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Fungal Alchemy in Action: Scalable, Cell-Free Mycoherbicides for Sustainable Agriculture

Ajay Kumar Singh* and Akhilesh Kumar Pandey

Mycological Research Laboratory, Department of Biological Science, Rani Durgawati University, Jabalpur – 482001, Madhya Pradesh, India

Abstract

Weeds are a major contributor to global agricultural losses, and conventional chemical herbicides have long dominated weed control strategies. However, their excessive use has led to widespread herbicide resistance, necessitating higher dosages and causing persistent environmental residues that hinder natural degradation. In response, fungal-based herbicides—known as mycoherbicides—have emerged as eco-friendly alternatives. Despite their potential, commercial deployment remains limited due to inconsistent efficacy and scalability challenges. This review highlights the emerging promise of cell-free fungal metabolites as a next-generation solution for sustainable weed management. These phytotoxic compounds, derived from submerged fermentation—primarily bioreactors—offer advantages in handling, formulation, and application. The use of industrial waste as carbon and nitrogen sources aligns with circular economy principles, enhancing process sustainability. Advances in bioprospecting and in silico screening are accelerating metabolite discovery and optimization. Fungal genera such as Alternaria sp., Colletotrichum sp., Curvularia sp. Fusarium sp., Drechslera sp., and Phoma sp. exhibit notable bioherbicidal activity. However, narrow host specificity, environmental persistence, and regulatory constraints continue to impede widespread adoption. The integration of cell-free metabolites with effective adjuvants alongside chemical herbicides is discussed as a strategy to enhance field efficacy and commercial viability.

Keywords: *Mycoherbicide · Fungal metabolites · Weed management · Bioprocess optimization · Sustainable agriculture.*

1. Introduction

Weeds pose a significant threat to global agriculture by competing with crops for essential resources such as nutrients, water, and light, often thriving under adverse conditions. Their unchecked proliferation leads to reduced yields, elevated production costs, and diminished market profitability. In India and the United States alone, annual weed-related losses in major crops like soybeans and dry beans are estimated at USD 11 billion and USD 17.2 billion, respectively[1,2,3].

Chemical herbicides have long served as the primary tool for weed management, targeting critical metabolic pathways in plants. Glyphosate, the most widely used herbicide globally, inhibits 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) in the shikimic acid pathway, thereby disrupting aromatic amino acid biosynthesis[4, 5]. By 2019, herbicides accounted for 47.5% of the 2 million tons of pesticides used worldwide, with China, the USA, Brazil, and India among the top consumers[6,7]. However, excessive and indiscriminate use has led to the emergence of herbicide-resistant weed populations—particularly against glyphosate and chlorsulfuron—alongside environmental persistence and potential health risks due to their recalcitrant nature[8,9,10].

To counter resistance, novel synthetic herbicides have been developed, including cinmethylin (targeting acyl-ACP thioesterase), cyclopyrimorate (inhibiting homogentisate solanesyltransferase), and tetflupyrolimet (acting on dihydroorotate dehydrogenase)[11,12,13]. However, high genetic homology between crops and weeds complicates target specificity, raising concerns about crop safety and unintended phytotoxicity[14,15]

As a sustainable alternative, bioherbicides—biological agents derived from plants, bacteria, fungi, or viruses—have gained increasing attention[16,17]. Fungi, in particular, are favored for industrial-scale production due to their host specificity, potent bioactivity, and environmentally benign profiles[18,19]. Additionally, bioherbicides offer lower discovery and development costs compared to synthetic herbicides, with relatively fewer regulatory hurdles. Nonetheless, commercial adoption remains constrained by challenges in efficacy, formulation stability, and regulatory compliance[20,21,22]

Recent research underscores the potential of cell-free phytotoxic fungal metabolites to enhance weed suppression while reducing environmental persistence and off-target effects[23,24,25,26,27]. Moreover, the use of industrial residues as fermentation substrates supports cost-effective production and aligns with circular economy principles.

This review critically evaluates the current landscape of fungal herbicides, with a particular focus on cell-free phytotoxic metabolites as scalable, sustainable solutions for weed management. It also examines commercially available fungal products and identifies key scientific and industrial barriers to market success. The novelty lies in bridging cutting-edge scientific innovation with industrial feasibility to chart a path toward commercially viable bioherbicidal technologies.

