



Bioherbicide Formulation Engineering: Advances in Delivery Systems, Stability and Field Performance

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Abstract:

Bioherbicides offer complementary tools for integrated weed management, but variable field efficacy, limited storage stability and application-system incompatibility continue to restrict adoption. This critical narrative review examines formulation engineering as an integrated development process linking biological active ingredients, carrier architecture, critical quality attributes and field delivery. Conventional aqueous suspensions, grain carriers, alginate and Pesta granules, oil-based and multiple emulsions are compared with biodegradable polymer systems, micro- and nanoencapsulation, cell-free fermentation products, multi-component formulations and precision application. Emphasis is placed on the mechanisms by which formulations influence moisture retention, ultraviolet tolerance, adhesion, rainfastness, release, biological potency and spray performance. Evidence is differentiated by laboratory, greenhouse, field and commercial context; findings from conventional herbicides and other biological crop-protection agents are treated as transferable formulation principles rather than direct proof of bioherbicide efficacy. Cell-free systems avoid the requirement to maintain propagule viability but introduce challenges in chemical characterization, potency standardization and degradation control. Advanced carriers and precision delivery are promising when they address measurable product limitations, although evidence for sustained field advantages remains uneven. We propose a stage-gated, dose-normalized reporting framework integrating active production, formulation, packaging, application, safety and cost per treated hectare. Comparative multi-environment field trials, realistic stability programs and validated potency measures are priorities for translating formulation advances into reliable weed control.

Keywords: Bioherbicide; formulation; encapsulation; cell-free metabolites; precision application

1. Introduction

Weeds reduce crop productivity by competing for light, water, nutrients and space, interfering with harvest, contaminating produce, and acting as reservoirs for pests and pathogens. Chemical herbicides remain indispensable in many production systems, but the evolution of herbicide resistance, restrictions on some active ingredients, public concern about pesticide residues and biodiversity, and the need for diversified integrated weed management have intensified interest in biologically based weed control. Bioherbicides broadly include microorganisms, microbial products and naturally derived phytotoxic substances applied in a manner analogous to herbicides. In the classical microbial approach, a pathogen is mass produced and applied inundatively at a dose sufficient to initiate disease rapidly in the target weed. Fungi have historically dominated this field, giving rise to the term mycoherbicide, although bacteria and microbial metabolites are also important (Charudattan 2001; Hallett 2005; Cordeau et al. 2016; Singh and Pandey 2019b; Morin 2020; Hasan et al. 2021; Roberts et al. 2022; Singh and Pandey 2022b; Duke 2024; Islam et al. 2024).

Auld et al. (2003) presented formulation as a major constraint to commercialization. A useful product was expected to combine low cost, long shelf life, ease of application and reliable efficacy, while many fungal candidates were limited by their requirement for prolonged humidity or leaf wetness. Early commercial products demonstrated that biological weed control could work, but also showed that the development pathway was much more demanding than simply identifying a pathogenic isolate. Biological specificity, fermentation yield, propagule quality, environmental dependence, storage stability, application technology, regulatory cost and market size all influence commercial feasibility (Auld and Morin 1995; Auld et al. 2003; Duke 2024).

Formulation is therefore not an inert final step. It determines the physical state of the active ingredient, protects it during storage and transport, governs rehydration or release, affects spray atomization and deposition, and modifies the microenvironment experienced by the organism or metabolite after application. For a living fungal propagule, formulation must preserve viability while permitting rapid germination and infection. For a bacterial cell, osmotic stress, desiccation and nutrient status may be critical. For a cell-free metabolite, the dominant issues may instead be solubility, oxidation, hydrolysis, adsorption, photolysis and controlled delivery. Consequently, a formulation that is optimal for one biological active ingredient may be unsuitable for another.

The formulation landscape has changed markedly since 2003. Research on biological plant-protection agents increasingly uses natural and synthetic polymers, microcapsules, nanoparticles, lipid carriers and hybrid materials. A systematic review of micro- and nanocarriers for biological plant-protection agents found extensive use of naturally sourced polymers such as chitosan and alginate, alongside synthetic polymers and inorganic materials (Campos et al. 2022; Pérez-de-Luque 2024). In parallel, bioherbicide research has expanded toward cell-free phytotoxic fermentation products, which can avoid some ecological and storage constraints associated with living inocula (Duke 2024; Bendejacq-Seychelles et al. 2024; Ocán-Torres et al. 2024). Precision application, sensors, robotic platforms and unmanned aerial vehicles also create opportunities to reduce application volume and target biological products more accurately (Hewitt et al. 2024). Recent syntheses further emphasize that formulation reliability, production scale-up and application technology remain decisive constraints on commercialization (Roberts et al. 2022; Islam et al. 2024; Marrone 2024).

This review updates the formulation framework established by Auld et al. (2003) while deliberately narrowing the focus to formulation–delivery relationships rather than repeating broad inventories of bioherbicide organisms or products. The distinctive contribution is a function-based synthesis that connects carrier architecture and critical quality attributes to field delivery, cell-free actives, precision application, scale-up, environmental fate, and techno-economics. This framing addresses a persistent gap between laboratory formulation studies and the operational requirements that determine commercial field reliability (Duke 2024; Hewitt et al. 2024; Marrone 2024).

2. Scope and literature-selection approach

This review is a critical narrative review focused on formulation and delivery technologies for microbial and metabolite-based bioherbicides. Priority was given to peer-reviewed studies and reviews published from 2015 through 2026, complemented by landmark formulation papers needed to trace the development of alginate carriers, Pesta granules, oil emulsions, invert emulsions, and polymer systems. Recent sources were selected to represent four rapidly developing interfaces: micro/nanocarriers, cell-free phytotoxic metabolites, application engineering and precision delivery, and commercialization. Because the objective is mechanistic synthesis rather than bibliometric enumeration, evidence is interpreted according to the formulation function demonstrated (e.g., viability preservation, moisture retention, ultraviolet protection, release control, deposition, or field efficacy) and the experimental scale at which it was tested. Laboratory improvements are therefore not treated as evidence of field superiority unless supported by realistic application or environmental testing.

2.1. Evidence appraisal and limits of the narrative approach

This article is a critical narrative review, not a systematic review or meta-analysis. The synthesis emphasizes literature published during 2015–2026 together with foundational formulation studies. The authors did not retain a reproducible database search history, full search strings, screening counts or formal risk-of-bias ratings; consequently, the cited studies must not be interpreted as an exhaustive or statistically representative sample. We group evidence by active type (living propagule versus cell-free biochemical), formulation function (stability, moisture, deposition or release) and level of validation (physicochemical testing, controlled bioassay, greenhouse, field or commercial use). Reports

on botanical herbicides, synthetic-herbicide carriers and agents used against plant diseases are explicitly treated as transferable material-science evidence, not direct demonstrations of field efficacy against weeds. This distinction guides the comparative evidence matrix presented below (Table 8).

