



REVIEW ARTICLE

# Revolutionizing agriculture: Innovative bioinoculant formulation technologies for sustainability

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## Abstract

Microbes are essential for sustainable agriculture, helping to reduce dependency on harmful chemical fertilizers and pesticides. Researchers are actively seeking solutions as researchers become increasingly aware of the adverse effects of urbanization and population growth. Studies on plant disease management highlight the benefits of biocontrol agents and biofertilizers for enhancing plant growth and development alongside their delivery mechanisms. While laboratory formulations show potential, their effectiveness often diminishes in field applications due to a lack of understanding of delivery systems. A comprehensive examination of the entire bioformulation process-from isolating beneficial microorganisms to production-is necessary. Various bioformulations exist, each with distinct advantages and limitations. Innovative formulation strategies are being explored to extend shelf life and improve delivery efficiency, enhancing field productivity and reducing environmental impact. The interplay between bioformulation technology and precision agriculture further emphasizes opportunities for optimizing resource use and minimizing costs. A key challenge remains in developing advanced bioformulation technologies that yield environmentally safe, user-friendly products with optimal field performance, ultimately replacing harmful chemicals. This review critically evaluates the latest developments in formulation types, field efficacy and the factors hindering widespread adoption.

## Keywords

biocontrol; biofertilizer; formulations; microorganisms; PGPR

## Introduction

The extensive application of chemical fertilizers and pesticides to boost food production has led to numerous health risks for humans and animals while degrading agroecosystems (1). Conversely, microorganisms are vital in managing plant diseases, weeds, and pests that threaten crops and forestry. For example, certain fungi can colonize the aerial parts of plants, enhancing their resilience against drought, heat, insect infestations and various diseases (2). Recent studies indicate that biofertilizers significantly enhance the solubilization of essential minerals in the soil, improve nutrient uptake and facilitate the availability of micronutrients in forms more accessible to plants. The term "plant growth-promoting microorganisms" (PGPM) encompasses a range of beneficial organisms, including cyanobacteria, phosphobacteria, rhizobia and Azospirillum, which have been shown to enhance plant growth and crop yields across various regions (3). Microorganisms such as *Bacillus* sp.,

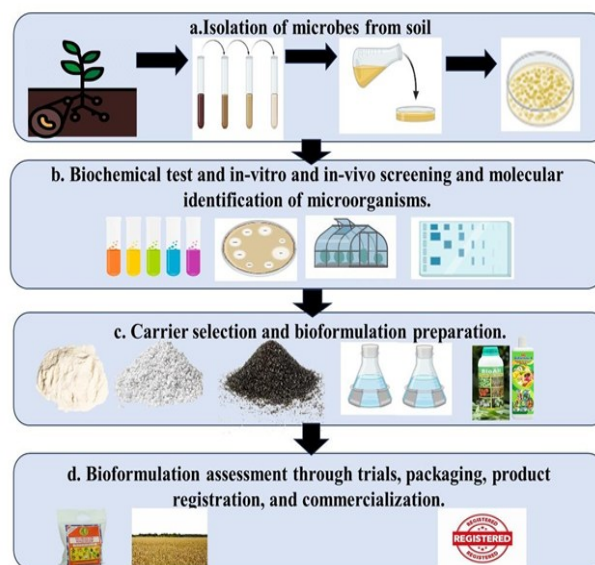
*Methylobacterium* sp., *Pseudomonas* sp., and Arbuscular Mycorrhizae (AM) are gaining recognition in agricultural practices due to their positive influence on plant development (4). Bioformulations cost-effective preparations containing beneficial microbes or their metabolites are employed to promote plant growth, enhance fertility and combat phytopathogens (5). These formulations often consist of active microbial ingredients combined with inert carriers such as diluents and surfactants, which help maintain the physical integrity of the microorganisms and boost their stability and biological efficacy (6).