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Ajay Kumar Singh | drajay2009@gmail.com

2. Production Strategies for Fungal-Based Herbicides Derived from Metabolite Extracts

The development of fungal herbicides typically involves two core stages: the first stage is screening and isolation of microorganisms with phytotoxic activity, and the second stage is large-scale cultivation for formulation and application. However, when targeting cell-free metabolites, additional steps are required—particularly the extraction and purification of bioactive compounds—depending on their chemical nature, which may simplify or complicate downstream processing.

The first phase, Isolation and Screening, begins with identifying dominant weed species in the target environment and evaluating the chemical herbicides currently used for their control to understand potential resistance mechanisms. Fungal candidates are then isolated either from infected weed tissues or from rhizospheric soils where weed-crop interactions are evident[28,29,30]. Alternatively, reference strains from established fungal culture banks may be utilized. These isolates are screened using Petri dish assays with weed seeds, assessing phytotoxic responses such as inhibition of germination, reduction in shoot and root growth, and other indicators of herbicidal activity.

The second phase of bioherbicide development involves selecting the most promising microbial strains and subjecting them to metabolite characterization. This step is critical for elucidating the bioherbicide's mode of action (MOA). Advanced analytical tools such as mass spectrometry and computational techniques like molecular docking are commonly employed for profiling and identifying active phytotoxic compounds.

In the third phase, the focus shifts to evaluating the mycoherbicide's efficacy and target spectrum across various weed species. This assessment can be conducted using both isolated metabolites and whole fungal cultures. Greenhouse trials are recommended to simulate natural conditions and generate ecologically relevant data. Toxicity and ecotoxicity assays are also essential, particularly on crop species related to the target weeds, to determine selectivity and minimize off-target effects.

Once efficacy and selectivity are confirmed, the production process is optimized to enhance metabolite yield. This involves adjusting fermentation parameters based on recent literature and experimental data. The resulting product is then formulated and subjected to further testing to validate its performance across a broader weed spectrum and its safety profile for crops[31,32]

The fourth and final phase involves scaling up production through fermentation, followed by purification of the active metabolites for field trials. Successful field validation paves the way for product registration, patenting, and commercialization.

2.1 Production Dynamics and Strategic Optimization

Fermentation is a pivotal stage in mycoherbicide production, directly influencing yield and cost-efficiency. Currently, one of the major limitations of biological herbicides is their higher production cost compared to chemical alternatives. A techno-economic analysis by Mupondwa estimated that a facility with two 33,000 L fermenters producing 3,602 tons annually would require a capital investment of USD 17.55 million and incur annual operating costs of USD 14.76 million. Despite this, the payback period is under one year, with a net present value (NPV) of 7%, indicating commercial viability.

To further enhance cost-effectiveness in microbial bioherbicide production, several strategies can be implemented, including the use of agro-industrial residues as alternative carbon and nitrogen sources to reduce raw material costs and promote circular economy principles[33,34,35]; optimization of fermentation processes through statistical tools such as response surface methodology; deployment of microbial consortia to broaden the spectrum of target weed species; and careful selection of fermenter types and operational modes aligned with the metabolic profile and growth kinetics of the producing organism[10,36,37]. Collectively, these approaches improve production efficiency and economic viability, thereby supporting the wider adoption of microbial bioherbicides in sustainable agriculture.

2.1.1. Preliminary Process Development

The upstream development of microbial bioherbicides based on cell-free metabolites begins with the selection of pre-characterized microbial strains from established culture banks. These strains must demonstrate high efficacy in controlling one or more target weed species, as validated through greenhouse or field trials. Optimizing fermentation conditions—including nutritional, chemical, physical, and biological parameters—is essential to accelerate microbial growth and metabolite production. Prior to scale-up, inoculum quality must be rigorously assessed for viability, and its quantity should be determined using mathematical models and microbial growth kinetics to minimize the lag phase and promote rapid exponential growth.

Fungal bioherbicides, upstream optimization focuses on sporulation conditions, light exposure as an abiotic stimulus, carbon source concentration, and incubation duration[38,39]

An emerging strategy involves the use of fungal consortia—combinations of multiple fungal species—which can enhance herbicidal efficacy through the production of diverse phytotoxic metabolites. This approach broadens the spectrum of target weeds and improves economic feasibility by consolidating production into a single fermentation batch[40,41,42,43]

However, successful implementation requires thorough laboratory testing to ensure strain compatibility and avoid antagonistic interactions during fermentation.