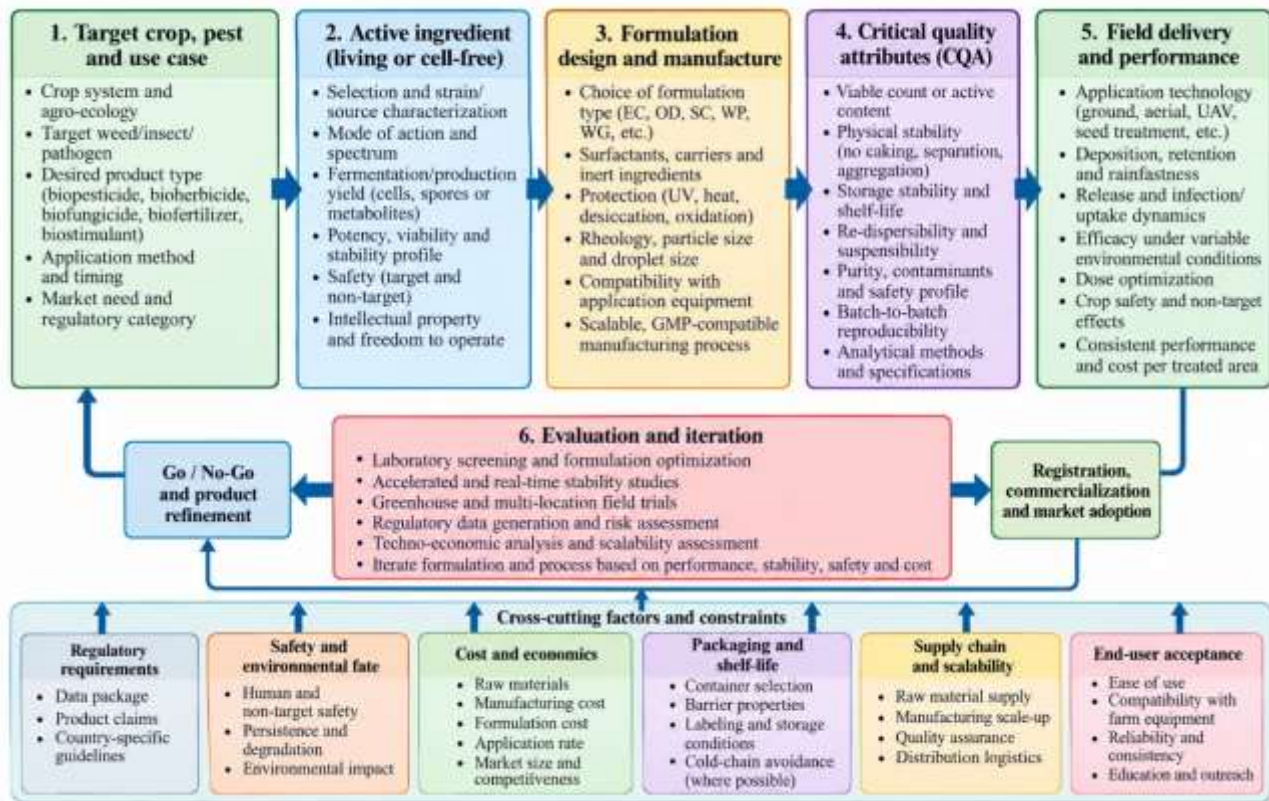


Fig. 1. Proposed integrated development framework linking active selection, formulation, quality attributes, delivery and commercial constraints

3. Formulation objectives and constraints

A bioherbicide formulation must simultaneously satisfy biological, physical, agronomic and commercial requirements. The active ingredient must remain viable or chemically active for an acceptable period, disperse or reconstitute reproducibly, tolerate handling, and be delivered at a dose that produces consistent weed suppression. At the same time, the formulation should be compatible with standard farm equipment, avoid excessive viscosity or foaming, minimize nozzle blockage, remain stable during mixing, and not injure the crop or beneficial organisms. These requirements create a multi-objective optimization problem in which improving one attribute may compromise another.

Moisture is the classic example. Many fungal pathogens require a dew period for conidial germination, appressorium formation, penetration and establishment (Auld and Morin 1995; Bailey et al. 2011). Adding surfactant may improve leaf wetting and coverage but can also accelerate evaporation. Adding a highly viscous polymer may retain water but mechanically restrict germ tubes or reduce oxygen transfer. Increasing oil content may slow water loss and enhance cuticular penetration but may cause phytotoxicity, high cost or sprayability problems. The key is therefore to engineer the microenvironment at the target surface rather than maximize any single physical property (Auld et al. 2003).

Environmental stresses continue after deposition. Solar ultraviolet radiation can rapidly reduce propagule viability and degrade phytotoxic metabolites. High temperature can destabilize proteins and membranes; low humidity accelerates desiccation; rainfall causes wash-off; and leaf architecture influences retention. Epicuticular waxes, trichomes, stomatal density and leaf angle affect droplet spreading and adhesion. These interactions help explain why greenhouse efficacy often overestimates field performance. Recent reviews continue to identify humidity, temperature, UV radiation, production cost and scale-up as major barriers to bioherbicide reliability (Hasan et al. 2021; Duke 2024).

Commercial formulation must also accommodate variability in the biological raw material. Fermentation batches may differ in propagule size, physiological age, lipid reserves, metabolite concentration, pH and residual nutrients. Downstream operations such as filtration, centrifugation, drying and milling can alter viability and particle-size distribution. Quality-by-design principles are therefore relevant: critical quality attributes should be defined early and linked to critical process parameters. For living products these attributes may include viable propagule count, germination rate, infectivity and contamination level; for cell-free products they may include marker-metabolite concentration, potency, pH, water activity and degradation profile.

4. Historical evolution of bioherbicide formulations

Early mycoherbicide research relied heavily on aqueous suspensions of spores or mycelial fragments. These simple systems remain useful experimental standards because they reveal the intrinsic activity of the biological agent. Wetting agents such as polysorbates can improve spreading, but aqueous formulations provide little protection against rapid drying. Commercial experience with DeVine and Collego showed that fermentation, harvest, drying, rehydration and application are integral parts of product design (Greaves et al. 1998; Zidack and Quimby 1999; Daigle and Connick 2002). Auld et al. (2003) distinguished two broad pathways: solid formulations that survive until favorable moisture occurs and liquid formulations intended to initiate infection soon after application.

Solid carriers included colonized rice, barley, millet and wheat; dried and milled grain could function both as fermentation substrate and application material. Calcium alginate encapsulation was another major innovation. Fungal propagules suspended in sodium alginate are dropped into calcium chloride, producing ionically cross-linked beads that can be dried and applied directly or used as a sporulation substrate (Walker and Connick 1983). The Pesta process combined fungal inoculum with wheat flour and kaolin to create a dough that was sheeted, dried and granulated (Connick et al. 1991; Quimby et al. 1999). These technologies anticipated modern encapsulation by combining protection, nutrient provision and controlled hydration.

Oil-based formulations were developed largely to address dew dependence. Simple vegetable-oil emulsions sometimes improved disease under controlled conditions (Auld 1993; Shabana 1997; Boyette 1994), while invert emulsions placed water droplets in a continuous oil phase, strongly reducing evaporation. Their disadvantages included high oil content, viscosity, specialized spraying requirements, cost and potential crop injury. Water-in-oil-in-water multiple emulsions represented an elegant compromise: an inner aqueous phase is enclosed in oil droplets that are dispersed in an external aqueous phase, allowing moisture retention with much lower total oil. Auld et al. (2003) reported formulations containing only a few percent oil that could be sprayed with conventional equipment.

The historical lesson is that successful formulation depends on matching carrier architecture to the biology of infection and the intended application niche. Wound-applied products for woody weeds can use simple pastes or slurries because the wound bypasses cuticular barriers and provides a protected infection court. Aquatic weeds may require floating or surface-retentive formulations. Soil-applied agents benefit from granules or encapsulated propagules that localize inoculum near germinating weed seeds. These niche-specific approaches remain highly relevant to contemporary product development.

The major formulation platforms, their advantages, limitations and best-fit application niches are summarized in Table 1.