The development of effective bioformulations necessitates the inclusion of viable microbes, spores, or their derivatives as active components alongside inert ingredients like peat, talc, or polymers such as xanthan gum (7). Without appropriate carriers, the viability of microbial populations can diminish rapidly after application to soil, limiting the establishment of a robust community of PGPM in the rhizosphere. This challenge is compounded by factors such as microbial biomass production, rhizosphere management complexities, and the physiological state of microorganisms at the time of application (8). Achieving a favourable plant response requires reaching a specific threshold of microbial cells, which varies by species. A primary objective of inoculant formulation is to create a conducive microenvironment that provides sustained physical protection for the introduced microorganisms, thereby preventing rapid population decline. On a field scale, these inoculants must reliably supply beneficial bacteria that can persist in the soil and be readily available to crops as needed (9). Research indicates that advancements in bioformulation technology must overcome several scientific challenges, including selecting superior strains to enhance crop diversity, ensuring microbial survival during seed coating and soil application and improving competitiveness against native rhizobacteria.

Furthermore, understanding environmental factors such as soil pH, nutrient deficiencies, salinity, temperature and pollution that affect nodulation, nitrogen fixation and biocontrol abilities is crucial. Addressing variability in efficacy across different locations and years and comprehending the complex interactions within rhizosphere environments are also essential for improving the consistency of field performance (10). Innovative technologies, including hydrogel-based, nano and encapsulated formulations, are emerging as effective alternatives in agriculture, often outperforming traditional formulations through enhanced slow-release capabilities and overall performance. This review explains the production process of various formulations, emphasizing their types, newer formulations, their field efficacy, their effectiveness over conventional formulations, the factors limiting widespread usage and the advanced technologies in bioformulations.

#### Bioformulation production process:

Commercializing a microbial formulation entails a series of steps, as shown in Fig. 1, which includes the isolation, identification and characterization of specific microorganisms and their biological functions, along with optimizing fermentation and storage conditions, designing the formulation, securing patents and ultimately launching the



**Fig 1:** Bioformulation production process.

a. Isolate beneficial microbes from soil. b. Conduct biochemical tests to identify microbial characteristics and assess capabilities in both lab and greenhouse conditions, including genetic identification. c. Select suitable carriers for microbial stabilization and mix them with selected microbes to create bioformulations. d. Assess bioformulations through trials, packaging, product registration and commercialization.

product. The process begins with isolating beneficial microorganisms such as bacteria, fungi, viruses, or nematodes that can disrupt the life cycles of plant pathogens or pests. Effective sampling is essential for enhancing the chances of discovering valuable strains, leading to the collection of samples from environments that indicate the presence of these microorganisms, such as decomposed arthropods, disease-suppressive soils, or healthy plants in outbreak areas. Successfully isolating these microorganisms in pure cultures necessitates the application of suitable cultivation techniques in synthetic media or through enrichment in biological systems (11).

Microbial formulations utilize a carrier-based approach that enables the retention of microorganisms with high survival rates and cell concentrations over extended periods. Carriers play a crucial role in the formulation process. Selected bacterial strains are stored in appropriate containers for prolonged durations and to enhance cell viability under conditions of osmotic stress, dehydration and elevated temperatures, the culture medium often includes protective agents such as glycerol, disaccharides, or amino acids. It is equally essential to determine optimal culture parameters such as dissolved oxygen levels, temperature, pH and mixing speed-as it is to select the suitable media. Maintaining 90% to 100% dissolved oxygen and a 25-28°C temperature range in liquid fermentation systems is essential for optimal bacterial growth. Furthermore, the chosen carrier should facilitate the controlled release of bioactive compounds tailored to specific applications, thereby improving bioavailability. Harvesting cells during the stationary phase is advantageous as it enhances their resilience to environmental stresses compared to the lag and exponential phases, which are associated with nutrient depletion and the accumulation of harmful metabolites (12).

## Types of Bioformulations:

Formulations can be broadly categorized into two main types, as illustrated in Fig. 2 & 3 and detailed with examples in Table 1: liquid and solid bioformulations. Liquid bioformulations include suspension concentrates, oil miscible flowable concentrates, ultra-low-volume suspensions and oil dispersions, as extensively described by (13). These liquid formulations offer several advantages over solid carrier formulations, such as higher cell counts, a reduced risk of contamination, an extended shelf life and enhanced virulence. It is important to note that microbial bio-agents remain dormant within liquid formulations, becoming activated upon application to the soil rhizosphere in the field. This characteristic helps extend the shelf life of liquid formulations, as noted by (14) and ensures optimal efficacy in agricultural applications.

