultimate indicator of weed management success and commercial viability.

### 8.3 Environmental Influences

Temperature: Most fungi exhibit optimal activity within 20-30°C range (Auld and Morin 1995).

Relative Humidity: High humidity promotes prolonged droplet persistence and metabolite uptake.

UV Radiation: Major limiting factor for metabolite persistence (Daub and Ehrenshaft 2000).

Rainfall and Moisture: Precipitation can wash metabolites from leaf surfaces before absorption (Vurro et al. 2009).

## X. COMMERCIALIZATION AND REGULATORY CONSIDERATIONS

Commercial development requires regulatory approval, safety assessment, market acceptance, and economic feasibility. Cell-free products offer advantages over conventional microbial bioherbicides but must satisfy rigorous regulatory requirements (Marrone 2019; Bailey 2014).

### 9.1 Product Registration and Regulatory Frameworks

United States: Regulated under EPA’s FIFRA and Biochemical Pesticide Program with reduced data requirements for lower-risk products (USEPA 2023).

European Union: Regulated under Regulation (EC) No. 1107/2009 with emphasis on environmental protection and biodiversity (EFSA 2021).

India: Regulated by CIBRC with growing emphasis on sustainable agriculture (CIBRC 2023).

### 9.2 Safety Evaluation

Toxicological Assessment: Evaluates acute/chronic toxicity, genotoxicity, and reproductive effects (Dayan et al. 2009).

Ecotoxicological Assessment: Evaluates impacts on beneficial insects, aquatic organisms, and soil microbes (Bailey 2014).

Environmental Fate: Most fungal metabolites exhibit relatively short environmental half-lives (Marrone 2019).

### 9.3 Market Opportunities

Organic Agriculture: Fastest-growing agricultural sector creating demand for biological alternatives (Willer et al. 2023).

Herbicide-Resistant Weeds: Expanding global problem creating market demand for alternative technologies (Heap 2025).

Specialty Crops: High-value crops justify investment in sustainable weed management.

## X. EMERGING TECHNOLOGIES

Emerging technologies are accelerating fungal metabolite discovery, improving production efficiency, and enhancing formulation performance. Integration of metabolic engineering, synthetic biology, AI, and precision agriculture is expected to transform bioherbicide development (Marrone 2019; Dayan and Duke 2014).

### 10.1 Metabolic Engineering

Targeted modification of cellular metabolic pathways enhances phytotoxin production through overexpression of biosynthetic genes, deletion of competing pathways, and optimization of precursor availability. Recent studies demonstrate substantial improvements through pathway engineering (Nielsen and Keasling 2016).

### 10.2 Synthetic Biology

Synthetic biology enables design of entirely new biological systems, reconstructing fungal biosynthetic pathways in alternative hosts and creating synthetic metabolite analogues. CRISPR-Cas genome editing accelerates pathway manipulation and discovery of silent biosynthetic clusters (Cairns et al. 2018; Awan et al. 2017).

### 10.3 Artificial Intelligence

Machine learning algorithms analyze large omics datasets to predict biosynthetic gene clusters, novel metabolites, structure-activity relationships, and toxicological properties.

AI-assisted metabolite discovery reduces development costs and improves research efficiency (Stokes et al. 2020).

### 10.4 Precision Agriculture and Smart Systems

Advanced sensing technologies including multispectral imaging, LiDAR, and machine vision enable real-time weed detection and mapping. Integration with GIS, GNSS, variable-rate application, and IoT sensors facilitates targeted bioherbicide deployment (Gebbers and Adamchuk 2010).

Drone-based application systems enable high-resolution weed mapping and precise herbicide delivery to localized infestations, particularly valuable for row crops, specialty crops, and aquatic weed management (Huang et al. 2021).

## XI. FUTURE PERSPECTIVES

Cell-free fungal metabolite-based mycoherbicides represent one of the most promising frontiers in sustainable weed management. Several scientific, technological, regulatory, and commercial challenges must be addressed before widespread adoption (Keller 2019).

### 11.1 Discovery of Novel Metabolites

Less than 10% of fungal species have been characterized, suggesting enormous untapped potential. Future research should prioritize exploration of under-investigated taxa, endophytic fungi, marine species, and discovery of metabolites with novel modes of action (Blackwell 2011).