Table 1. Major formulation platforms for bioherbicides and their principal attributes

Formulation platform	Typical active ingredient	Main advantages	Main limitations	Best-fit applications
Aqueous suspension	Spores, cells, mycelial fragments	Simple; low formulation cost; easy spraying	Rapid drying; short shelf-life; dew dependence	Research standard; irrigated or humid conditions

Formulation platform	Typical active ingredient	Main advantages	Main limitations	Best-fit applications
Wettable powder / water-dispersible granule	Dried propagules or metabolites	Lower shipping weight; reconstitutable; reduced contamination	Drying injury; dust or dispersibility issues	Foliar spray after reconstitution
Granules / colonized grain	Fungal biomass or spores	Localized delivery; nutrient support; simple soil application	Slow activation; field survival before wetting	Soil, pasture, pre-emergence use
Alginate biopolymer beads /	Cells, spores, metabolites	Mild encapsulation; protection; controlled hydration	Release may be slow; bead-size constraints	Soil, aquatic, controlled-release use
Oil-in-water emulsion	Spores or hydrophobic metabolites	Improved spreading and penetration; lower evaporation	Emulsion instability; oil compatibility	Foliar delivery
Invert W/O emulsion	Aqueous microbial inoculum	Strong moisture conservation; reduced dew requirement	High oil, viscosity, cost, phytotoxicity risk	Dew-sensitive foliar mycoherbicides
W/O/W multiple emulsion	Spores or aqueous actives	Moisture reservoir with lower oil content	Complex manufacture; phase instability	Foliar mycoherbicides
Micro/nanocapsules	Metabolites; selected microbial systems	Protection; controlled release; UV shielding	Cost; scale-up; regulatory complexity	Labile natural products and advanced delivery
Cell-free concentrate	Microbial metabolites/enzymes	No viability requirement; standardized dosing	Metabolite instability; purification/standardization	Foliar and soil biochemical bioherbicides

5. Solid and dry formulations

Dry formulations are attractive because they generally reduce shipping weight, microbial contamination risk and dependence on a cold chain. They can also provide longer shelf life when water activity is sufficiently low. However, drying is itself a major stress. Membrane damage, protein denaturation, oxidative injury and loss of germination can occur during air drying, spray drying, freeze drying or fluid-bed drying. Protective sugars, proteins, polyols and polymers may reduce injury, but their effects are species- and propagule-specific.

Granules are particularly suitable for soil application, pre-emergence weed suppression, rangeland treatment and situations in which immediate foliar activity is not required. Grain-based granules can provide nutrients that support establishment, whereas mineral carriers such as clays improve handling and flow. Starch-based matrices can absorb water and create a hydrated microhabitat. The Pesta concept remains relevant because flour, clay, oil and other additives can be adjusted to control density, disintegration and nutrient release. Modern extrusion and fluid-bed processes could improve uniformity compared with early hand-prepared granules.

Encapsulated dry beads provide additional control over release. Alginate is especially useful because gelation occurs under mild aqueous conditions that are compatible with living cells. The matrix can be blended with cellulose, starch, clays or other biopolymers to modify porosity, swelling and mechanical strength. Research on agricultural encapsulation more broadly shows that alginate and chitosan remain among the most frequently used natural polymers (Campos et al. 2022). Although much of this literature concerns biocontrol agents against pests and diseases rather than weeds, the material principles are directly relevant to bioherbicide delivery. Alginate-cellulose beads developed for the slow release of a conventional herbicide illustrate how porosity and swelling can be tuned (Nornberg et al. 2019), although such data represent transferable material science rather than evidence of bioherbicide efficacy.

Powders and wettable powders can provide high active-ingredient concentration and rapid dispersion but require careful control of dust, wettability, suspensibility and particle size. For living fungi, milling must not destroy propagules. Water-dispersible granules can reduce dust while retaining the logistical advantages of dry products. A formulation intended for reconstitution should disperse rapidly without forming persistent aggregates and should maintain viable propagules in suspension long enough for application.

A persistent limitation of dry formulations is activation. Once applied, the organism may remain dormant until sufficient moisture becomes available. During this waiting period it is exposed to UV radiation, temperature fluctuations, predation and microbial competition. Thus, the best dry formulation is not necessarily the one with the longest warehouse shelf-life; it must also protect the organism after field placement. Controlled-release coatings, UV-screening pigments and hygroscopic components are potential solutions, but each must be assessed for effects on germination and non-target safety.

6. Liquid formulations and adjuvant systems

Liquid products are convenient for foliar application because they can be metered, mixed and sprayed using familiar equipment. Their major disadvantages are generally shorter shelf-life, higher shipping weight and greater risk of contamination or phase instability. Liquid formulations range from simple aqueous suspensions to concentrated suspensions, oil dispersions, emulsions and multiple emulsions. The choice depends on whether the active ingredient is a living cell, a spore, a mycelial fragment, a soluble metabolite or a hydrophobic natural product.

Surfactants are among the most important excipients. Nonionic surfactants can improve wetting and spreading while minimizing ionic stress to cells, but compatibility must be tested because surfactants can disrupt membranes or alter germination. Organosilicone surfactants produce very low surface tension and can enhance stomatal infiltration, but excessive spreading can reduce deposit thickness and accelerate drying. The optimal surfactant concentration therefore depends on leaf surface properties, droplet volume and the moisture requirement of the biological agent.

Humectants such as glycerol, sorbitol and glycols reduce water activity and evaporation, yet high concentrations can impose osmotic stress; Tween 20 and glycerol, for example, have been evaluated as additives to mycoherbicide suspensions (Klein and Auld 1995). Polysaccharides and hydrophilic polymers can increase retention and adhesion. The Auld et al. (2003) review highlighted an important negative result: polymers that retained more water did not necessarily improve disease, and some appeared to impede germ-tube growth (Shabana et al. 1997; Chittick and Auld 2001). This remains a valuable warning against selecting adjuvants solely from physical measurements.

Stickers and film-forming agents improve rainfastness by anchoring deposits to the leaf. Oils can increase cuticular penetration, reduce evaporation and alter droplet spreading. Nutrients may stimulate rapid germination and shorten the period during which propagules are vulnerable to desiccation, but nutrient-rich formulations can also encourage contaminants during storage. Buffers maintain pH, antioxidants protect sensitive metabolites, antifoams improve manufacturing and tank mixing, and rheology modifiers reduce sedimentation. Formulation design should evaluate

the full mixture because interactions among excipients can be non-additive. The principal functional excipient classes and their compatibility considerations are summarized in Table 2.

Table 2. Functional formulation ingredients and selection considerations

Ingredient class	Examples	Primary function	Key compatibility concern
Surfactants	Polysorbates, lecithin, selected nonionics	Wetting, spreading, dispersion	Membrane toxicity; excessive evaporation
Humectants	Glycerol, sorbitol, glycols	Moisture retention	Osmotic stress; tackiness
Stickers/film formers	Gums, latex-like polymers, polysaccharides	Adhesion and rainfastness	Restricted germination or gas exchange
Oils	Vegetable oils, paraffinic oils	Evaporation control, penetration	Phytotoxicity; viscosity; oxidation
Biopolymers	Alginate, chitosan, starch, cellulose derivatives	Encapsulation and controlled release	Release rate; microbial compatibility
UV protectants	Lignin derivatives, pigments, selected antioxidants	Protection from solar radiation	Optical residues; non-target effects
Nutrients	Sugars, amino acids, yeast-derived nutrients	Stimulate germination and early growth	Contamination and reduced storage stability
Rheology modifiers	Xanthan, cellulose gums, clays	Suspension stability	Nozzle blockage; high viscosity
Buffers	Phosphate/citrate systems where compatible	pH control	Ionic stress; precipitation
Antifoams	Silicone or food-grade antifoams	Manufacturing and tank-mix control	Effects on spreading and viability

7. Emulsion technologies

Emulsions are particularly valuable when moisture retention and delivery of hydrophobic components are required. Oil-in-water emulsions are comparatively easy to spray and can contain vegetable oils that act as penetrants or evaporation retardants. Their performance depends on oil identity, surfactant hydrophilic-lipophilic balance, droplet size, emulsion stability and compatibility with the biological agent. Essential oils and other botanical phytotoxins are also commonly formulated as emulsions because of their low water solubility (Kostina-Bednarz et al. 2023).

Invert water-in-oil emulsions place the aqueous inoculum in dispersed droplets surrounded by a continuous oil phase. This architecture can substantially reduce water loss and has historically reduced dew requirements and inoculum thresholds for some mycoherbicides (Quimby et al. 1988; Amsellem et al. 1990; Boyette et al. 1993; Womack et al. 1996). However, high oil fractions can increase viscosity and cost, complicate atomization and create phytotoxicity risks. These drawbacks stimulated interest in multiple emulsions.