Once inoculants are prepared and fermentation conditions optimized at the laboratory scale, scale-up can proceed for both bacterial and fungal strains. The choice of a bioreactor is critical and should align with the production goals. Submerged fermentation is the predominant mode due to its scalability and process control. For example, Brun et al.[44] achieved 100% germination inhibition of *Cucumis sativus* and *Sorghum bicolor* using a *Phoma* sp.-based bioherbicide produced in stirred tank bioreactors under optimized conditions: 40–60 rpm agitation, 3 vvm aeration, 10% (v/v) inoculum, and pH 6.0 over 7 days.

Common bioreactor types for SmF include stirred tank and pneumatic bioreactors. Stirred tank bioreactors are favored for their high volumetric mass transfer, achieved through mechanical agitation that ensures uniform gas dispersion and continuous oxygen supply. Pneumatic bioreactors, such as airlift systems, use gas injection to distribute oxygen with minimal shear stress, making them suitable for shear-sensitive organisms, though less efficient for filamentous fungi[45,46]

Solid-state fermentation offers a viable alternative, particularly for fungal strains, by mimicking natural habitats with low moisture content and utilizing agro-industrial residues as nutrient sources—an economically attractive option[47,48]. de Bastos et al[49] demonstrated enhanced bioherbicidal activity against *Cucumis sativus* using *Diaphorte* sp. under SSF, while De Oliveira et al.[50] achieved high cutinase yields (16.22 U/g) with *Fusarium verticillioides*.

SSF systems may operate statically or with air circulation, with tray bioreactors offering practical advantages in gas exchange and CO_2 removal. However, challenges such as humidity control, scalability, and complex downstream processing may limit broader industrial adoption.

Fermentation mode selection—batch, fed-batch, or continuous—is another critical factor. While batch and fed-batch cultures are commonly used for kinetic studies, continuous fermentation offers industrial advantages by maintaining steady-state conditions and reducing downtime, making it attractive for metabolite production[33,51,52.]

To address economic constraints, the use of industrial residues as alternative carbon and nitrogen sources is a proven strategy. This not only reduces production costs but also supports circular economy principles. For instance, Cavaleante et al.[35] used orange and shrimp peels in SmF with *Rhizopus stoloniferse*, yielding bioherbicidal extracts effective against *Crocus sativus*. Camargo et al.[33] utilized microalgae biomass from biogas digestate to produce *Fusarium Fusarium*-based bioherbicides, achieving 80–100% foliar damage in *Cucumis sativus*. Mitchell et al.[53] identified bean brine as an optimal carbon source for *Gloeocercospora sorghi* sporulation, while Brun et al.[44] successfully employed corn mash liquor for *Phoma* sp. fermentation, demonstrating effective control of cucumber and sorghum.

2.1.2. Product Finishing Operations

Following fungal cultivation, the downstream process focuses on recovering and concentrating the bioactive metabolites while eliminating impurities. The first step in downstream processing is separating microbial biomass from the bioactive metabolites. In solid-state fermentation (SSF), this involves recovering all components attached to the solid substrate. de Bastos et al.[49] used distilled water at a 1:10 (w/v) ratio with agitation (100 rpm, 28 °C for 1 h) to extract phytotoxic compounds from *Diaporthe* sp., followed by filtration and storage for concentration.

In submerged fermentation (SmF), where both biomass and metabolites coexist in a liquid medium, extraction is generally unnecessary. Most phytotoxic metabolites are extracellular, so cell lysis is not required. Biomass separation is typically achieved through centrifugation or membrane filtration (e.g., 0.45 μm). Centrifugation is suitable for small-scale operations, while filtration is more cost-effective for industrial-scale production.

Once separated, further purification may be necessary to remove residual impurities that could affect herbicidal performance. Techniques such as membrane filtration (ultrafiltration, microfiltration, nanofiltration) or solvent extraction are selected based on the physicochemical properties of the metabolites. For instance, Chaves et al.[24] concentrated *Phoma dimorpha* broth using polymeric membranes, yielding fractions with strong phytotoxicity against *Echinochloa*, *Amaranthus cruentus*, *Senna obtusifolia*, and *Bidens pilosa*. Similarly, Brun et al.[44] employed methanol, ethanol, and ethyl acetate for liquid–liquid extraction of *Phoma* sp. metabolites, identifying pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl) as the most potent compound against *Cucumis sativus* and *Sorghum bicolor*.

At the industrial level, purification and metabolite identification remain underdeveloped, as most commercial bioherbicides are formulated using live microbial cells. Consequently, purification is primarily conducted at the laboratory scale.

