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Research Paper

Advances in Microbial Herbicides for Control of the Invasive Weed *Lantana camara*

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Abstract: The invasive shrub *Lantana camara* has emerged as one of the most problematic weeds worldwide, threatening biodiversity, reducing agricultural productivity, and altering ecosystem dynamics. Conventional management strategies—mechanical removal, chemical herbicides, and classical biological control—have often proven costly, environmentally unsustainable, or only partially effective. In this context, cell-free broth bioherbicides, derived from microbial metabolites clarified of viable cells, represent a promising and eco-friendly alternative. These formulations harness the phytotoxic potential of microbial secondary metabolites while eliminating risks associated with the release of live organisms.

This review synthesizes recent advances in the development of microbial bioherbicides, with a particular focus on their application against *Lantana camara*. We highlight the advantages of metabolite-based approaches, including target specificity, reduced environmental persistence, and compatibility with integrated weed management programs. Critical stages in the development pipeline are examined—ranging from microbial strain selection and metabolite discovery to characterization, formulation optimization, and deployment strategies. Special attention is given to efficacy assessments under greenhouse and field conditions, safety evaluations for non-target species, and regulatory frameworks governing biopesticide approval.

Furthermore, we identify key research gaps, such as the need for improved formulation stability under variable field conditions, deeper insights into modes of action, and strategies to mitigate potential resistance development. By consolidating current knowledge and outlining future directions, this review underscores the potential of cell-free microbial bioherbicides as sustainable, field-ready solutions for the effective management of *Lantana camara* and other invasive weeds.

Keywords: *Lantana camara*, Phytotoxic compounds, Biological weed control, Bioherbicide formulation, Environmental safety.

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Introduction:

Lantana camara is recognized as one of the most aggressive invasive shrubs worldwide, with profound ecological, agricultural, and socioeconomic impacts. Originally native to Central and South America, it has spread extensively across tropical and subtropical regions, where it disrupts native plant communities, reduces biodiversity, and alters ecosystem processes such as nutrient cycling and fire regimes (Day et al., 2003; Sharma et al., 2005). Its ability to form dense thickets suppresses the regeneration of indigenous species, thereby threatening ecological balance and agricultural productivity (Sharma et al., 2005).

The success of *L. camara* as an invader is partly attributed to its production of diverse bioactive compounds, including allelochemicals, which inhibit the germination and growth of neighboring plants (Gentle & Duggin, 1997). This chemical arsenal not only enhances its competitive advantage but also complicates management efforts, underscoring the need for effective and targeted control strategies. Conventional approaches such as mechanical removal and chemical herbicides have been widely employed; however, these methods are often labor-intensive, costly, and environmentally unsustainable, with risks of collateral damage to non-target species and ecosystems (Day et al., 2003).

Biological control options have therefore gained increasing attention. Classical biocontrol using insects and pathogens has shown partial success but is limited by ecological unpredictability and regulatory concerns (Day & Neser, 2000). Against this backdrop, microbial bioherbicides—particularly those based on cell-free phytotoxic metabolites—offer an innovative and environmentally aligned alternative. Unlike live microbial agents, cell-free broth formulations harness the phytotoxic potential of microbial secondary metabolites while eliminating risks associated with the release of viable organisms into ecosystems (Hoagland, 2001). This approach enhances safety, regulatory acceptability, and consistency in application.

Furthermore, the allelopathic potential of plant-derived chemicals highlights the importance of developing selective and well-characterized bioherbicidal agents. Such agents must be carefully evaluated to prevent unintended effects on crops, native vegetation, and beneficial organisms. By integrating microbial metabolite-based bioherbicides into weed management programs, it is possible to achieve sustainable suppression of *Lantana camara* while minimizing ecological disruption.

Rationale for Cell-Free Broth Bioherbicides

Mechanistic selectivity

Microbial metabolites often act through specific biochemical pathways, disrupting essential plant processes such as membrane integrity, photosynthesis, respiration, or hormone signaling. Unlike broad-spectrum synthetic herbicides, these natural compounds can be fine-tuned for target specificity by adjusting concentration, timing, and formulation design. This selectivity reduces collateral damage to non-target plants and enhances compatibility with integrated weed management programs. For invasive weeds like *Lantana camara*, which thrive through allelopathic interactions and competitive dominance, metabolite-driven selectivity offers a way to suppress growth without destabilizing surrounding ecosystems (Hoagland, 2001; Duke et al., 2000).

Safety profile

One of the most significant advantages of cell-free broth bioherbicides is the absence

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of viable microbial cells. By removing living organisms, risks associated with persistence in the environment, unintended colonization, or horizontal gene transfer are minimized. This makes cell-free formulations more acceptable to regulatory agencies and safer for use in diverse ecological settings. At the same time, the phytotoxic chemistry of microbial metabolites remains intact, ensuring effective weed suppression. The clarified nature of these products also reduces concerns about pathogenicity or unintended microbial interactions with crops, soil microbiota, or beneficial insects (Boyette et al., 2012; Bailey, 2014).