Water-in-oil-in-water systems encapsulate small aqueous droplets inside oil droplets that are themselves suspended in water. The architecture provides an internal moisture reservoir while allowing the external phase to remain water-rich. The classic example reviewed by Auld et al. (2003) used Tween 20, Span 80, paraffin oil and water and achieved low total oil content. Multiple emulsions are thermodynamically unstable and can fail through coalescence, creaming,

osmotic swelling or leakage, so modern development should quantify droplet-size distribution, phase separation, viscosity and storage stability.

Nanoemulsions extend the emulsion concept to submicron droplets. Their small droplet size can improve kinetic stability, dispersion and contact with plant surfaces. Taban et al. (2020) demonstrated that nanoencapsulation of savory essential oil in biopolymer-based systems could slow release and enhance phytotoxic activity, illustrating the potential of nano-enabled natural herbicides. For microbial bioherbicides, however, a nanoemulsion carrier must remain compatible with much larger living propagules; the nano-scale component may therefore function as an adjuvant or metabolite carrier rather than directly encapsulating fungal spores.

8. Microencapsulation and biodegradable polymer carriers

Microencapsulation separates the active ingredient from the external environment using a protective matrix or shell. Benefits can include reduced desiccation, UV protection, controlled release, improved handling and separation of incompatible ingredients. Techniques relevant to biological products include ionic gelation, extrusion, spray drying, coacervation, emulsification, fluid-bed coating and interfacial polymerization. The choice must consider process temperature, solvent exposure, shear and osmotic stress.

Alginate remains a benchmark material because it is biodegradable, inexpensive and gelled by divalent cations under mild conditions. Chitosan provides film-forming, mucoadhesive and antimicrobial properties and can be combined with alginate to create polyelectrolyte complexes. Starch, cellulose derivatives, pectin, gums, proteins and lignin-derived materials offer additional renewable options. Campos et al. (2022) concluded that naturally sourced polymers represent a major fraction of the encapsulation literature for biological plant-protection agents.

Carrier composition can be tailored to the target environment. A highly porous bead may rehydrate quickly but release the active ingredient too rapidly. A dense matrix may provide long protection but delay germination beyond the agronomically useful period. Inclusion of clay can improve mechanical strength and adsorption, whereas sugars or proteins can protect cells during drying. For cell-free metabolites, the polymer can be selected to modulate diffusion and photostability rather than cell viability.

Encapsulation efficiency, loading capacity, particle size, morphology, swelling, release kinetics and storage stability are essential characterization parameters. For living products, these measurements must be linked to viable count, germination and infectivity. For metabolites, chemical assays should measure both total content and biological potency because a compound may remain analytically detectable after losing herbicidal activity.

9. Nanotechnology and nano-enabled bioherbicides

Nanotechnology has become a prominent theme in crop-protection formulation. Nano-enabled carriers can increase apparent solubility, protect labile molecules, improve adhesion and provide controlled release. Pérez-de-Luque (2024) argued that nanotechnology may help address bioherbicide limitations such as low solubility, short shelf life and loss of viability, while emphasizing that production cost, safety and possible toxic effects must be considered. Broader encapsulation literature similarly supports biodegradable polymer carriers, especially chitosan and alginate, while underscoring the need to connect carrier properties with biological performance (Campos et al. 2022). The promise is therefore conditional rather than automatic.

Potential carriers include chitosan nanoparticles, alginate nanogels, polymeric nanocapsules, lipid nanoparticles, liposomes, silica-based materials and nanoemulsions. For botanical or microbial metabolites, nanoscale encapsulation can reduce volatilization and photodegradation and may increase leaf contact. For living microorganisms, direct encapsulation at the nanoscale is generally not physically appropriate for whole cells or fungal spores; instead, nano-materials may form coatings, protective matrices or co-delivery systems.

The nanoherbicide literature using conventional herbicidal active ingredients provides useful formulation principles but cannot be transferred uncritically to biological products. Carrier surface charge influences adhesion to the cuticle, particle size affects transport and deposition, and release rate governs exposure. Yet nanomaterials may behave differently in soil and water, and their ecotoxicological profiles can differ from those of bulk materials. Biodegradability and transformation products must therefore be part of design criteria.

Recent reviews of biodegradable nanoherbicide carriers emphasize polysaccharide-based systems and controlled release as routes to reduce off-target exposure. The same logic is relevant to natural-product bioherbicides. Nevertheless, field evidence for nano-enabled microbial bioherbicides remains much thinner than laboratory evidence. Claims of improved efficacy should be supported by realistic dose comparisons, appropriate controls, environmental fate studies and cost analysis.

A practical near-term role for nanotechnology may be the stabilization of cell-free metabolites, essential oils or synergistic adjuvants rather than the replacement of all conventional microbial formulations. Hybrid systems in which a microbial propagule is delivered in a conventional carrier while a nanoencapsulated metabolite or protectant is co-applied could provide complementary functions, but compatibility and regulatory complexity will increase.

9.1. Interpreting nano-enabled formulation evidence

Comparative evaluation should distinguish a carrier effect from differences in active dose, adjuvant loading and application volume. At minimum, studies should include untreated, blank-carrier and dose-matched nonencapsulated-active controls; report particle-size distribution and active loading; and measure potency after environmentally realistic ultraviolet, temperature and rainfall exposure. Improvements in encapsulation efficiency or in vitro release are intermediate endpoints, not proof of greater field weed suppression. Where evidence comes from essential oils or non-bioherbicide pesticides, extrapolation to microbial propagules requires separate compatibility and field testing (Campos et al. 2022; Pérez-de-Luque 2024).

10. Cell-free microbial bioherbicide formulations

Cell-free fermentation products have attracted renewed attention because they can retain phytotoxic activity without requiring establishment of a living pathogen on the target weed. Filtrates or supernatants may contain organic acids, peptides, polyketides, terpenoids, alkaloids, proteins, extracellular enzymes and other secondary metabolites. Duke (2024) highlighted several potential advantages of killed or cell-free microbial products, including reduced dependence on viability and potentially simpler control of dose and composition. This direction is consistent with recent work by Singh and Pandey (2019a, 2020, 2022b, 2025a, 2025b), who emphasized fungal metabolites, foliar pathogenic metabolites and cell-free fungal broth as practical routes for natural-herbicide development.

The formulation problem changes fundamentally when living propagules are removed. Dew may still influence penetration and retention, but germination and infection are no longer prerequisites. Instead, stability of the active metabolite becomes dominant. Oxidation, hydrolysis, photolysis, volatilization and adsorption can reduce potency. Proteinaceous phytotoxins and enzymes may denature at interfaces or high temperature. Hydrophobic compounds may precipitate or require emulsification. A cell-free product therefore resembles a biochemical pesticide formulation more than a classical inoculum.

Recent analysis of cell-free microbial bioherbicides identifies submerged fermentation in stirred-tank bioreactors as a common production platform and notes opportunities to use low-cost industrial residues as carbon and nitrogen sources (Ocán-Torres et al. 2024). Such integration with circular-bioeconomy principles may improve economics, but crude fermentation broths are chemically complex. Product standardization therefore requires marker compounds or validated bioassays, and batch-to-batch variation in metabolite profiles must be controlled.

Formulation options include soluble concentrates, emulsifiable systems, suspension concentrates, dried powders, microcapsules, polymeric nanoparticles and lipid carriers. Crude broth may also be concentrated by membrane filtration, evaporation or adsorption onto a carrier. Purification can improve reproducibility but increases cost; therefore, the optimal commercial product may be a standardized enriched fraction rather than a pure compound.

Cell-free formulations are particularly attractive when the producing organism poses regulatory or ecological concerns, when infection is strongly humidity-dependent, or when the phytotoxic metabolite has broad activity. Conversely, metabolite production yield may be low, and the active spectrum may be less selective than the living pathogen. These trade-offs should be considered early in product development, especially because recent reviews show that chemical identification, potency-linked markers, and scalable fermentation are prerequisites for defensible cell-free products (Bendejacq-Seychelles et al. 2024; Ocán-Torres et al. 2024).