### 11.2 Nano-Enabled Delivery Systems

Nanotechnology offers innovative solutions for protecting fungal metabolites. Development of smart nanoparticles, stimuli-responsive systems, UV-protective formulations, and targeted delivery mechanisms will significantly enhance field performance (Kah et al. 2018; Fraceto et al. 2016).

### 11.3 Climate-Resilient Formulations

Climate change impacts weed dynamics and herbicide performance. Development of thermally stable, UV-resistant, rainfast formulations with controlled-release technologies capable of broad environmental adaptability will be essential for global adoption (Matzrafi et al. 2021).

### 11.4 Regulatory Harmonization

Harmonization of international registration requirements, establishment of science-based risk assessment frameworks, and streamlined approval procedures for biological products would reduce development costs and facilitate global market access (Singh & Pandey 2026; Marrone 2019).

### 11.5 Commercial-Scale Optimization

Economic feasibility requires development of low-cost fermentation substrates utilizing agricultural wastes, process intensification strategies, continuous fermentation systems, and advanced bioreactor design. Artificial intelligence-assisted optimization is expected to improve metabolite yields and production consistency (Pandey et al. 2019).

## XII. CONCLUSION

Cell-free fungal metabolite-based mycoherbicides have emerged as a promising next-generation approach for sustainable weed management. By harnessing the herbicidal potential of fungal-derived phytotoxins, secondary metabolites, extracellular enzymes, and other bioactive compounds, these products circumvent limitations of living fungal formulations while offering improved stability, enhanced biosafety, simplified regulatory requirements, easier standardization, and greater formulation flexibility.

Recent advances in fungal biotechnology, fermentation engineering, metabolomics, proteomics, and natural product discovery have substantially enhanced understanding of fungal phytotoxic metabolites and their mechanisms of action. Progress in formulation science—including encapsulation technologies, controlled-release systems, and nanoformulations—has improved stability, efficacy, and field applicability. The multifaceted modes of action provide valuable opportunities for managing herbicide- resistant weed populations and reducing reliance on conventional herbicides.

Integration of emerging technologies including metabolic engineering, synthetic biology, artificial intelligence-assisted discovery, precision agriculture, and drone-based applications is expected to accelerate development and commercialization. These innovations offer potential for improving metabolite production efficiency, identifying novel compounds, enhancing formulation performance, and enabling highly targeted weed management.

Despite considerable progress, challenges remain including large-scale production economics, metabolite yield variability, formulation stability, regulatory complexities, and comprehensive safety assessments. Addressing these challenges requires coordinated interdisciplinary efforts among microbiologists, plant pathologists, weed scientists, formulation chemists, fermentation engineers, regulatory authorities, and industry stakeholders.

As global agriculture faces increasing pressures from herbicide resistance, environmental concerns, and regulatory restrictions, cell-free fungal metabolite- based mycoherbicides are poised to become integral components of integrated weed management programs. Their successful development and adoption could contribute significantly to sustainable agricultural intensification, environmental conservation, and global food security in coming decades.

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## REFERENCES

[1] Aebersold R, Mann M. 2016. Mass-spectrometric exploration of proteome structure and function. Nature 537:347–355.

[2] Agrios GN. 2005. Plant Pathology. 5th ed. Burlington (MA): Elsevier Academic Press.

[3] Andolfi A, Boari A, Evidente M, Cimmino A, Vurro M, Evidente A. 2014. Gulypyrones A and B and phomentrioloxin: fungal metabolites with herbicidal activity. J Nat Prod 77:2352–2360.

[4] Apel K, Hirt H. 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 55:373–399.

[5] Auld BA, Morin L. 1995. Constraints in the development of bioherbicides. Weed Technol 9:638–652.

[6] Awan AR, Blount BA, Bell DJ, Shaw WM, Ho JC, McKiernan RM, Ellis T. 2017. Biosynthesis of natural products through synthetic biology. Trends Biotechnol 35:947–959.

[7] Bacon CW, Porter JK, Norred WP, Leslie JF. 1996. Production of fusaric acid by Fusarium species. Appl Environ Microbiol 62:4039–4043.

[8] Bailey KL. 2014. The bioherbicide approach to weed control using plant pathogens. In: Abrol DP, editor. Integrated Pest Management. San Diego (CA): Academic Press. p.245–266.

[9] Berestetskiy AO. 2008. A review of fungal phytotoxins: from basic studies to practical use. Appl Biochem Microbiol 44:453–465.