Manufacturing consistency

Fermentation technology provides a scalable and reproducible platform for producing microbial metabolites. Once active strains are identified, they can be cultivated under controlled conditions to yield consistent quantities of bioactive compounds. Downstream clarification processes—such as filtration, centrifugation, or solvent extraction—remove cells and impurities, resulting in standardized cell-free products. This consistency is critical for quality control, regulatory approval, and farmer confidence, as it ensures predictable performance across different batches and environments. Furthermore, metabolite-based formulations can be stabilized with carriers and adjuvants, extending shelf life and improving field applicability (Hoagland, 2001; Bailey, 2014).

Sustainability

Cell-free broth bioherbicides align strongly with sustainability goals in agriculture and ecosystem management. By reducing reliance on synthetic herbicides, they lower chemical residues in soil and water, mitigate risks to human health, and preserve biodiversity. Their natural origin and biodegradability make them compatible with organic farming systems and environmentally conscious weed management strategies. In addition, they can be integrated with mechanical removal, crop rotation, and restoration practices to form holistic management programs. For invasive species such as *Lantana camara*, which are notoriously difficult to control, sustainable bioherbicides provide a long-term solution that balances efficacy with ecological stewardship (Day et al., 2003; Sharma et al., 2005).

Target Weed Context for *Lantana camara*

Ecological impact

Lantana camara is considered one of the world's most invasive weeds, with widespread ecological consequences across tropical and subtropical regions. Its invasiveness is driven by a combination of traits, including prolific seed production, vegetative propagation, and the release of diverse bioactive compounds that suppress the growth of neighboring plants. These allelochemicals contribute to the displacement of native vegetation, reduce species richness, and alter ecosystem processes such as nutrient cycling, hydrology, and fire regimes (Gentle & Duggin, 1997; Sharma et al., 2005). Dense thickets of *L. camara* can prevent the regeneration of native tree seedlings, thereby impeding forest succession and biodiversity recovery (Day et al., 2003). In addition, its ability to colonize disturbed habitats makes it a persistent threat to both natural ecosystems and agricultural landscapes.

Management challenges

The resilience of *L. camara* complicates its control through conventional methods. Mechanical removal is labor-intensive and often ineffective due to its capacity to resprout from cut stems and rootstocks

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(Day & Neser, 2000). Chemical herbicides, while effective in the short term, pose risks of environmental contamination, non-target damage, and resistance development. Moreover, the allelopathic effects of *L. camara* exacerbate its competitive dominance, making restoration of invaded sites particularly challenging (Sharma et al., 2005). These limitations highlight the need for innovative strategies such as microbial bioherbicides. Cell-free broth formulations, derived from microbial metabolites, offer a promising solution by providing potent yet selective phytotoxic activity while minimizing risks to non-target species and ecosystems (Hoagland, 2001; Boyette et al., 2012). Such approaches could complement integrated weed management programs, facilitating ecological restoration and long-term suppression of *L. camara*.

Development pipeline

Discovery and screening

Microbial source selection: Prospect diverse habitats for phytotoxic microbes, prioritizing soils, leaf litter, rhizospheres, and phyllosphere communities associated with dense Lantana stands, invasion fronts, and sites showing natural suppression. Include endophytes, epiphytes, and rhizosphere-associated bacteria and fungi; target genera historically rich in herbicidal metabolites (e.g., Fusarium, Alternaria, Colletotrichum, Phoma/Boeremia, Pseudomonas, Bacillus, Streptomyces). Employ enrichment culturing, dilution plating, and high-throughput microfermentations to capture metabolite diversity (Hoagland, 2001; Duke et al., 2000; Boyette et al., 2012; Bailey, 2014).

Primary assays: Use tiered bioassays to detect activity against Lantana: seed germination and radicle elongation tests; hypocotyl/coleoptile growth assays; detached leaf puncture assays; and mini-pot seedling screens. Apply clarified, cell-free culture filtrates at graded doses to build dose–response curves and estimate IC50/EC50 values. Standardize inoculum-free filtrate preparation (filtration, 0.22 µm sterilization) to avoid confounding by live cells (Charudattan, 2001; Boyette et al., 2012; Green et al., 1998).

Bioactivity profiling: Record symptomology kinetics and signatures (chlorosis, necrosis, wilting, desiccation, growth arrest), and measure biochemical endpoints such as electrolyte leakage, chlorophyll fluorescence (Fv/Fm), respiration rate, and ROS accumulation to infer likely modes of action and prioritize fractions for isolation (Duke & Dayan, 2016; Dayan et al., 2012; Inderjit & Duke, 2003).