In contrast, solid bioformulations include granules, micro granules, wettable powders, water-dispersible granules and dust, as highlighted by Jeet and Baldi (12). The carriers used for solid bioformulation delivery can vary widely, encompassing biological materials such as peat, charcoal, sawdust and rice husk, as well as inorganic options like aluminium silicate, clay, perlite, talc powder and vermiculite (13). To create these products, binders,

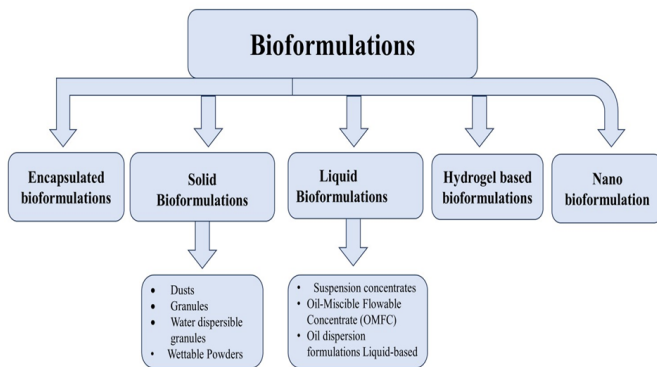


Fig 2: Types of bioformulations.

dispersants and wetting agents are mixed in (15). Various bioformulations are applied in farmers' fields to promote crop growth as biofertilizers, as indicated in Table 2.

## Conventional Bioformulations:

### Dusts:

Dusts, among the oldest types of formulations, consist of a finely ground mixture of the active ingredient (typically 10%) with particle sizes ranging from 50 to 100  $\mu\text{m}$ . Despite their long history of use, they can be more effective in certain applications for pest control (16). According to Merchant (17), dust can be applied to targets either manually or mechanically. Inert substances used in dust formulations may include anticaking agents, ultraviolet protectants and sticky materials to enhance adhesion. Typically, dust formulations contain 10% microorganisms by weight. Dust can be blended on-site with locally available carriers to facilitate transportation and storage. The production of dust involves sorbing an active ingredient onto a finely powdered solid inert material, such as talc,

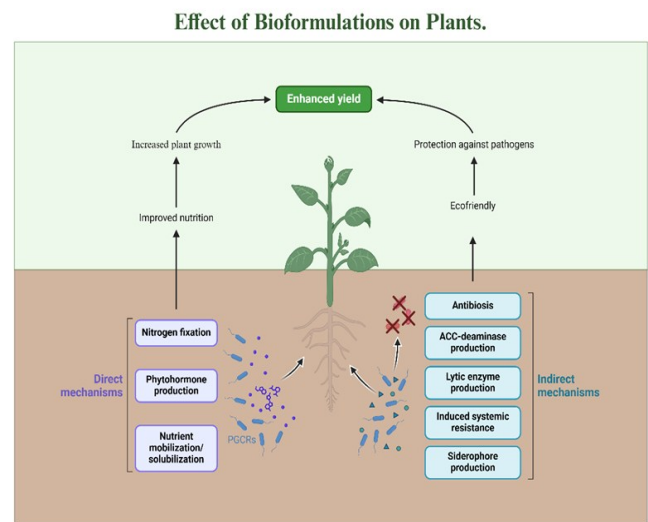


Fig 3: Effect of bioformulations on plants.

