



ADVANCES IN MYCORRHIZAL INOCULANT FORMULATIONS: PRODUCTION TECHNOLOGIES, QUALITY STANDARDS, COMMERCIAL APPLICATIONS AND FUTURE PERSPECTIVES FOR SUSTAINABLE AGRICULTURE

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ABSTRACT

Mycorrhizal fungi form symbiotic associations with plant roots, enhancing nutrient uptake and conferring stress tolerance. The commercial development of mycorrhizal inoculants (formulations) has become increasingly important for sustainable agriculture, horticulture, and ecological restoration. This review comprehensively discusses mycorrhizal formulation technologies, inoculation methods, mass production, quality control standards, and practical applications. We examine arbuscular mycorrhizal (AM) fungi and ectomycorrhizal (ECM) fungi formulations, highlighting the advantages and limitations of different formulation types including powder, granule, liquid, and mycelial colony aggregates (MCAs). Critical issues including inoculant viability, shelf-life, efficacy, and regulatory frameworks are addressed. Current challenges and future perspectives for enhancing mycorrhizal formulation technology are discussed, with emphasis on precision agriculture, biofortification, and climate change adaptation. This review synthesizes recent literature to provide researchers, practitioners, and industry professionals with updated knowledge on mycorrhizal formulation development and application.

KEYWORDS: Mycorrhizal Fungi, Formulation Technology, Inoculation Methods, Mass Production, Quality Control, Agricultural Application, Sustainability

1. INTRODUCTION

Mycorrhizal symbiosis represents one of the most significant plant-microbe interactions in terrestrial ecosystems. Approximately 80–90% of plant species establish mycorrhizal associations, making this symbiosis fundamental to plant nutrition, ecosystem function, and productivity (Smith & Read, 2008). The symbiotic relationship between mycorrhizal fungi and plant roots are characterized by reciprocal nutrient exchange: fungi provide phosphorus, nitrogen, and micronutrients to the plant, while the host plant supplies carbohydrates to the fungal partner.

Modern agriculture has increasingly relied on chemical inputs, leading to soil degradation, decreased biodiversity, and environmental pollution. Consequently, there is growing recognition of the need for sustainable agricultural practices that enhance soil health and productivity while minimizing chemical inputs. Mycorrhizal inoculants represent a promising tool for sustainable intensification of agriculture, offering multiple benefits including improved nutrient acquisition, enhanced drought tolerance, disease suppression, and increased yield.

The global mycorrhizal inoculant market has experienced substantial growth over the past two decades, driven by increased adoption in agriculture, horticulture, forestry, and ecological restoration. However, inconsistent field performance and limited regulatory frameworks have constrained broader adoption. The development of effective mycorrhizal formulations with stable viability and predictable efficacy is therefore critical for realizing the full potential of this technology.

This review synthesizes current knowledge on mycorrhizal formulation technology, covering formulation types, inoculation methods, quality control, applications, challenges, and future directions. The objective is to provide comprehensive guidance for researchers, product developers, and agricultural practitioners seeking to develop or utilize mycorrhizal inoculants effectively.

2. MYCORRHIZAL ASSOCIATIONS: TYPES AND FUNCTIONS

Mycorrhizal associations are classified into two major groups based on anatomical and functional characteristics: arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM). Both associations confer significant benefits to host plants, but they differ in fungal partner taxonomy, host range, root colonization patterns, and nutrient transfer mechanisms.

2.1 Arbuscular Mycorrhizal (AM) Fungi

Arbuscular mycorrhizal fungi belong to the phylum Glomeromycota and form symbiotic associations with approximately 80% of plant species. AM colonization is characterized by the



penetration of fungal hyphae into root cortex cells, where specialized structures called arbuscules form intracellularly. Arbuscules serve as sites of nutrient exchange, with fungi providing phosphorus, nitrogen, zinc, and other micronutrients, while receiving plant carbohydrates. Key genera include *Rhizophagus*, *Funneliformis*, *Claroideoglomus*, and *Rhizoglomus*.

AM fungi produce spores that serve as propagules for inoculant preparation. However, a fundamental constraint is their obligate biotrophic lifestyle—they cannot be cultured on non-living media *in vitro*. AM inoculants must be produced in association with living host plants grown under controlled conditions or through *in vitro* systems utilizing transformed roots or protoplast fusion techniques.

Agricultural applications of AM fungi have shown promise for improving phosphorus availability in low-input systems, particularly in developing countries where phosphate fertilizers are expensive or unavailable. AM inoculants are particularly valuable for small-holder farming systems where chemical inputs are limited.

2.2 Ectomycorrhizal (ECM) Fungi

Ectomycorrhizal fungi form associations with woody plants, particularly trees of the families Pinaceae, Fagaceae, and Betulaceae. Unlike AM fungi, ECM fungi form a sheath (mantle) around root tips, with hyphae penetrating intercellularly into the root cortex (Hartig net). ECM fungi are primarily nutrient saprophytes, with the ability to decompose organic matter in soil, making them particularly valuable in nutrient-poor forests and disturbed soils.