Metabolite characterization:

Fractionation: Partition crude broths using immiscible solvents (e.g., ethyl acetate, n-butanol) to separate polar/non-polar actives; employ solid-phase extraction and bioassay-guided chromatography (flash, HPLC). Track recovery of phytotoxicity at each step, reconstituting fractions to original volumes for comparable dosing (Macías et al., 2008; Dayan et al., 2000; Rimando & Duke, 2003).

Structural elucidation: Use LC–MS/MS for dereplication, accurate-mass profiling, and annotation of known metabolite classes (polyketides, peptides, terpenoids, alkaloids). Confirm structures via NMR (1D/2D), UV/Vis, and IR; assess stereochemistry when relevant to bioactivity. Map structure–activity relationships (SAR) by testing analogs and semi-synthetic derivatives (Duke et al., 2010; Gross & König, 2006; Clardy & Walsh, 2004).

Mode-of-action studies: Evaluate impacts on photosystem II (chlorophyll fluorescence parameters), carotenoid and

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chlorophyll content, membrane integrity (electrolyte leakage), oxidative stress markers (MDA, H2O2), and hormone signaling (auxin/ABA/ethylene pathways). Complement plant assays with isolated chloroplasts, thylakoids, and enzyme targets (e.g., ALS, HPPD, PPO) to triangulate mechanisms (Dayan & Duke, 2014; Dayan et al., 2010; Peterson, 2001).

Formulation and stability:

Carrier systems: Develop aqueous solutions, emulsifiable concentrates, and micro-/nanoemulsions using safe solvents and adjuvants (non-ionic surfactants, stickers/spreaders) to enhance leaf wetting, cuticle penetration, and rainfastness. Consider encapsulation (cyclodextrins, liposomes, biopolymer microcapsules) to protect labile metabolites and modulate release (Green & Beestman, 2007; Damodaran et al., 2013; Mishra et al., 2018).

Shelf-life optimization: Characterize chemical and biological stability under thermal, photolytic, and pH stress. Incorporate antioxidants, UV absorbers, and buffers where compatible; use amber packaging, oxygen barriers, and desiccants to minimize degradation. Establish storage conditions and retest intervals with validated stability-indicating assays (Bailey, 2014; Dayan et al., 2012; Moshrefi et al., 2019).

Application methods: Calibrate spray volumes and droplet spectra for canopy architecture of Lantana; time applications to phenological windows with high susceptibility (active vegetative growth, post-pruning flush). Integrate cut-stump or basal bark exposure tactics where systemic or contact action is limited; consider sequential applications to address re-sprouting (Day et al., 2003; Gentle & Duggin, 1997).

Efficacy assessment:

Greenhouse trials: Use randomized designs with sufficient replication; standardize plant age, pot size, substrate, and nutrition. Quantify endpoints including biomass reduction, SPAD/Chl content, photosystem efficiency, mortality, and regrowth over 2–6 weeks. Compare against untreated controls and conventional herbicide benchmarks; perform dose–response modeling (log-logistic) to estimate potency (Seefeldt et al., 1995; Ritz et al., 2015).

Field trials: Conduct multi-site, replicated plot trials across infested landscapes representing variability in climate, soils, and invasion density. Track knockdown, re-sprouting frequency, cover reduction, and community recovery over seasons; pair with mechanical removal for woody stems and follow-up treatments to deplete the propagule bank (Day et al., 2003; Sharma et al., 2005).

Selectivity testing: Screen representative native species and regional crops at equal and elevated doses; include germination and early-growth assays plus mature plant tolerance tests. Map selectivity windows and establish safety margins; evaluate soil residual effects via bioassays and short-term microcosm studies (Inderjit & Nilsen, 2003; Boyette et al., 2012).

Safety and regulatory considerations:

Environmental fate: Determine degradation kinetics in soil and water (DT50), sorption (K_d/K_oc), mobility, and photolysis. Assess transformation products and their toxicity; evaluate impacts on soil microbiomes via amplicon sequencing and functional assays. Emphasize the absence of viable cells and lack of persistence in product dossiers (USEPA, 2016; OECD, 2002; Bailey, 2014).

Non-target effects: Test acute and chronic toxicity to beneficial insects (pollinators, predators), earthworms, aquatic organisms,

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and non-target plants. Include exposure scenarios relevant to label directions (spray drift, runoff) and establish appropriate restrictions and mitigations (USEPA, 2016; Chandler et al., 2011; ECPA, 2013).

Compliance pathways: Align data packages with biopesticide regulatory frameworks focusing on metabolite identity, purity, manufacturing consistency, and risk assessment rather than organism containment. Prepare chemistry, toxicology, efficacy, and environmental fate modules; adopt Good Laboratory Practice (GLP) and Quality by Design (QbD) principles for credibility (USEPA, 2016; EU Regulation 1107/2009; APVMA, 2014).