11. Synbiotic and multi-component formulations

An emerging concept is to combine living microorganisms with botanically derived components or other biological actives in a single formulation. Razafindralambo et al. (2026) proposed a synbiotic framework in which microbial and plant-derived components provide complementary or synergistic weed suppression. The concept is attractive because a botanical phytotoxin could provide rapid initial injury while a pathogen establishes infection, potentially reducing the environmental window required for disease development.

Multi-component products may also combine two compatible microorganisms, a microorganism with its own fermentation metabolites, or a bioherbicide with a reduced dose of a conventional herbicide. Such combinations can broaden the spectrum or improve consistency, but formulation becomes substantially more complex. Each component may have different pH, solvent, surfactant and storage requirements. A botanical essential oil that is herbicidal may also be antimicrobial and therefore kill the microbial partner.

Compatibility testing should therefore proceed hierarchically: in vitro viability or chemical stability, mixture stability, plant-surface behavior, greenhouse efficacy, crop safety and finally field performance. Synergy should be demonstrated statistically rather than inferred from higher activity of a mixture. Regulatory evaluation may also be more demanding because toxicology and environmental fate must address the complete formulation and possible interaction products (Fig. 2).

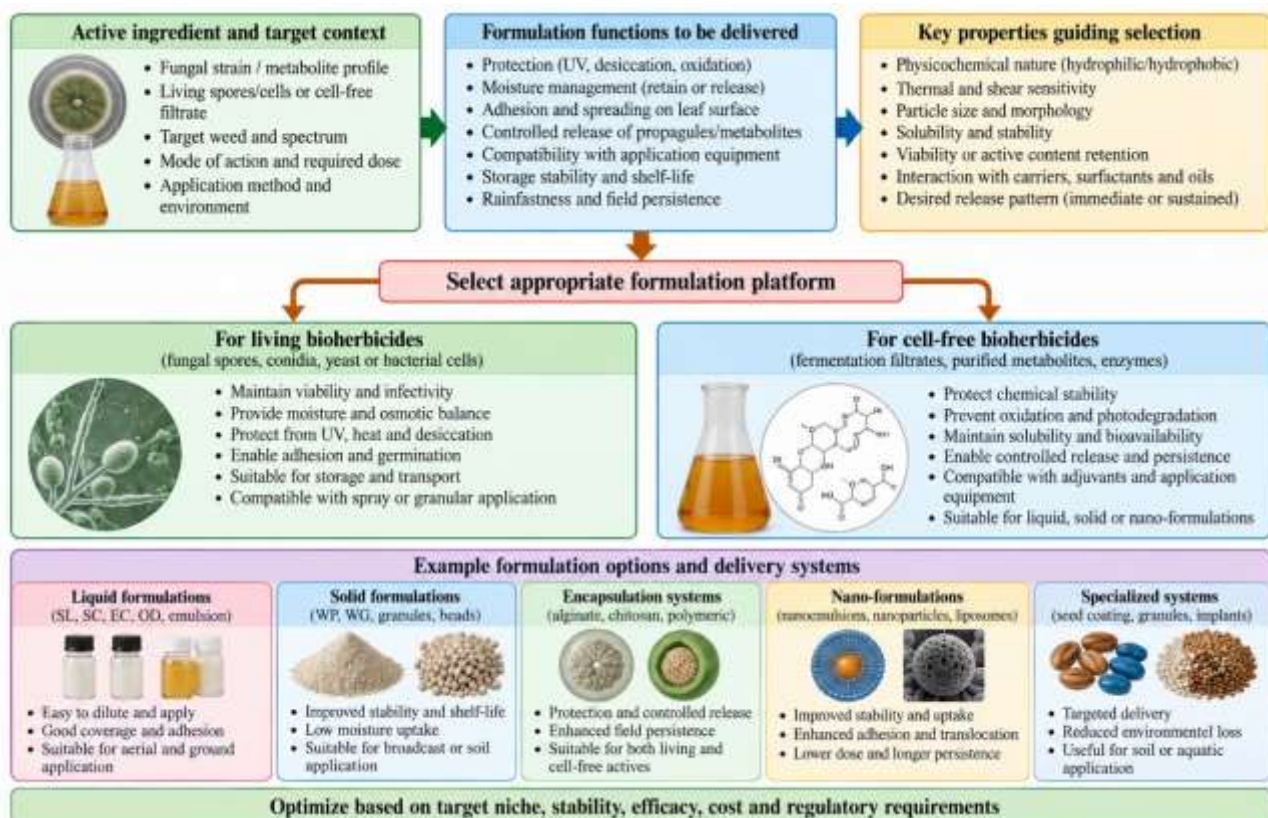


Fig. 2. Function-led formulation selection for living and cell-free bioherbicides.

11.1. Compatibility and synergy: minimum evidentiary requirements

For living–botanical combinations, evaluate viability and germination of the microbial partner after mixing and storage before testing efficacy. Report the botanical dose, inoculum dose, mixture ratio and each component alone. An observed mixture response exceeding either single treatment does not by itself establish synergy; an explicit additivity model, confidence intervals and crop-safety controls are needed. The resulting formulation should be tested for phase separation, photostability, packaging interactions and non-target effects before claims of improved field reliability are made.

12. Formulation characterization and quality control

Publication-quality formulation research should report sufficient physicochemical data to permit comparison and reproducibility. For liquid products, relevant measurements include pH, density, viscosity, surface tension, droplet or particle size, polydispersity, zeta potential when meaningful, sedimentation, creaming, emulsion separation, redispersibility and foaming. For dry products, moisture content, water activity, particle-size distribution, bulk density, flowability, wettability, dispersibility and rehydration are important.

Biological quality control must be tailored to the active ingredient. For fungal propagules, colony-forming units alone may be misleading because clumps can generate single colonies. Germination percentage, germ-tube development and infectivity provide complementary information. For bacteria, viable count, physiological state and toxin production may be relevant. For cell-free products, chemical marker concentration should be paired with a standardized phytotoxicity assay.

Shelf-life should be defined by a pre-established specification rather than by visual appearance. Real-time storage at intended commercial temperatures is essential, although accelerated studies can help screen formulations. Freeze-thaw cycles, elevated temperature, light exposure and packaging interactions may reveal failure modes. Packaging can adsorb hydrophobic metabolites, permit oxygen or moisture transfer, or interact with surfactants; therefore, container-closure systems should be included in stability programs.

Quality control also requires microbiological purity. Nutrient-rich biological formulations are vulnerable to contamination by bacteria, yeasts and molds. Preservatives may protect the product but can harm the intended organism. Aseptic processing, low water activity, refrigerated storage or selective preservatives may be needed. Specifications should include identity of the production strain, absence of specified contaminants and acceptable limits for adventitious organisms.

For advanced carriers, release kinetics should be measured under conditions relevant to the intended use. Release in distilled water may not predict behavior on a leaf or in soil. Temperature, pH, ionic strength, rainfall and UV exposure can alter polymer swelling and degradation. Formulation characterization should therefore connect laboratory metrics to biological performance through mechanistic hypotheses. A practical set of critical quality attributes for living and cell-free products is presented in Table 3.