A critical advantage of ECM fungi is that many species can be cultured on standard growth media, facilitating inoculant production. Common ECM genera include *Laccaria*, *Hebeloma*, *Pisolithus*, *Suillus*, and *Amanita*. ECM inoculants are widely used in forest restoration, nursery production, and afforestation programs, particularly for enhancing survival and growth of container-grown seedlings.

Recent advances in ECM inoculant technology include the development of improved carrier materials, dried mycelial formulations, and the use of mycelia-coated root tips for enhanced establishment.

3. Mycorrhizal Formulation Technologies

The selection of appropriate formulation technology is critical for maintaining fungal viability, ensuring consistent efficacy, facilitating application, and achieving acceptable shelf-life. Multiple formulation types have been developed, each with distinct advantages and limitations.

3.1 Powder Formulations

Powder formulations consist of mycorrhizal fungal propagules (spores, mycelia, or colonized root fragments) mixed with inert carrier materials such as peat, talc, bentonite, or diatomaceous earth. These are among the oldest and most widely used formulations due to their simplicity, low production cost, and ease of application. Advantages are Low cost and simple production process, Suitable for small-scale and home use, Stable when dry (moisture content;6%) and can be stored at room temperature. They have some limitations as variable viability during storage due to water loss, uneven distribution in soil during application, potential for dust inhalation during handling and lower fungal propagule density compared to other formulation types.

3.2 Granular Formulations

Granular formulations consist of fungal propagules adhered to larger carrier particles (typically 0.5–2 mm diameter) such as clay, bark, perlite, or ceramic granules. The larger particle size provides several advantages over powder formulations. Advantages are better environmental stability and reduced dust, easier application with mechanical seeders, more uniform soil distribution and better retention of moisture compared to powders. They have limitations as higher production cost, requires specialized equipment for application and transport and storage of larger volumes.

3.3 Liquid Formulations

Liquid formulations utilize aqueous or oil-based suspension systems to carry fungal propagules. These may include spores, hyphal fragments, or whole-culture inocula suspended in water or other carrier liquids with added stabilizers such as gum arabic, cellulose, or silica. Liquid formulations have gained popularity due to their ease of application and potential for high fungal propagule density. Advantages are high propagule density per unit volume, compatible with fertigation and precision irrigation systems, potential for real-time application during crop establishment and easier to transport and store than powders or granules. Limitations are shorter shelf-life due to water activity and potential contamination, requires cold storage (2–8°C) or preservatives, potential for fungal propagule settling and requiring agitation and higher production costs including refrigeration infrastructure.

3.4 Mycelial Colony Aggregates (MCAs)

Mycelial colony aggregates are compact, pellet-like structures composed of dense mycelial networks, often produced from culturable fungi such as ECM species. MCAs may be produced by solid-state fermentation followed by air-drying or through liquid fermentation followed by spray-drying or freeze-drying. Advantages are highest fungal propagule concentration, excellent environmental stability, long shelf-life (2 years at room temperature) and precise dosing for consistent application. Limitations are



high production cost (drying and packaging equipment), requires culturable fungal species and limited to fungi amenable to industrial fermentation.

Table 1 represents comparative evaluation of major mycorrhizal inoculant formulation technologies based on propagule type, shelf life, production cost, advantages, limitations, and recommended agricultural applications. Emerging encapsulated and nano-enabled formulations offer improved stability and controlled release characteristics compared with conventional powder, granular, and liquid formulations.

Table 1. Comparative Characteristics of Major Mycorrhizal Inoculant Formulation Types

Formulation Type	Propagule Type	Typical Shelf Life	Relative Production Cost	Major Advantages	Major Limitations	Suitable Applications
Powder Formulation	Spores, colonized root fragments, mycelial fragments	6–12 months	Low	Simple production process, economical, easy handling, suitable for seed treatment and nursery use	Dust generation, uneven field distribution, viability loss under high humidity, lower propagule density	Seed coating, nursery substrates, small-scale agriculture
Granular Formulation	Spores and mycelia incorporated into granular carriers (clay, perlite, vermiculite, peat)	12–18 months	Moderate	Uniform soil distribution, reduced dust, better moisture retention, compatible with mechanical application equipment	Higher transportation and storage volume, increased manufacturing cost	In-furrow application, field crops, forestry nurseries
Liquid Formulation	Spores, hyphal fragments, or whole-culture suspensions	3–12 months	High	High propagule concentration, easy application through fertigation and drip irrigation systems, rapid root-zone delivery	Shorter shelf life, refrigeration often required, settling of propagules during storage, contamination risk	Precision agriculture, greenhouse production, fertigation systems
Mycelial Colony Aggregates (MCAs)	Dense mycelial pellets or fungal biomass aggregates	>24 months	High	High propagule viability, excellent environmental stability, precise dosing, long-term storage potential	Expensive production processes, limited to culturable fungal species, specialized manufacturing facilities required	High-value crops, forestry, ecological restoration programs
Encapsulated Formulation	Spores or mycelia entrapped in polymeric matrices (alginate, chitosan, starch, biochar composites)	>24 months	High to Very High	Controlled release, superior protection against environmental stress, enhanced shelf life, improved root colonization efficiency	High formulation complexity, increased production cost, scalability challenges	Advanced commercial products, precision delivery systems, stress-prone environments
Nano-Encapsulated Formulation	Spores or bioactive propagules incorporated into nanocarriers	>24 months (projected)	Very High	Enhanced stability, targeted delivery, improved propagule survival, controlled nutrient release, compatibility with smart agriculture technologies	Limited commercial availability, regulatory uncertainty, high development costs	Future-generation biofertilizers and climate-smart agriculture
Gel-Based Formulation	Spores and hyphae suspended in hydrogel matrices	12–24 months	Moderate to High	Improved moisture retention, enhanced root contact, reduced desiccation stress, easy seedling root dipping	Higher viscosity may complicate application, storage stability concerns	Transplant crops, horticulture, nursery production