Challenges and Future Directions

Achieving field robustness

A major challenge for cell-free broth bioherbicides is maintaining efficacy under variable field conditions. Environmental stresses such as ultraviolet radiation, rainfall, fluctuating temperatures, and soil pH can rapidly degrade microbial metabolites, reducing persistence and potency. Many natural compounds are chemically unstable compared to synthetic herbicides, necessitating formulation strategies that enhance stability without compromising safety. Encapsulation technologies, UV protectants, and controlled-release carriers have shown promise in extending shelf life and improving field performance (Green & Beestman, 2007; Mishra et al., 2018; Bailey, 2014). Robust formulations will ultimately determine whether bioherbicides can transition from experimental success to practical weed management solutions.

Resistance management

As with conventional herbicides, repeated use of bioherbicides with a single mode of action risks selecting for tolerant or resistant weed populations. *Lantana camara*, with its genetic variability and adaptive capacity, could evolve mechanisms to detoxify or evade metabolite effects if exposed consistently to one compound. Diversified mixtures of microbial metabolites, rotation with other herbicidal modes of action, and integration with mechanical or cultural practices are essential to mitigate this risk. Research into synergistic combinations of metabolites and monitoring for early signs of tolerance will be critical to sustaining long-term efficacy (Duke & Dayan, 2016; Heap, 2024; Dayan et al., 2019).

Precision targeting

Ensuring minimal impact on non-target plants and beneficial organisms remains a central concern. Restoration contexts, where native vegetation is reintroduced after *Lantana* removal, demand bioherbicides that selectively suppress the invader without harming regenerating species. Precision targeting requires deeper insights into metabolite modes of action, dose refinement, and application timing. Advances in plant physiology, metabolomics, and ecological modeling can help predict selectivity windows and guide safe deployment. Given *Lantana's* allelopathic interactions with surrounding flora, bioherbicides must be carefully evaluated to avoid exacerbating ecological imbalance (Gentle & Duggin, 1997; Inderjit & Duke, 2003; Boyette et al., 2012).

Integrated strategies

No single method is likely to eradicate *Lantana camara* due to its resilient propagule bank, capacity for vegetative regrowth, and widespread distribution. Cell-free bioherbicides should therefore be integrated into broader management frameworks. Pairing metabolite-based treatments with mechanical removal of

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woody stems, prescribed burning, or habitat restoration can accelerate ecosystem recovery and reduce reinvasion pressure. Long-term success will depend on adaptive management, where bioherbicides are deployed strategically alongside ecological restoration practices to rebuild native plant communities and suppress *Lantana* resurgence (Day et al., 2003; Sharma et al., 2005; Pimentel et al., 2005).

Conclusion:

Cell-free broth bioherbicides represent a novel and environmentally responsible strategy for weed management, harnessing the potency of microbial phytotoxic metabolites while avoiding the ecological risks associated with releasing live agents. For *Lantana camara*, a globally recognized invasive shrub with severe ecological and agricultural impacts, this approach offers a selective, scalable, and sustainable control option. By removing viable microbial cells, clarified broth formulations reduce concerns about persistence, pathogenicity, and unintended ecological interactions, while maintaining strong herbicidal activity, as highlighted by Hoagland (2001) and Boyette et al. (2012).

The successful development of such bioherbicides requires a rigorous pipeline—from microbial strain discovery and metabolite characterization to formulation optimization, efficacy testing, and safety evaluation. When integrated with broader management practices such as mechanical removal, habitat restoration, and ecological monitoring, cell-free bioherbicides can contribute significantly to long-term suppression of *Lantana camara* (Day et al., 2003; Sharma et al., 2005).

Recent studies by Singh and Pandey have emphasized the importance of microbial metabolites in sustainable weed management, noting their role as eco-friendly alternatives to synthetic herbicides and their potential in integrated weed control frameworks (Singh & Pandey, 2014). Their work underscores the promise of microbial-derived phytotoxins not only for *Lantana camara* but also for other invasive weeds, reinforcing the need for continued research into metabolite diversity, stability, and field applicability.

Looking ahead, future research should focus on enhancing field robustness through advanced formulations (Mishra et al., 2018), mitigating resistance risks via metabolite mixtures or rotations (Duke & Dayan, 2016; Heap, 2024), and ensuring precision targeting to safeguard non-target species (Gentle & Duggin, 1997; Inderjit & Duke, 2003). By consolidating current advances and addressing these challenges, cell-free microbial bioherbicides can be translated into practical, field-ready solutions that align with global goals of sustainable agriculture and biodiversity conservation (Pimentel et al., 2005).

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