Table 3. Critical quality attributes for living and cell-free bioherbicides

Attribute	Living microbial product	Cell-free metabolite product	Typical method
Identity	Strain identity and purity	Chemical/metabolomic fingerprint	Molecular chromatography ID;
Potency	Viability, germination, infectivity	Phytotoxic activity / marker concentration	CFU/germination; bioassay; HPLC/LC-MS
Physical stability	Suspension, dispersibility, integrity	Precipitation, emulsion or suspension stability	Visual/centrifugal tests; particle sizing
Moisture/water activity	Critical for dry survival	Critical for degradation and microbial contamination	Moisture analyzer; aw meter
pH	Affects viability and contaminant growth	Affects chemical stability	Calibrated pH meter
Particle/droplet size	Sprayability and deposition	Release, stability, bioavailability	Laser diffraction/DLS as appropriate

Attribute	Living microbial product	Cell-free metabolite product	Typical method
Shelf-life	Retention of viable infective units	Retention of chemical potency	Real-time and accelerated stability
Microbial contamination	Specified limits essential	Specified limits essential for crude broths	Plate counts/selective media
Release profile	Rehydration and propagule liberation	Diffusion/dissolution of active metabolites	Release studies under relevant media

12.1. Stage-gated product specifications

An initial product specification should define identity, potency, microbial contamination limits, storage conditions and packaging compatibility. Living products additionally require viable propagule counts, germination and infectivity after storage and spray passage. Cell-free products require a reproducible chromatographic fingerprint, potency-linked marker or validated bioassay, and a degradation profile; analytical detectability alone is insufficient to establish retained herbicidal activity. Advance to field testing only when the formulated product meets predefined acceptance criteria under intended handling and weather-stress conditions.

13. Application technology and precision delivery

Formulation and application cannot be optimized independently. Hewitt et al. (2024) emphasized that bioherbicide success depends on matching formulation and delivery to the target weed and climatic region. Contact-active biological products often require high coverage, whereas systemic or infection-based agents may depend more on retention at susceptible infection courts. Droplet size influences coverage, evaporation, drift, bounce, shatter and runoff.

Nozzle selection should reflect formulation rheology and particle size. Suspensions containing large spores or microcapsules require orifices that avoid blockage and shear damage. Very fine droplets increase coverage but are more vulnerable to drift and evaporation. Coarser droplets reduce drift but may provide insufficient target coverage. Narrow droplet-size spectra and validated spray models can help optimize deposition.

Unmanned aerial vehicles are increasingly relevant for site-specific weed management. Their low carrier volumes and rotor downwash create a different deposition environment from conventional boom sprayers. Biological formulations intended for UAV use must tolerate high concentration, short mixing times and potentially rapid droplet evaporation. Sensors and machine vision can identify weed patches, enabling treatment only where needed and potentially offsetting the higher cost per unit of bioherbicide active ingredient.

Robotic direct implantation is another promising niche, especially for woody invasive weeds. Capsules or inoculum plugs placed into stems bypass spray losses and can create protected infection sites. Hewitt et al. (2024) discuss direct implantation and future robotic platforms as routes to reduce labor. Such delivery demonstrates that the best bioherbicide formulation may not resemble a conventional herbicide spray.

Precision application also creates opportunities for decision-support systems. Weather forecasts can identify periods of favorable humidity and temperature, while canopy sensing can estimate target density. A formulation with moderate dew dependence may become commercially useful if applied only during predicted infection windows. Thus, digital agriculture can partly compensate for biological variability.

14. Formulation strategies for aquatic, soil and woody weeds

Aquatic weeds require formulations that resist rapid dilution and maintain contact with floating leaves or submerged tissues. Floating granules, oil-containing droplets, stickers and buoyant carriers can localize the active ingredient at

the air-water interface. For water hyacinth, for example, a formulation should wet the waxy leaf, resist wash-off and maintain moisture around fungal propagules without releasing environmentally persistent carrier materials into water.

Soil-targeted weeds and parasitic weeds favor different architectures. Granules, seed coatings and encapsulated propagules can place the biological agent near weed seeds or host roots. The success of *Fusarium oxysporum* f. sp. *strigae* against *Striga* illustrates how delivery with crop seed can create a favorable spatial relationship between the bioherbicide and the parasitic weed (Ciotola et al. 2000; Baker et al. 2024). Cell-free metabolites may also be delivered in controlled-release soil matrices, although sorption and microbial degradation must be considered.

Woody weeds often permit wound-based delivery. Cutting, drilling or implanting creates an entry point and protected moist environment, reducing the need for sophisticated foliar moisture management. Products based on *Chondrostereum purpureum* and other fungi have exploited cut-stump application. The approach is labor intensive but highly targeted and minimizes non-target exposure, making it attractive for invasive-plant management in natural areas.

These examples reinforce a broader principle: formulation should begin with the ecological and operational context. A universal formulation is unlikely to be optimal across foliar annual weeds, aquatic weeds, parasitic weeds and woody perennials. A niche-based formulation selection framework is summarized in Table 4.

Table 4. Formulation selection according to weed ecology and application niche

Target niche	Preferred formulation concepts	Critical performance requirement	Representative challenge
Annual foliar weeds	Liquid suspensions, O/W emulsions, W/O/W, cell-free sprays	Coverage, adhesion, moisture retention	Rapid evaporation and rain wash-off
Perennial/woody weeds	Wound paste, implant, capsule	Protected infection court and persistence	Labor and access to individual plants
Aquatic weeds	Floating granules, buoyant carriers, adhesive emulsions	Surface localization and wash-off resistance	Dilution and aquatic safety
Soil/pre-emergence weeds	Granules, encapsulated propagules, controlled-release matrices	Persistence near seed/root zone	Competition with soil microbiota
Parasitic weeds	Seed coating, rhizosphere delivery	Spatial proximity to host-parasite interface	Maintaining viability on seed
Precision/UAV treatment	Concentrated stable liquids, low-volume compatible formulations	Atomization, deposition, high-concentration stability	Evaporation and small carrier volume

15. Scale-up, manufacturing and economics

Commercialization requires a formulation that can be manufactured reproducibly at scale. Fermentation yield, raw-material cost, downstream recovery, drying, formulation ingredients, packaging and distribution all contribute to cost of goods. Highly host-specific bioherbicides may address small markets, making expensive specialized manufacturing difficult to justify. This economic reality was recognized in early formulation literature and remains a central explanation for the limited number of widely successful microbial bioherbicides (Auld et al. 2003; Duke 2024).

Process scale-up can alter the biological active ingredient. Oxygen transfer, shear, mixing time, nutrient gradients and morphology change with bioreactor size. A fungus that produces infective spores in a shake flask may produce mainly mycelial biomass in a large stirred tank. Formulation scientists should therefore define which propagule is required and design fermentation to produce it consistently. Downstream steps must preserve the relevant physiological state.

Drying is often one of the most expensive and damaging operations. Spray drying is scalable but exposes cells to heat and interfacial stress; freeze drying is gentle for some organisms but costly; fluid-bed drying may suit granules. Liquid concentrates avoid drying but increase transport cost and may require refrigeration. Techno-economic analysis should compare complete supply chains rather than individual unit operations.

Low-cost agricultural and industrial residues can reduce fermentation-media costs and support circular-economy objectives (Ocán-Torres et al. 2024; Singh and Pandey 2026), but variable composition may complicate quality control. Crude cell-free broths can reduce purification costs, yet regulatory specifications still require consistent potency. The optimal balance between purification and standardization will depend on the active chemistry and target market.

Commercial product design should also consider user behavior. A formulation requiring complicated mixing, strict timing, special nozzles or prolonged irrigation is less likely to be adopted unless it provides a clear advantage. Compatibility with existing equipment and farm routines is therefore a design requirement, not merely a marketing consideration.

15.1. Dose-normalized techno-economic comparison

Economic comparisons should be based on cost per successfully treated hectare rather than cost per kilogram of formulation. Include active yield, recovery loss, carrier cost, packaging, transport, storage, application volume, required repeat applications and achieved weed suppression. A higher-cost carrier may be justified only if the additional stability or efficacy is demonstrated at a matched active dose and persists in realistic field conditions. Sensitivity analysis should test fermentation yield, storage loss, application frequency and target-market size (Auld et al. 2003; Duke 2024; Marrone 2024).