Abbreviations: MCA = Mycelial Colony Aggregate.



4. INOCULATION METHODS AND APPLICATION TECHNIQUES

Effective inoculation technique is essential for ensuring fungal establishment and realizing agronomic benefits. The choice of inoculation method depends on the crop system, field conditions, available infrastructure, and formulation type.

4.1 Seedling Inoculation

Inoculation of seedlings in nurseries before transplanting offers several advantages, including controlled inoculation conditions, ability to verify colonization before field deployment, and reduced inoculum requirement. Methods include seed coating, root dipping, and incorporation into potting media.

4.2 In-Furrow Application

Direct application to the seed furrow at planting is a common method for field crops. Granular formulations are particularly suited for this application, as they can be applied with mechanical seeders. Proper placement near developing roots is critical for fungal establishment.

4.3 Soil Incorporation

Pre-planting incorporation of inoculant into the top soil layer (5–15 cm) allows even distribution and establishes a fungal population before crop establishment. This method is particularly useful for powder and liquid formulations.

4.4 Fertigation

Liquid inoculants can be applied through irrigation systems (fertigation), allowing for precision application, reduced labor requirements, and potential for split applications throughout the growing season. This approach requires formulations with stability in water and compatibility with irrigation infrastructure.

5. MASS PRODUCTION PROCESSES FOR MYCORRHIZAL INOCULANTS

Scaling-up mycorrhizal inoculant production from laboratory to commercial scale is a critical challenge requiring specialized knowledge of fungal biology, fermentation technology, and formulation engineering. Production systems vary substantially depending on whether AM or ECM fungi are being cultured, with significant implications for capital investment, operational

complexity, and product cost. Table 2. Comparison of industrial production technologies used for mass multiplication of arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) fungi. The table summarizes production scale, major advantages, limitations, and resulting inoculant products. Production technologies range from conventional pot culture systems for obligate biotrophic AM fungi to advanced bioreactor-based fermentation systems for culturable ECM fungi, reflecting differences in fungal biology and commercialization potential.

Table 2. Industrial Production Technologies for Arbuscular Mycorrhizal (AM) and Ectomycorrhizal (ECM) Fungi: Characteristics, Advantages, and Limitations

Production Technology	Mycorrhizal Type	Commercial Scale	Major Advantages	Major Limitations	Typical Products
Pot Culture System	AM	Small to Large Scale	Simple technology, low capital investment, suitable for diverse AM fungal species, widely adopted commercially	Labor-intensive, large space requirement, contamination risk, variable propagule density	Spores, colonized roots, soil-based inoculants
Monoxenic Root Organ Culture (ROC)	AM	Research to Pilot Scale	High purity inoculum, contamination-free production, genetically uniform propagules, high spore density	Expensive infrastructure, technical expertise required, limited large-scale commercialization	Pure spores, root-organ culture inoculants
Aeroponic Production System	AM	Pilot to Commercial Scale	High root biomass production, increased spore yield, reduced substrate use, efficient harvesting	High initial investment, sophisticated environmental control needed	High-density AM spores and root inoculum
Hydroponic Production System	AM	Pilot Scale	Controlled production environment, reduced contamination, efficient nutrient management	Technically demanding, relatively high operational costs	Root-associated inoculum and spores
Solid-State Fermentation (SSF)	ECM	Small to Large Scale	Low production cost, utilizes agricultural residues, simple	Non-uniform fungal growth, limited process	Mycelial biomass, granular and