[10] Blackwell M. 2011. The fungi: 1, 2, 3 ... 5.1 million species? Am J Bot 98:426–438.

[11] Boyette CD, Hoagland RE, Weaver MA. 2015. Biocontrol efficacy of fungal pathogens for weed management. Biol Control 89:123–132.

[12] Bünemann EK, Schwenke GD, Van Zwieten L. 2006. Impact of agricultural inputs on soil organisms. Aust J Soil Res 44:379–406.

[13] Cairns TC, Nai C, Meyer V. 2018. How a fungus shapes biotechnology: advances in Aspergillus systems biology. Biotechnol Adv 36:739–751.

[14] Calvo AM, Wilson RA, Bok JW, Keller NP. 2002. Relationship between secondary metabolism and fungal development. Microbiol Mol Biol Rev 66:447–459.

[15] Campos EVR, de Oliveira JL, Fraceto LF, Singh B. 2014. Chitosan nanoparticles as delivery systems for agricultural applications. Int J Biol Macromol 75:45–52.

[16] Charudattan R, Dinoor A. 2000. Biological control of weeds using plant pathogens. Plant Dis 84:1159–1170.

[17] Charudattan R. 2001. Biological control of weeds by means of plant pathogens. Crit Rev Plant Sci 20:539–570.

[18] Chauhan BS, Mahajan G. 2014. Recent Advances in Weed Management. Cham (Switzerland): Springer.

[19] CIBRC. 2023. Biopesticide Registration Guidelines. Central Insecticides Board and Registration Committee, Government of India.

[20] Daub ME, Ehrenshaft M. 2000. The photoactivated toxin cercosporin: contributions to plant disease and fundamental biology. Annu Rev Phytopathol 38:461–490.

[21] Dayan FE, Cantrell CL, Duke SO. 2009. Natural products in crop protection. Bioorg Med Chem 17:4022–4034.

[22] Dayan FE, Duke SO. 2014. Natural compounds as next-generation herbicides. Plant Physiol 166:1090–1105.

[23] Del Valle EMM. 2004. Cyclodextrins and their uses: a review. Process Biochem 39:1033–1046.

[24] Délye C, Jasieniuk M, Le Corre V. 2013. Deciphering the evolution of herbicide resistance in weeds. Trends Genet 29:649–658.

[25] Dewick PM. 2009. Medicinal Natural Products: A Biosynthetic Approach. 3rd ed. Chichester (UK): Wiley.

[26] Duke SO, Powles SB. 2008. Glyphosate: a once-in-a-century herbicide. Pest Manag Sci 64:319–325.

[27] EFSA. 2021. Guidance on the assessment of the relevance of metabolites in groundwater. EFSA Journal 19:6768.

[28] Evans HC. 1997. Parthenium hysterophorus: a review of its weed status and possibilities for biological control. Biocontrol News Inf 18:89N–98N.

[29] Evidente A, Andolfi A, Vurro M, Zonno MC. 2006. Fungal phytotoxins and their potential as bioherbicides. Nat Prod Commun 1:511–520.

[30] Fraceto LF, Grillo R, de Medeiros GA, Scognamiglio V, Rea G, Bartolucci C. 2016. Nanotechnology in agriculture: which innovation potential does it have? Front Environ Sci 4:20.

[31] Gebbers R, Adamchuk VI. 2010. Precision agriculture and food security. Science 327:828–831.

[32] Gill SS, Tuteja N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance. Plant Physiol Biochem 48:909–930.

[33] Girard V, Joubert DA. 2019. Contribution of secreted carbohydrate-active enzymes in fungal virulence. Curr Opin Microbiol 52:1–8.

[34] Gomez KA, Gomez AA. 1984. Statistical Procedures for Agricultural Research. 2nd ed. New York (NY): Wiley.

[35] Heap I. 2025. The International Survey of Herbicide Resistant Weeds. Available from: www.weedscience.org

[36] Hoagland RE. 2001. Microbial allelochemicals and pathogens as bioherbicidal agents. Weed Technol 15:835–857.

[37] Huang Y, Hoffmann WC, Lan Y, Wu W, Fritz BK. 2021. Development of agricultural drones for precision crop protection. Biosyst Eng 203:38–55.

[38] Javaid A, Ali S. 2011. Biocontrol of *Parthenium hysterophorus* L. using *Alternaria alternata* culture filtrates. Pak J Weed Sci Res 17:153–163.