16. Biosafety, environmental fate and regulation

Biological origin does not automatically imply safety. Living bioherbicides require assessment of host range, pathogenicity to non-target plants, persistence, dispersal and potential effects on beneficial organisms. Production strains should be taxonomically well characterized and screened for undesirable toxin production. Cell-free products require toxicological and ecotoxicological evaluation of active metabolites and relevant impurities.

Advanced carriers introduce additional questions. Biodegradable polymers are generally preferred, but degradation products and additives must be considered. Persistent inorganic nanoparticles may accumulate or move differently from conventional formulation ingredients. Nano-enabled products should therefore be evaluated on the basis of actual exposure, transformation and hazard rather than assumed to be environmentally benign because the active ingredient is natural.

Regulatory frameworks vary among jurisdictions. In the European Union, microbial and other biological plant-protection products are evaluated within the broader plant-protection-product framework, including approval of active substances and authorization of formulated products. In the United States, microbial pesticides and biochemical pesticides are regulated by the Environmental Protection Agency under pesticide law. Registration dossiers generally require identity, manufacturing process, product chemistry, efficacy or performance information as applicable, toxicology, non-target organism data and environmental fate information.

Regulatory development should begin early because formulation changes can trigger new data requirements. Replacing a carrier, adding a surfactant or moving from living cells to a cell-free metabolite can alter the regulatory identity of the product. A formulation platform should therefore be selected with both technical performance and registration strategy in mind.

17. Commercialization status and lessons from past products

The history of bioherbicides contains both technically successful niche products and many candidates that failed to achieve sustained markets. Reviews have documented products based on *Colletotrichum*, *Chondrostereum*, *Fusarium*, *Xanthomonas* and other microorganisms, but broad adoption in major field crops remains limited (Cordeau et al. 2016; Morin 2020; Hasan et al. 2021; Roberts et al. 2022; Kremer 2023; Duke 2024; Székács 2024). Narrow host range can

be an advantage for crop safety but a disadvantage for market size, while environmental dependence can produce inconsistent field performance even when laboratory efficacy is excellent. Market analyses likewise emphasize the difficulty of matching the spectrum, cost and operational simplicity of conventional herbicides (Marrone 2024).

Candidates for graminaceous weeds continue to be developed, as shown by studies of *Exserohilum monoceras* against *Echinochloa* species in rice and of *Bipolaris yamadae* strain HXDC-1-2 in rice and dry fields (Zhang and Watson 1997; Tan et al. 2024). Indigenous-isolate and metabolite-based programs, including work on invasive weeds such as *Lantana*, have likewise been reviewed (Singh and Pandey 2019b, 2022a), and recent editorial perspectives stress the need for fit-for-purpose products and realistic adoption pathways (Duke et al. 2024).

Successful niches share several features: a well-defined target, a delivery method that fits the biology, limited alternatives or strong demand for non-chemical control, and an application environment that can be managed. Cut-stump fungal products for woody weeds and seed-delivered agents against parasitic weeds illustrate this principle. The Striga “Toothpick Project” illustrates this niche logic by coupling a virulence-selected *Fusarium* product with a locally workable delivery and commercialization model (Baker et al. 2024). These cases suggest that commercialization strategy should identify situations in which biology provides a unique operational advantage rather than attempting to imitate broad-spectrum chemical herbicides in every context.

Cell-free products may broaden opportunities because they can be standardized and combined more easily with conventional formulation technologies. However, they lose the self-amplifying potential of a living pathogen and may require higher manufacturing throughput. Likewise, nanoformulations may improve stability but can increase cost and regulatory complexity. Commercial success therefore depends on the value delivered per treated hectare, not technological novelty. Selected recent literature that defines the current formulation landscape is summarized in Table 5.

Table 5. Selected literature relevant to modern bioherbicide formulation

Reference	Topic	Key relevance to formulation
Cordeau et al. 2016	Bioherbicide status and constraints	Modern synthesis of microbial and natural-product bioherbicides
Campos et al. 2022	Micro/nanocarriers for biological plant protection	Polymeric encapsulation materials including chitosan and alginate
Hasan et al. 2021	Achievements and commercialization	Production, environmental and commercial constraints
Pérez-de-Luque 2024	Nanotechnology for bioherbicides	Opportunities and safety/cost limitations of nano-enabled delivery
Hewitt et al. 2024	Application technology	Dry inoculum and liquid formulations with spray engineering and UAVs
Duke 2024	Commercial limitations	Technical barriers and potential advantages of cell-free products
Singh and Pandey 2022b	Foliar fungal pathogenic metabolites	Fungal metabolite herbicides: development and commercialization prospects
Singh and Pandey 2025a	Cell-free fungal broth formulation	Recent work on metabolite-rich, non-viable mycoherbicide concepts
Singh and Pandey 2026	Fungus-based bioherbicides	Synthesis of formulation, scale-up and precision technologies

Reference	Topic	Key relevance to formulation
Razafindralambo et al. 2026	Synbiotic bioherbicides	Combined microbial-botanical formulations

18. Research gaps and future perspectives

The most important research gap is the weak linkage between formulation characterization and multi-environment field performance. Many studies demonstrate improved stability or greenhouse phytotoxicity but do not test whether the formulation remains superior under variable humidity, rainfall, UV exposure and weed growth stage. Future work should use standardized controls and report active dose, carrier dose, application volume and environmental conditions in sufficient detail for comparison. Recent systematic and critical reviews reinforce the need for stronger mechanistic characterization, standardized reporting and field validation (Raza et al. 2025; Zhang et al. 2025).

Formulation-by-design offers a path forward. High-throughput screening can evaluate matrices of surfactants, humectants, polymers and protectants, while statistical experimental design can identify interactions. Mechanistic models can connect evaporation, droplet spreading, release and germination. Machine learning may eventually predict promising formulations from physicochemical descriptors, but robust training data will require standardized reporting.

Biodegradable micro- and nanocarriers deserve continued investigation, particularly for cell-free metabolites and botanical actives. The priority should be function rather than particle size: protection from UV, controlled release, adhesion, rainfastness and reduced dose. Environmental fate and cost should be evaluated at the same stage as efficacy.

Synbiotic formulations combining microorganisms and plant-derived components represent a new frontier (Razafindralambo et al. 2026). Their greatest potential may lie in complementary kinetics, with a fast-acting phytotoxin weakening the weed and a pathogen providing sustained damage. However, compatibility, mixture stability and regulatory complexity remain major challenges.

Precision agriculture may be equally transformative. UAVs, robotic applicators, weed-detection cameras and weather-based decision support can reduce treated area, optimize timing and create application methods specifically suited to biological products. A bioherbicide that is too expensive for blanket application may be economically viable when applied only to mapped weed patches.

Finally, living and cell-free products should not be treated as competing paradigms. A development program can evaluate both. If a pathogen is highly effective but environmentally fragile, its metabolites may provide a more robust product. If metabolite yields are too low, a living formulation may exploit in planta production. The best route should be selected using biological efficacy, manufacturing economics, safety and target-market requirements. Priority research questions and measurable development endpoints are consolidated in Table 6.

Table 6. Research priorities for next-generation bioherbicide formulations

Priority	Research question	Suggested endpoints
Dew independence	Can the formulation maintain infection under short or absent dew?	Disease progress, leaf wetness duration, field mortality
UV protection	Does protection preserve viability/potency without crop injury?	UV survival, photodegradation kinetics, crop safety
Controlled release	Is release synchronized with infection or phytotoxic action?	Release kinetics, bioavailability, efficacy duration
Shelf-life	Can potency be retained under realistic distribution conditions?	12–24-month stability, packaging compatibility

Priority	Research question	Suggested endpoints
Low-volume application	Can products function at UAV-compatible carrier volumes?	Droplet spectrum, deposition, viability, efficacy
Cell-free standardization	Which markers predict biological potency?	LC-MS/HPLC markers plus validated bioassay
Biodegradable carriers	Do carriers degrade safely after performing their function?	Degradation, soil/water fate, ecotoxicity
Economics	Does improved efficacy justify formulation complexity?	Cost of goods, dose/ha, application cost, sensitivity analysis
Synbiotic systems	Are combinations genuinely synergistic and stable?	Mixture stability, synergy models, non-target safety
Field robustness	Does laboratory superiority persist across environments?	Multi-location, multi-season trials and weather-linked analysis

18.1. Comparative evidence and interpretation across formulation platforms

A useful comparison of formulation platforms must separate established performance mechanisms from the strength of bioherbicide-specific evidence. Classical oil emulsions and granules have direct mycoherbicide precedents, whereas some modern polymer and nanoscale carrier claims rely primarily on controlled assays or adjacent crop-protection applications. A carrier's improvement in encapsulation efficiency, photostability or release profile should not be equated with superior weed suppression at an equivalent active dose.