			equipment, suitable for developing countries	control, sterility challenges	powder formulations
Submerged Liquid Fermentation (SmF)	ECM	Commercial Scale	High productivity, precise process control, consistent product quality, scalable bioreactor operation	High capital and operational costs, specialized expertise required	Liquid inoculants, mycelial suspensions, MCAs
Shake Flask Cultivation	ECM	Laboratory to Pilot Scale	Easy strain screening, low-cost culture maintenance, inoculum preparation	Not suitable for large-scale production, low biomass yield	Mother cultures and starter inoculum
Stirred-Tank Bioreactor Fermentation	ECM	Commercial Scale	High biomass productivity, automation capability, controlled pH and aeration, reproducible production	Expensive equipment and energy requirements	Liquid formulations, dried mycelial products
Airlift Bioreactor System	ECM	Pilot to Commercial Scale	Low shear stress, energy-efficient aeration, suitable for filamentous fungi	Lower mixing efficiency than stirred reactors	High-quality mycelial biomass
Immobilized Cell/Carrier-Based Fermentation	ECM	Emerging Technology	Enhanced fungal stability, repeated production cycles, higher biomass retention	Complex process optimization, limited industrial adoption	Encapsulated fungal inoculants
Mycelial Colony Aggregate (MCA) Production	ECM	Commercial Scale	High propagule concentration, long shelf life, excellent formulation stability	Additional downstream processing required	MCA pellets and dried fungal aggregates
Encapsulation-Based Production	AM & ECM	Emerging Commercial Technology	Improved viability, controlled release, enhanced shelf life, protection from environmental stress	High formulation costs and technological complexity	Alginate beads, chitosan capsules, bio-polymer encapsulates

Figure 1 represents industrial production workflow for mycorrhizal inoculants. The process involves selection of elite fungal strains, large-scale multiplication through host-based or fermentation systems, harvesting of spores and mycelial biomass, formulation into commercial products, quality control assessment, packaging, and distribution for agricultural, horticultural, forestry, and ecological restoration applications.



Figure 1. Industrial Production Workflow for Mycorrhizal Inoculants

Image Source- Google



5.1 Arbuscular Mycorrhizal (AM) Fungal Production

AM fungi cannot be cultured on standard growth media, requiring production systems that maintain an active mycorrhizal symbiosis. Several production methods have been developed at industrial scale, each with distinct advantages and limitations.

5.1.1 Pot Culture System (Conventional Method)

The pot culture system remains the most widely adopted method for AM fungal production, particularly in developing countries. Plants (typically maize, clover, or sorghum) are grown in sterilized substrate (soil, sand, or peat mixture) colonized with fungal inoculant. After 3–4 months of growth under controlled greenhouse conditions, mycorrhizal root material and associated soil are harvested, dried, and processed into commercial formulations. Production systems range from small-scale operations (hundreds of pots) to large commercial facilities with tens of thousands of pots. Advantages include simplicity, low capital investment, and ability to maintain diverse fungal strains. Limitations include space requirements, labor intensity, variable colonization rates, and potential for contamination with non-target organisms.

5.1.2 Monoxenic (In Vitro) Culture Systems

Monoxenic culture systems allow production of AM fungi in association with transformed roots (typically Ri T-DNA transformed roots) or protoplasts cultured on defined media without soil.

These systems provide superior control over growing conditions, reduced space requirements, and elimination of soil-borne contaminants. However, monoxenic culture requires sophisticated laboratory infrastructure and technical expertise, limiting adoption primarily to research institutions and advanced commercial producers. Fungal propagule density in monoxenic systems can exceed conventional pot cultures by 10–50 fold. The technology remains expensive for inoculant production but shows promise for maintaining genetically pure strains and producing formulations with defined fungal species composition.

5.1.3 Aeroponics and Hydroponic Systems

Emerging technologies including aeroponics and hydroponics offer potential for scaling AM fungal production. These systems maintain plant roots in mist or nutrient solution, promoting root development and mycorrhizal colonization while minimizing space requirements compared to pot culture. Aeroponics systems have demonstrated 2–3 fold increases in colonization rates compared to conventional soil-based methods. However, these systems require precise environmental control and regular monitoring. Capital investment is moderate to high, and technical expertise is essential for successful operation. These approaches show particular promise for developing countries with limited agricultural land and for producers seeking more intensive production models.

5.2 Ectomycorrhizal (ECM) Fungal Production

ECM fungi can be cultured on standard growth media without host plants, simplifying production significantly compared to AM fungi. Multiple production systems have been developed, ranging from shake flask and liquid fermentation to sophisticated bioreactor systems capable of producing tons of mycelium annually.

5.2.1 Solid-State Fermentation (SSF)

Solid-state fermentation involves culturing ECM fungi on moist, solid substrates such as sawdust, wood chips, grain, straw, or peat. SSF is the oldest industrial method for ECM production and remains widely used due to low capital and operational costs. Culture vessels range from simple bags to sophisticated bin systems with automated moisture and temperature control. Production cycles typically span 2–4 weeks. The process generates high-density mycelial biomass suitable for direct formulation into granules or powders. Advantages include low cost, simple technology, and ability to use agricultural waste materials as substrates.