[39] John RP, Tyagi RD, Brar SK, Surampalli RY, Prevost D. 2011. Bio-encapsulation technologies in agriculture. Crit Rev Biotechnol 31:211–226.

[40] Kah M, Hofmann T. 2014. Nanopesticide research and development. Environ Int 63:224–235.

[41] Kah M, Kookana RS, Gogos A, Bucheli TD. 2018. A critical evaluation of nanopesticides and nanofertilizers against conventional products. Nat Nanotechnol 13:677–684.

[42] Keller NP, Turner G, Bennett JW. 2005. Fungal secondary metabolism: from biochemistry to genomics. Nat Rev Microbiol 3:937–947.

[43] Keller NP. 2019. Fungal secondary metabolism: regulation and function. Nat Rev Microbiol 17:167–180.

[44] Knowles DA. 2008. Recent Developments of Safer Formulations of Agrochemicals. Cambridge (UK): Royal Society of Chemistry.

[45] Kudsk P, Mathiassen SK. 2020. Pesticide regulation and sustainable weed management. Crop Prot 134:105204.

[46] Lam E, Kato N, Lawton M. 2001. Programmed cell death, mitochondria and the plant hypersensitive response. Nature 411:848–853.

[47] Liebermann B, Ellinger A, Günther W. 1977. Tentoxin and chloroplast development. Phytochemistry 16:1881–1884.

[48] Liu Y, Bassham DC. 2012. Autophagy: pathways for self-eating in plant cells. Annu Rev Plant Biol 63:215–237.

[49] Maffi D, Mazzucco M, Lametti G, Cioni P, Ronga C, Bianchi S, Mecca D. 2018. Ultra-high performance liquid chromatography with photodiode array detection for analysis of fungal metabolites. J Chromatogr A 1577:112–119.

[50] Marrone PG. 2019. Pesticidal natural products: current status and future potential. Pest Manag Sci 75:2325–2334.

[51] Matzrafi M, Brunharo CACG, Tehranchian P, Hanson BD. 2021. Climate change effects on weed management and herbicide efficacy. Pest Manag Sci 77:2223–2231.

[52] Mitchell DA, Berovic M, Krieger N. 2006. Solid-state fermentation bioreactors. Biotechnol Adv 24:185–194.

[53] Nielsen J, Keasling JD. 2016. Engineering cellular metabolism. Cell 164:1185–1197.

[54] Norsworthy JK, Ward SM, Shaw DR, et al. 2012. Reducing the risks of herbicide resistance. Weed Sci 60(SP1):31–62.

[55] Nothias LF, Petras D, Schmid R, et al. 2020. Feature-based molecular networking in the GNPS environment. Nat Commun 11:1946.

[56] Oerke EC. 2006. Crop losses to pests. J Agric Sci 144:31–43.

[57] Pandey A, Negi S, Soccol CR. 2019. Current Developments in Biotechnology and Bioengineering. Amsterdam (Netherlands): Elsevier.

[58] Pandey A, Soccol CR, Mitchell D. 2000. New developments in solid-state fermentation. Process Biochem 35:1153–1169.

[59] Patti GJ, Yanes O, Siuzdak G. 2012. Metabolomics: the apogee of the omics trilogy. Nat Rev Mol Cell Biol 13:263–269.

[60] Powles SB, Yu Q. 2010. Evolution in action: plants resistant to herbicides. Annu Rev Plant Biol 61:317–347.

[61] Room PM, Harley KLS, Forno IW, Sands DPA. 1981. Successful biological control of salvinia. Nature 294:78–80.

[62] Schulz B, Baranska M. 2007. Raman spectroscopy of plant tissue – an emerging alternative to molecular diagnostics? ChemMedChem 2:30–37.

[63] Schulz B, Wanke U, Draeger S, Aust HJ. 2002. Endophytes of Ricinus communis: a contribution to the indophytic mycota of *Jatropha curcas* and *Manihot esculenta*. Mycopathologia 158:335–342.

[64] Shabana YM. 2005. Biological control of water hyacinth with fungi. Biocontrol Sci Technol 15:1–17.

[65] Singh, A. K., Pandey, A. K. 2019. Fungal metabolites as natural sources of herbicides: A novel approach to weed management. Journal of Applied and Natural Science, 11(1), 158–163.