Table 8 is a qualitative evidence map derived from the literature discussed in this review; it does not report pooled effect sizes, rank products by efficacy or imply that all cited studies tested the same organism, weed, dose or environment. A quantitative meta-analysis would require comparable active-dose data, defined controls, replicate variance, weather exposure and common field endpoints, which are not consistently available across the reviewed platforms.

Table 8. Qualitative evidence map for formulation approaches (not a quantitative efficacy comparison).

Platform	Direct bioherbicide evidence discussed	Mechanistic advantage	Important qualification
Aqueous inoculum	Historical microbial bioherbicide standard	Simple delivery and dose reference	Rapid drying and environmental dependence
Grain, Pesta and alginate granules	Classical mycoherbicide formulation studies	Local delivery, hydration and inoculum protection	Activation depends on moisture and target niche
Oil and invert emulsions	Classical foliar mycoherbicide studies	Moisture conservation and leaf contact	Viscosity, crop safety, cost and spray compatibility
Biopolymer microcapsules	Some biological crop-protection evidence; variable weed-specific validation	Protection and release tuning	Bioherbicide field superiority not established by material tests alone

Nano-enabled carriers	Emerging natural-product and related carrier evidence	Solubility, photoprotection and controlled release	Dose-matched field tests and carrier-fate data needed
Cell-free concentrates	Emerging metabolite-based bioherbicide evidence	No viable propagule requirement; measurable chemical potency	Complex metabolite mixtures and degradation must be standardized
Precision/UAV delivery	Application-engineering literature and developing use cases	Targeted deposition and timing	Formulation-specific field validation and economic comparison needed

18.2. Minimum reporting framework for comparative formulation trials

Comparative formulation studies are essential for identifying delivery systems that provide consistent biological activity, adequate storage stability, and reliable field performance. However, meaningful comparison among formulations requires standardized reporting of the active ingredient, formulation composition, applied dose, physicochemical properties, biological potency, application conditions, and environmental variables. Differences in these parameters can substantially influence apparent efficacy and may lead to misleading conclusions when formulations are compared only on the basis of nominal product concentration. Therefore, formulation trials should be designed around dose-normalized and field-relevant comparisons, with sufficient information to distinguish the effects of the active ingredient from those of carriers, surfactants, protectants, and other formulation components.

A minimum reporting framework should first provide an unambiguous description of the active ingredient and formulation system. For microbial products, this includes organism and strain identity, propagule type, viable count, germination or other relevant potency measurements, and the concentration present at application. For cell-free metabolites, botanical extracts, enzymes, or other biochemical actives, the reported information should include active or marker-compound concentration, analytical method, and, where appropriate, a chromatographic or chemical fingerprint. Formulation composition should identify the major functional classes of excipients, including carriers, oils, surfactants, dispersants, rheology modifiers, humectants, protectants, and preservatives. Such information is necessary to interpret whether improved performance results from increased biological potency or from enhanced delivery and persistence.

Dose normalization is particularly important when comparing EC, OD, SC, WP, WG, encapsulated, or other formulation platforms because products may contain substantially different concentrations of active material. Results should therefore report both product application rate and actual active dose per unit area, expressed using an appropriate metric such as viable propagules, CFU, spores, active or marker compound, or biologically defined potency units. Spray volume, dilution factor, application equipment, nozzle characteristics, droplet properties, number of applications, and application timing should also be documented. Untreated controls and suitable formulation-blank or carrier controls are valuable for separating active-ingredient effects from those caused by formulation auxiliaries.

Physicochemical and stability characteristics should be reported together with biological efficacy. Depending on formulation type, relevant measurements may include pH, moisture or water activity, particle-size distribution, viscosity or rheological behavior, suspensibility, emulsion stability, dispersibility, redispersibility, sedimentation, and active-content or viable-count retention. Storage temperature, duration, packaging system, and sampling intervals should also be specified. Linking these measurements with efficacy provides a stronger basis for establishing critical quality attributes and product specifications than reporting physical stability or biological activity independently.

Finally, field-relevant studies should document environmental and experimental conditions that can modify product performance, including temperature, relative humidity, rainfall or irrigation, UV exposure, crop and target growth stage, and relevant characteristics of the application site. Experimental design, replication, statistical analysis, efficacy endpoints, crop safety, and non-target observations should be reported sufficiently to permit interpretation and reproducibility. Wherever possible, efficacy should be evaluated across multiple environments and related to storage stability, formulation characteristics, and cost per treated area. The minimum elements proposed in Table 7 therefore provide a practical framework for generating formulation data that are scientifically comparable and more useful for scale-up, regulatory evaluation, and commercial translation.

Table 7. Proposed minimum reporting checklist for dose-normalized and field-relevant formulation studies.

Domain	Minimum reportable information	Primary interpretation
Active and target	Strain/metabolite identity, target weed, weed stage, crop context	Reproducibility and selectivity
Dose and controls	Viable units or chemically defined active per hectare; blank carrier; unformulated dose-matched control	Separates carrier benefit from dose effects
Physical performance	Water activity, viscosity, particle/droplet size, adhesion, rainfastness, release	Connects mechanism to delivery
Stability	Storage time/temperature, packaging, potency or germination retention	Commercial shelf-life relevance
Application	Nozzle, pressure, carrier volume, droplet spectrum, deposition	Equipment compatibility
Field robustness	Humidity, leaf wetness, UV, rainfall, replicate sites/seasons	Generalizability
Safety and economics	Crop injury, non-target endpoints, carrier fate, cost/treated hectare	Practical adoption

19. Conclusions

Formulation remains one of the decisive determinants of whether a bioherbicide progresses from laboratory discovery to practical weed management. The foundational technologies reviewed by Auld et al. (2003)—aqueous suspensions, grain carriers, alginate encapsulation, Pesta granules, polymers, oil emulsions, invert emulsions and water-in-oil-in-water systems—established principles that remain valid: protect the active ingredient, create a favorable microenvironment, match delivery to the infection process, and minimize dependence on unpredictable field conditions.

The modern formulation toolbox is substantially broader. Biodegradable polymers, microencapsulation, nanoemulsions, polymeric and lipid carriers, cell-free fermentation products, multi-component formulations and precision application platforms provide new ways to address stability, moisture, solubility, UV sensitivity, retention and targeting. Yet technological sophistication does not guarantee commercial value. Every formulation must ultimately be judged by reproducible field efficacy, shelf-life, safety, manufacturability, cost and ease of use.

Future progress will depend on integrating strain selection or metabolite discovery with fermentation, downstream processing, formulation, packaging and application engineering from the beginning of development. Product-specific solutions are likely to outperform universal platforms. For foliar mycoherbicides, reduction of dew dependence and improvement of rainfastness remain central. For cell-free products, chemical stabilization and controlled release are priorities. For aquatic, parasitic and woody weeds, ecological localization of the active ingredient may be more important than conventional spray performance.

The central implication is that formulation choice must be justified by an active-specific failure mode and verified through dose-matched, carrier-controlled comparisons. Integrated assessment of stability, delivery, multi-environment efficacy, non-target safety and cost per successfully treated hectare—not carrier novelty alone—offers the strongest route toward reliable and commercially viable biological weed management.

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