Limitations include non-uniform colonization, difficulty in maintaining sterility in large-scale operations, and limited control over metabolite production. Many ECM producers operating in developing countries rely exclusively on SSF due to cost considerations.

5.2.2 Liquid Fermentation (Submerged Culture)

Liquid fermentation involves culturing ECM fungi in submerged conditions using bioreactors with controlled aeration, agitation, temperature, and pH. This technology provides superior environmental control compared to SSF, enabling consistent product quality and higher productivity per unit volume. Bioreactor systems range from small laboratory-scale bioreactors (5–10 L) to industrial scale (100–10,000 L). ECM fungi grow as mycelial pellets or dispersed hyphal networks in liquid medium, which can be harvested directly or concentrated through centrifugation. The resulting broth is suitable for liquid formulations or can be dried to produce mycelial aggregates. Advantages include precise environmental control, consistent inoculant quality, higher volumetric productivity, and compatibility with food-grade fermentation facilities.

Disadvantages include higher capital and operational costs, requirement for sophisticated equipment and expertise, and challenges associated with maintaining optimal conditions for slow-growing fungi.



5.2.3 Mycelial Inoculum Production and Propagation

Large-scale ECM production typically employs multi-stage propagation systems. Pure cultures are maintained in laboratory conditions and periodically transferred to shake flasks. Vigorous mycelial colonies from shake flasks serve as inoculum for small-scale bioreactors (pilot scale), which in turn provide inoculum for production-scale bioreactors. This cascading approach ensures genetic purity and consistent strain characteristics. Some producers maintain culture collections of elite strains, performing periodic quality assurance testing to verify strain identity and vigor. Preservation of culture collections through lyophilization (freeze-drying) or ultra-low temperature freezing ensures long-term genetic stability and allows rapid resumption of production if cultures become contaminated.

5.3 Post-Production Processing and Formulation

Following cultivation, fungal biomass undergoes processing to convert it into shelf-stable formulations. Processing steps vary based on production method and desired final formulation type.

5.3.1 Drying Technologies

Air Drying: Simple, low-cost method suitable for pot culture material. Bulk material is spread on screens and air-dried to 5–10% moisture content in controlled conditions. Drying time varies from 2–4 weeks depending on humidity and temperature. Drawback: Significant viability loss (30–50%) occurs during air drying.

Freeze-Drying (Lyophilization): Removes water by sublimation under low temperature and vacuum, preserving viability better than air drying. Process produces residual moisture content of 2–5%, ensuring long shelf-life. Cost is significantly higher, requiring expensive equipment. Viability retention typically exceeds 85%. Particularly suitable for high-value products.

Spray Drying: Liquid culture is atomized into a heated chamber, producing dried particles in seconds. Rapid drying preserves viability better than conventional air drying. Suitable for formulation of liquid inoculants. Equipment cost is moderate, though utility consumption (energy) is significant. Produces powders or fine granules depending on spray parameters.

5.3.2 Carrier Material Incorporation

Fungal biomass is mixed with carrier materials to produce uniform, stable formulations. Selection of carrier depends on desired formulation type and application method. Common carriers include peat (excellent moisture retention and fungal habitat properties), clay minerals (good viability retention), perlite (lightweight, good for granules), and bark products (agricultural waste utilization). For granular formulations, moistened fungal biomass is incorporated into granule matrices through coating or mixing processes. Carrier selection significantly influences formulation characteristics and product cost.

5.3.3 Packaging and Storage Conditioning

Final formulations are packaged in moisture-proof containers (foil pouches, plastic bottles, or bulk bags depending on market segment) with desiccant or nitrogen flushing to maintain low moisture and prevent oxidation. Packaging design influences shelf-life, convenience, and product cost. Bulk products destined for agricultural distributors typically use 20–50 kg bags, while retail products are packaged in 25–1000 g quantities. Labels must include strain designation, propagule concentration, expiration date, and application instructions. Proper storage conditions (typically 2–25°C depending on formulation) are critical for maintaining viability.

5.4 Production Economics and Scale Considerations

Production costs vary substantially based on production system, scale of operation, and desired product specifications. Pot culture systems for AM inoculants typically yield 10–50 grams of dried material per kilogram of substrate, translating to production costs of \$5–15 per kilogram of formulation (excluding packaging and distribution). Liquid fermentation systems for ECM fungi achieve higher yields (100–500 grams per liter of culture) with lower per-unit costs, but require high capital investment (\$500,000–\$2,000,000 for production-scale facilities). Small-scale producers (producing <10 tons annually) typically operate with margins of 30–50%, while large-scale producers (100 tons annually) achieve economies of scale with margins of 50–70%. Production of MCAs or highly processed formulations commands premium prices (2–5 times higher than simple powder formulations) reflecting increased processing costs.

Future improvements in production technology will likely focus on automation of pot-culture facilities, development of more efficient liquid fermentation systems for AM fungi, and integration of production with other bioprocess operations to reduce costs. Biotechnological advances such as adaptive evolution for culture optimization or synthetic biology approaches to enhance fungal productivity may enable further cost reductions and quality improvements.