Chromatographic techniques such as HPLC and GC-MS are commonly used for metabolite profiling. For example, Zhang et al.[54] purified 4-hydroxy-3-methoxycinnamic acid and indole derivatives from *Pythium aphanidermatum* using HPLC with UV detection and a reversed-phase C18 column, achieving complete inhibition of *D. sanguinalis* root and coleoptile growth.

Advancing purification strategies requires robust bioprospecting and metabolite profiling, supported by in silico tools such as molecular docking. These technologies simulate interactions between phytotoxic metabolites and target proteins in weeds, helping predict binding affinity and guide the selection of appropriate purification methods. This approach reduces development costs and enhances production efficiency.

Following purification, the bioherbicide must be formulated according to its intended application. Solid formulations are generally more effective than liquid ones for spray applications. Incorporating adjuvants like surfactants can improve metabolite penetration through plant cell walls. Promising formulation strategies include biogranular solids using rice, wheat, soybean, or seed flours, and microemulsions—especially for fungal spore-based products. Once formulated, the product should be stored under defined conditions to preserve stability and efficacy, ready for research trials or commercial deployment.

3. Current Mycoherbicidal Technologies Using Fungal Cultures and Metabolite Extracts

Mycoherbicides for weed control are available in both commercial and experimental forms, derived primarily from fungal sources. While whole-cell fungal cultures have traditionally dominated the field, cell-free fungal metabolites—such as peptides, phytotoxins, enzymes, and other bioactive compounds—are gaining attention for their enhanced specificity, environmental compatibility, and ease of formulation. These metabolite-based products offer promising alternatives to conventional herbicides, although further research is needed to optimize their efficacy and scalability[20,55,56]

Fungi represent a vast biological reservoir for weed management, with approximately 8,000 of the 150,000 described species classified as phytopathogenic. These plant-associated fungi (PAF) exert herbicidal effects through mechanisms such as the secretion of cell wall-degrading enzymes, which hydrolyze plant polysaccharides to facilitate colonization, and the production of mycotoxins, hormones, and secondary metabolites that confer host specificity—an essential trait for selective weed suppression. Over the past decade, several fungal genera have demonstrated notable bioherbicidal potential. For instance, *Alternaria* species such as *A. cassiae*, *A. sonchi*, and *A. alternata* produce selective phytotoxic metabolites, with *A. sonchi* targeting *Sonchus arvensis*, *A. alternata* effective against *Echinochloa* spp. and *Eichhornia crassipes*, and *A. macrospora* showing strong activity against *Parthenium hysterophorus*. Similarly, *Phoma* species like *P. dimorpha* and *P. macrostoma* exhibit significant herbicidal efficacy, while *Fusarium* spp.—notably *F. oxysporum* f. sp. *strigae*—are effective against *Striga hermonthica*, a major parasitic weed in maize cultivation.

Despite extensive research, only a few fungal bioherbicides have reached commercial markets. These include LockDown® (*Colletotrichum gloeosporioides* f. sp. *aeschynomene*), originally registered as Collego™ in 1982 and reintroduced in 2006 for controlling *Aeschynomene virginica*; Bio-Phoma™ (*Phoma macrostoma*), registered with Canada's PMRA for broadleaf

weed control; Di-Bak™ Parkinsonia, based on *Lasiodiplodia pseudotheobromae*, *Macrophomina phaseolina*, and *Neoscytalidium novaehollandiae*, which targets woody weeds like *Parkinsonia aculeata*; and Kichawi Kill™ (*Fusarium oxysporum* f. sp. *strigae*), approved by the Kenya Pest Control Products Board in 2021 for controlling *Striga hermonthica*, though its commercial production remains limited pending further optimization.

Technological advancements have significantly improved fungal bioherbicide yields and specificity. *Alternaria* spp. are known to produce over 300 metabolites, many of which exhibit host-specific phytotoxicity. *Fusarium* biomass has long been recognized for its herbicidal potential, with Boyette et al.[57] reporting control rates of 95–98% against *Cassia occidentalis*, *Senna obtusifolia*, and *Sesbania herbacea*. Nzioki et al.[58] identified tyrosine as a key metabolite in *F. oxysporum* for suppressing *Striga hermonthica*, leading to the development of Kichawi Kill™, which achieved 88–93% field efficacy through seed-coating applications. These findings underscore the growing potential of cell-free fungal metabolites as scalable, selective, and environmentally sustainable tools for integrated weed management.