[66] Singh, A. K., Pandey, A. K. (2019a). Preliminary screening of isolated mycoherbicidal fungi for the management of the noxious weed *Xanthium strumarium*. Journal of Agri-Search, 6(2), 92–95.

[67] Singh, A. K., Pandey, A. K. 2020. Microbial phytotoxins as sources of herbicides: Current developments and future scope. Acta Scientific Biotechnology, 1(3), 6–11.

[68] Singh, A. K., Pandey, A. K. 2021. Phytotoxic secondary metabolites of fungi as potential sources of natural herbicides: A mini review. RASSA Journal of Science for Society, 3(3), 145–147.

[69] Singh, A. K., Pandey, A. K. 2022. Foliar fungal pathogenic metabolites as promising alternatives to chemical herbicides: Recent developments, future perspectives, and commercialization potential. International Journal of Plant and Environment, 8(1), 44–51.

[70] Singh, A. K., Pandey, A. K. 2023. Innovations in exploiting RNAi-based herbicidal compounds for invasive weed management: Strategies, challenges, and future prospects. International Journal of Research Publication and Reviews, 4(1), 2150–2155.

[71] Singh, A. K., Pandey, A. K. 2024. Exploitation of mycometabolites in weed management: Global scenario and future applications. In Applied Mycology: Entrepreneurship with Microorganisms (Chapter 9, pp. 179–188). Springer Nature.

[72] Singh, A. K., Pandey, A. K. 2025. Fungal metabolite-based cell-free filtrates: A new era of eco-conscious natural herbicides. NL Journal of Agriculture and Biotechnology, 2(4), 40–44.

[73] Singh, A. K., Pandey, A. K. 2026. Regulation of microbial metabolite-based mycoherbicides in India: Policy gaps, risk assessment challenges, and future directions. *International Journal Research Publication Analysis*, 2(2), 1–33.

[74] Singhania RR, Patel AK, Soccol CR, Pandey A. 2009. Recent advances in solid-state fermentation. Biochem Eng J 44:13–18.

[75] Steel RGD, Torrie JH, Dickey DA. 1997. Principles and Procedures of Statistics: A Biometrical Approach. 3rd ed. New York (NY): McGraw-Hill.

[76] Stokes JM, Yang K, Swanson K, et al. 2020. A deep learning approach to antibiotic discovery. Cell 180:688–702.

[77] Strobel GA, Ford E, Worapong J, Harper JK, Arif AM, Grant DM, Fung PCW, Chau RMW. 1991. Isomeric phytotoxins from *Alternaria alternata*. Phytochemistry 31:3669–3673.

[78] Stuart B. 2004. Infrared Spectroscopy: Fundamentals and Applications. Chichester (UK): Wiley.

[79] Tadros T. 2013. Formulation Science and Technology: Physicochemical Principles. Weinheim (Germany): Wiley-VCH.

[80] Templeton GE, Greaves MP, Leonard OK. 1986. Biological herbicides: discovery, development, commercialization. Weed Sci 34:15–21.

[81] Tiwari BS, Belenghi B, Levine A. 2002. Oxidative stress increased respiration and ATH1 expression in *Arabidopsis thaliana*. J Exp Bot 53:1855–1865.

[82] Tsuge T, Harimoto Y, Akimitsu K, Ohtani K, Kodama M. 2013. Host-selective toxins produced by *Alternaria alternata*. FEMS Microbiol Rev 37:44–66.

[83] USEPA. 2023. Biochemical Pesticides Registration Guidelines. Washington (DC): United States Environmental Protection Agency. van Doorn WG, Woltering EJ. 2005. Many ways to exit? Cell death categories in plants. Trends Plant Sci 10:117–122.

[84] Vurro M, Bonciani B, Vannacci G. 2009. Emerging infectious diseases and biological weed control. Plant Pathol 58:426–437.

[85] Walton JD. 1994. Host-selective toxins: agents of compatibility. Plant Cell 6:1723–1733.

[86] Willer H, Schlatter B, Trávníček J, Kemper L, Lernoud J. 2023. The World of Organic Agriculture: Statistics and Emerging Trends. Frick (Switzerland): FiBL

[87] Wolfender JL, Litaudon M, Touboul D, Queiroz EF. 2019. Innovative metabolomics approaches for natural product discovery. Nat Prod Rep 36:855–868.