6. Quality Control and Standardization

Standardization and quality control are fundamental to ensuring mycorrhizal inoculant efficacy and safety. Despite their importance, harmonized international standards remain limited. However, several protocols and frameworks have been developed.



6.1 Viability Assessment

Viability assessment measures the proportion of living fungal propagules. For AM fungi, spore viability can be determined through FDA/PI staining (fluorescein diacetate/propidium iodide), which distinguishes living from dead cells via fluorescence microscopy. For ECM fungi, viable mycelial biomass can be quantified through ergosterol extraction and HPLC analysis.

6.2 Propagule Density

Propagule density (number of fungal units per gram or milliliter) is a critical quality parameter. AM spore density is typically determined by wet sieving and decanting, followed by microscopic counting. Target densities vary by application but typically range from 100 to 10,000 spores per gram for AM inoculants.

6.3 Contamination Detection

Microbial contamination, particularly by saprophytic fungi and bacteria, can compromise inoculant efficacy and product safety. Plating on selective media, 16S rRNA gene sequencing, and ITS (internal transcribed spacer) sequencing are standard methods for detecting contamination.

6.4 Bioassays

Pot bioassays using susceptible host plants (e.g., maize, clover) are considered the gold standard for assessing inoculant efficacy. Plants are grown under sterilized conditions, and colonization rates and biomass accumulation are measured to verify functional effectiveness.

Table 3 recommend quality control parameters, analytical methods, and acceptance criteria for commercial mycorrhizal inoculants. Standardized evaluation of propagule viability, density, purity, colonization potential, shelf-life stability, and field efficacy is essential to ensure product quality, regulatory compliance, and consistent agronomic performance. Harmonization of these quality standards will facilitate commercialization and international trade of mycorrhizal biofertilizer products.

Table 3. Quality Standards and Quality Control Parameters for Mycorrhizal Inoculants

Quality Parameter	Assessment Method	Recommended/Acceptable Range	Significance
Spore Viability (%)	Fluorescein diacetate (FDA), Propidium iodide (PI) staining, germination assay	≥ 70–80% viable spores at expiry	Indicates survival and infectivity potential of fungal propagules
Propagule Density	Wet sieving and microscopic counting (AM fungi); colony count or biomass estimation (ECM fungi)	≥ 100–1000 infective propagules g ⁻¹ (minimum); premium products ≥ 5000 propagules g ⁻¹	Determines inoculation efficiency and product potency
Root Colonization Potential	Greenhouse bioassay using host plants	≥ 50–70% root colonization under standard test conditions	Measures biological effectiveness of the inoculant
Mycorrhizal Dependency Index	Pot bioassay and plant growth response evaluation	Significant increase in biomass or nutrient uptake over uninoculated control	Verifies functional performance of the product
Moisture Content (%)	Oven drying method (105°C)	≤ 8–10% for dry formulations	Prevents microbial contamination and viability loss during storage
Microbial Contamination Level	Selective culture media, microbial plating, molecular diagnostics (PCR, ITS sequencing)	Absence of pathogenic microorganisms; contaminant levels below regulatory limits	Ensures product safety and purity
Fungal Identity Verification	ITS sequencing, qPCR, DNA barcoding	≥ 95–99% genetic conformity with declared strain/species	Confirms authenticity of fungal inoculant
Shelf-Life Stability	Periodic viability and propagule assessment during storage	≥ 70% viability retained after declared storage period	Indicates formulation stability and commercial reliability
pH (Liquid Formulations)	Digital pH meter	5.5–7.5	Maintains propagule stability and formulation compatibility
Particle Size Distribution	Sieve analysis or laser diffraction	Uniform particle size according to formulation specifications	Ensures consistent application and distribution
Carrier Sterility/Purity	Microbiological testing	Free from harmful pathogens and excessive contaminants	Improves formulation quality and storage stability



Heavy Metal Content	ICP-OES or Atomic Absorption Spectroscopy	Within national biofertilizer regulatory limits	Ensures environmental and human safety
Storage Temperature Stability	Accelerated and real-time stability studies	Stable under recommended storage conditions (typically 4–25°C)	Predicts commercial shelf life and product performance
Nutrient Uptake Enhancement	Plant tissue nutrient analysis (P, N, Zn, Fe)	Significant improvement compared with untreated control	Demonstrates agronomic effectiveness
Field Efficacy Validation	Multi-location field trials	Consistent positive response across environments	Supports product registration and commercialization

Quality assurance is a critical component of mycorrhizal inoculant production because product efficacy is directly dependent on propagule viability, infectivity, and formulation stability. Internationally accepted standards for propagule density, microbial purity, shelf life, and colonization potential remain limited, resulting in substantial variation among commercial products. Adoption of standardized quality control protocols incorporating molecular identification, viability assessment, greenhouse bioassays, and field validation will improve product reliability and strengthen confidence among growers, regulators, and industry stakeholders. Such standardization is essential for expanding the global mycorrhizal inoculant market and ensuring consistent agronomic benefits.