4. Operational Limitations and Breakthrough Remedies

Despite extensive research into fungal-derived mycoherbicides, their commercial adoption remains limited, reflecting a disconnect between scientific innovation and industrial uptake. One major constraint is the narrow spectrum of activity exhibited by most mycoherbicides, which, unlike broad-spectrum chemical herbicides, often target specific weed species and may pose risks to non-target crops and ecosystems. Persistence of live fungal agents in the field is another concern, as some may form resistant structures or colonize unintended areas, complicating regulatory approval and ecological safety. Additionally, microbial products face challenges in storage and application, with viability affected by environmental conditions and genetic stability, leading to inconsistent field performance. Several strains, including *Alternaria destruens*, *Colletotrichum acutatum*, and *C. gloeosporioides* f. sp. *malvae*, have been discontinued due to poor field viability.

To address these limitations, innovative strategies are being explored, such as cell-free metabolite-based formulations that eliminate risks associated with live organisms and improve shelf stability. Encapsulation technologies like microemulsions and biogranules enhance delivery and protect active compounds from degradation, while integrated weed management (IWM) approaches combine bioherbicides with low-dose chemical herbicides to boost efficacy and delay resistance. In silico bioprospecting and molecular docking streamline metabolite discovery and formulation, and microbial consortia offer broader weed control through synergistic interactions. Regulatory frameworks also influence adoption; for instance, Brazil's agencies—ANVISA, IBAMA, and MAPA—impose strict controls on live microbial agents due to environmental concerns, whereas the European Commission's pesticide reduction goals and eased biopesticide registration requirements support metabolite-based innovations.

Cell-free fungal metabolites, being non-living and biodegradable, reduce persistence risks and support crop rotation without residual phytopathogen interference. Studies such as Chaves et al.[24] have demonstrated high phytotoxicity from *Phoma dimorpha* metabolites recovered via membrane filtration, highlighting both efficacy and the potential of scalable downstream processing.

However, further research is needed to determine whether metabolite production is host-induced, which may require incorporating weed-derived components into fermentation media. Seed coating and encapsulation technologies offer controlled release and preventive weed control, improving environmental resilience and sustained efficacy.

Integrated approaches combining fungal metabolites with chemical herbicides have achieved up to 100% weed control, various synergistic effects observed between fungal genera such as *Fusarium*, *Colletotrichum*, *Phoma*, and *Alternaria* and herbicides like glyphosate, glufosinate-ammonium, 2,4-D, thidiazuron, and MCPP. These combinations effectively suppress a wide range of weeds, including *Euphorbia heterophylla*, *Brachiaria plantaginea*, *Bidens pilosa*, *Conyza bonariensis*, *Abutilon theophrasti*, *Convolvulus arvensis*, and *Senna obtusifolia*, offering a scalable and sustainable solution for modern weed management.

5. Conclusions

Mycoherbicides offer a promising and environmentally sustainable alternative for weed management, yet a significant gap persists between scientific innovation and commercial implementation. Compared to other biologically based products in industrial sectors, the commercialization of mycoherbicides remains limited. In contrast, recent scientific literature has introduced a diverse array of novel strategies—particularly those centered on cell-free fungal metabolites—which demonstrate improved specificity, reduced environmental persistence, and greater regulatory compatibility.

This disparity reflects the industry's historical reluctance to invest in fungal biocontrol agents, often citing concerns over limited efficacy, high production costs, and narrow target ranges relative to chemical herbicides. However, as emphasized throughout this review, the transition toward metabolite-based mycoherbicides presents a viable and scalable path forward. Fungal genera such as *Alternaria*, *Fusarium*, *Phoma*, and *Colletotrichum* have emerged as key producers of potent phytotoxic metabolites with selective herbicidal activity.

Moreover, the integration of agro-industrial residues as fermentation substrates enhances cost-efficiency and aligns with circular economy principles, improving the economic feasibility of large-scale production. Technological advancements across the entire development pipeline—from strain selection and metabolite extraction to formulation and field application—are steadily overcoming previous limitations and paving the way for broader adoption.

By addressing critical challenges and embracing innovative solutions, mycoherbicides—particularly those based on cell-free fungal metabolites—have the potential to deliver effective, affordable, and ecologically responsible weed control. Their successful deployment could significantly reduce crop losses, mitigate herbicide resistance, and contribute to the resilience and sustainability of modern agricultural systems.

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