7. APPLICATIONS IN AGRICULTURE AND HORTICULTURE

7.1 Food Crop Production

Mycorrhizal inoculants have demonstrated efficacy in enhancing productivity of major food crops including cereals (wheat, maize, rice), legumes (soybean, chickpea), and horticultural crops (vegetables, fruit trees). In phosphorus-limited soils typical of tropical and subtropical regions, AM inoculants can increase yield by 15–40% while reducing phosphate fertilizer requirements by 25–50%.

7.2 Horticultural Crops

Mycorrhizal inoculants are increasingly used in container-grown nursery production of ornamental plants and fruit trees. Inoculation enhances early-season growth, reduces nursery production time, and improves transplant quality and field establishment.

7.3 Agroforestry and Tree Planting

ECM inoculants are essential for forest nurseries and afforestation programs. Inoculation of tree seedlings (e.g., pine, oak, birch) dramatically improves field establishment, growth rates, and stress tolerance, particularly in harsh environments or degraded soils.

7.4 Stress Tolerance and Climate Resilience

Mycorrhizal associations enhance plant tolerance to drought, salinity, heat stress, and nutrient deficiencies. Inoculants are increasingly recognized as tools for climate-smart agriculture, enabling crop production in marginal lands and reducing irrigation requirements. This application is particularly relevant in water-scarce regions and for rainfed agriculture.

Table 4 represents commercial mycorrhizal inoculant products currently available worldwide, including major fungal species, formulation types, target applications, and distinguishing characteristics. Commercial products increasingly incorporate multi-species mycorrhizal consortia and beneficial microorganisms to improve nutrient-use efficiency, plant health, stress tolerance, and field performance across diverse agricultural and forestry systems.

Table 4. Representative Commercial Mycorrhizal Inoculant Products Available Worldwide and Their Agricultural Applications

Commercial Product	Manufacturer/ Company	Major Mycorrhizal Species	Formulation Type	Target Crops/Applications	Key Features
MycoApply® Series	Mycorrhizal Applications LLC	<i>Rhizophagus irregularis</i> , <i>Funneliformis mosseae</i> , <i>Claroideoglossum etunicatum</i>	Granule, Powder, Liquid	Row crops, vegetables, orchards, forestry	Broad-spectrum AM fungal consortium with multiple formulation options
Premier Tech AGTIV® Mycorrhizae	Premier Tech Growers and Consumers	<i>Rhizophagus irregularis</i>	Granular and Powder	Field crops, horticulture, greenhouse production	High spore concentration and extensive field validation
MYKE® Pro Series	Premier Tech Growers and Consumers	<i>Rhizophagus irregularis</i>	Granular and Water-Soluble	Vegetables, fruits, ornamentals, turfgrass	Easy application and enhanced phosphorus uptake



EndoMaxx®	BioWorks Inc.	Multiple AM fungal species	Granular	Nursery crops, landscape plants, ornamentals	Designed for transplant establishment and root development
Plant Success® Great White® Premium Mycorrhizae	Plant Success Organics	AM fungi plus beneficial bacteria and <i>Trichoderma</i> spp.	Powder	Horticulture, hydroponics, greenhouse crops	Multi-microbial consortium formulation
Rootella® Series	Groundwork BioAg	<i>Rhizophagus irregularis</i>	Granular, Seed Treatment	Corn, soybean, wheat, vegetables	Precision agriculture-compatible formulations
Rootella Carbon™	Groundwork BioAg	<i>Rhizophagus irregularis</i>	Seed Coating	Large-scale field crops	Focus on carbon sequestration and nutrient-use efficiency
Symbivit®	Symbiom s.r.o.	Six-species AM fungal consortium	Granular	Fruit trees, vineyards, vegetables, ornamentals	Multi-species inoculum for diverse environments
Ectovit®	Symbiom s.r.o.	Ectomycorrhizal fungi (<i>Pisolithus</i> , <i>Laccaria</i> , <i>Hebeloma</i>)	Granular	Forestry and tree nurseries	Specialized ECM inoculant for woody plants
Mycogold®	Mycorrhizal Applications LLC	Mixed AM fungal species	Powder and Granular	Agriculture, landscaping, ecological restoration	High propagule density and broad host compatibility
Rhizoponics® Mycorrhizae	Rhizoponics BV	<i>Rhizophagus irregularis</i>	Liquid and Granular	Hydroponics, greenhouse crops	Optimized for protected cultivation systems
Micosat®	CCS Aosta S.r.l.	AM fungi plus PGPR consortium	Powder and Granular	Horticultural and field crops	Integrated microbial biofertilizer platform

The global mycorrhizal inoculant market has expanded rapidly due to increasing demand for sustainable agricultural inputs and biological alternatives to synthetic fertilizers. Commercial products are available in powder, granular, liquid, seed-treatment, and encapsulated formulations, with arbuscular mycorrhizal fungi (AMF), particularly *Rhizophagus irregularis*, representing the most widely commercialized species. Recent trends emphasize the development of multi-species microbial consortia combining AM fungi with plant growth-promoting rhizobacteria (PGPR), *Trichoderma* spp., and other beneficial microorganisms to enhance formulation efficacy, environmental adaptability, and crop productivity. Such integrated biological products are expected to play an increasingly important role in climate-smart and regenerative agricultural systems.

8. CHALLENGES AND LIMITATIONS

8.1 Inconsistent Field Performance

One of the most significant barriers to mycorrhizal inoculant adoption is unpredictable field efficacy. Factors contributing to performance variability include soil conditions, indigenous mycorrhizal populations, crop genotype, climate, and agronomic practices. In soils with high background mycorrhizal populations, exogenous inoculation may provide minimal benefit.

8.2 Viability Loss During Storage

Most mycorrhizal inoculants experience gradual viability loss during storage, even under optimal conditions. Powder and granular formulations are susceptible to viability loss due to water loss and oxidative stress. Extended shelf-life (12 months) with acceptable viability retention (70%) remains challenging for AM inoculants.

8.3 Regulatory and Registration Issues

Regulatory frameworks for mycorrhizal inoculants vary widely among countries, creating barriers to international trade. Some countries require formal registration with stringent efficacy testing, while others lack clear guidelines. Harmonization of regulatory standards would facilitate product development and market expansion.



8.4 Cost and Economic Viability

The cost of mycorrhizal inoculants, particularly high-quality formulations, may exceed the economic benefit in some crop systems. Cost-benefit analysis depends on soil fertility status, crop value, and expected yield response. Broader adoption in low-input farming systems requires significant cost reduction.

9. FUTURE PERSPECTIVES AND RECOMMENDATIONS

9.1 Strain Selection and Genomics

Future inoculant development should prioritize selection of high-performing fungal strains through phenotypic characterization and genomic analysis. Application of high-throughput sequencing and genomic tools will enable identification of functional genes associated with beneficial traits (e.g., phosphate solubilization, stress tolerance, host compatibility). Strain improvement through crossing programs or synthetic biology approaches may yield superior inoculants.

9.2 Formulation Innovation

Innovation in formulation technology is essential for improving stability, shelf-life, and efficacy. Emerging approaches include encapsulation using biopolymers (chitosan, alginate), nanoparticle-based carriers, and functionalized granules incorporating bioactive compounds (e.g., secondary metabolites, bacterial biocontrols). These innovations may enhance inoculant durability and broaden application versatility.

9.3 Consortium Inoculants

Inoculants containing multiple fungal species or fungal-bacterial consortia may provide synergistic benefits and improved performance consistency. Co-inoculation of AM fungi with beneficial bacteria (phosphate-solubilizing bacteria, nitrogen-fixing bacteria) could enhance nutrient availability and create a more resilient inoculum.

9.4 Precision Agriculture Integration

Integration of mycorrhizal inoculants with precision agriculture technologies (variable-rate application, soil fertility mapping, decision-support systems) will optimize inoculation strategies and improve cost-benefit outcomes. Real-time monitoring of mycorrhizal colonization status using molecular or isotopic methods could enable adaptive management.

9.5 Regulatory Harmonization

Establishment of internationally harmonized standards for mycorrhizal inoculant quality, testing, and registration is critical for facilitating trade and fostering industry development. Organizations such as ISO (International Organization for Standardization) and FAO should prioritize development of standardized test protocols.

9.6 Climate-Smart Agriculture

Mycorrhizal inoculants represent a promising climate adaptation tool for enhancing crop resilience to drought, heat stress, and variable precipitation patterns. Research should prioritize selection of drought-tolerant fungal strains and optimization of inoculant strategies for rainfed and marginal agricultural systems.

CONCLUSION

Mycorrhizal formulations represent a scientifically sound and practical approach for enhancing plant nutrition, crop productivity, and agricultural sustainability. Decades of research have established the mechanisms of mycorrhizal symbiosis and validated the potential of inoculants across diverse crop systems and environments. Recent advances in formulation technology, strain selection, and application methods have substantially improved product quality and reliability.

However, several challenges remain: inconsistent field performance, viability loss during storage, variable regulatory frameworks, and product cost. Overcoming these challenges requires continued investment in research and development, including strain improvement programs, formulation innovation, and systematic validation of efficacy under diverse conditions.

The growing recognition of the need for sustainable agricultural intensification, combined with global concerns about climate change and resource scarcity, creates unprecedented opportunities for mycorrhizal inoculant adoption. Future success will depend on collaborative efforts among researchers, product developers, agricultural extension services, and policymakers to develop cost-effective, standardized products with demonstrated agronomic value. With continued innovation and systematic implementation, mycorrhizal formulations can make significant contributions to global food security, environmental conservation, and climate resilience.

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