Table 1. Overview of bioformulation types, characteristics and examples

| Main Categories                | Subcategories                            | Characteristics   | Examples  | Reference |
|--------------------------------|--|---|---|-----------|
| Solid                          | Granular                                 | Dry particles containing non-dusty active substances (5-20%), with coarse particles (100-1000 $\mu\text{m}$ ).              | MET52® ( <i>M. anisopliae</i> var. <i>anisopliae</i> strain F52), Certis LLC ( <i>Trichoderma virens</i> ). | (70, 73)  |
|                                | Wettable Powder                          | Contains 15-45% filler, 1-10% dispersant, 3-5% surfactant and 50-80% powder.  | Trichox WP ( <i>Trichoderma harzianum</i> ).  | (5, 33)   |
|                                | Dust                                     | Active substance (typically 10%) is finely pulverized, with particles ranging from 50 to 100 $\mu\text{m}$ .                | Biofox C ( <i>Fusarium oxysporum</i> ).   | (16)      |
|                                | Water-Dispersible Granules               | Utilizes dispersion and wetting agents similar to wettable powders, often with a higher concentration of dispersing agents. | Contans WG ( <i>Coniothyrium minitans</i> ), AQ 10 WG ( <i>Ampelomyces quisqualis</i> AQ10).                | (35, 74)  |
| Liquid                         | Suspension Concentrate                   | Dust-free, quantifiable and easy to pour for spraying.  | Cerall ( <i>Pseudomonas chlororaphis</i> MA342).  | (40)      |
|                                | Oil Emulsion Formulations                | Suspension of active components in a water-insoluble solvent.   | DiTera.   | (29)      |
|                                | Oil-Miscible Flowable Concentrate (OMFC) | Water-dispersible and safe for use.   | BioOilFlow Pro ( <i>Trichoderma afroharzianum</i> T22 and <i>Azotobacter</i>                                | (29)      |
| Encapsulated Bioformulations   | Macro and Microencapsulation             | Microbial cells coated within a polymeric material, with macro beads (mm-cm) and microbeads (1-1000 $\mu\text{m}$ ).        | Molt-X.   | (75)      |
| Hydrogel-Based Bioformulations |  | Polymeric hydrogels with hydrophilic functional groups that absorb large amounts of water.                                  | BioGelPro, MicroGelBoost ( <i>Lactobacillus bulgaricus</i> ).   | (68)      |

**Table 2.** Overview of bioformulations, microorganisms and their functions

| Trade Name           | Microorganism Employed  | Assigned Function   | References |
|----------------------|---|---|------------|
| Inagro Bio Gold      | Cocktail of over 30 microbes: <i>Azotobacter chroococcum</i> + <i>Pseudomonas fluorescens</i> | Nitrogen fixation, drought resistance, disease resistance and bionematicide   | (84)       |
| Bamil and Omug       | <i>Bacillus</i> , <i>Micrococcus</i> , <i>Clavibacter</i> and symbiotic bacteria              | Promote plant growth and alleviate salt stress by providing minerals  | (85, 86)   |
| Rizotorphin          | <i>Rhizobium</i> sp.  | Colonizes root zones and stimulates plant growth through various mechanisms   | (87)       |
| JumpStart            | <i>Penicillium bilaii</i>   | Enhances phosphate availability for plants  | (84)       |
| Cell-Tech            | Nitrogen-fixing bacteria  | Provides nitrogen for plant use   | (84)       |
| BoveMax®             | <i>Beauveria bassiana</i>   | Controls Broca ( <i>Hedypates betulinus</i> ) infestations in Erva-mate plantations   | (84)       |
| CataPult Nodulest 10 | Two strains of <i>Bradyrhizobium japonicum</i> + Sphagnum peat moss                           | N <sub>2</sub> -fixing biofertilizers containing various beneficial bacteria, enhancing nutrient availability and stress mitigation | (88-90)    |
| Micofert             | <i>Glomus intraradices</i> , <i>G. etunicatum</i> , <i>Gigaspora</i> sp.                      | Increases height, shoot diameter, dry biomass and leaf area   | (88)       |
| FOSFORINA®           | <i>Pseudomonas fluorescens</i>  | Promotes plant growth and mitigates stress factors  | (91)       |
| BioAgri              | <i>Pseudomonas chlororaphis</i> strains   | Controls seed-borne diseases in grains  | (84)       |
| TagTeam              | Combination of rhizobia strains with <i>Penicillium bilaii</i>                                | Multi-action inoculant for legumes, improving phosphate utilization and nitrogen fixation   | (84)       |
| Biopower             | Multi-strain nitrogen-fixing bacteria   | Facilitates nitrogen fixation in plants   | (92)       |
| Nitragin Gold®       | <i>Rhizobia</i> strains   | Achieves high levels of nitrogen fixation for optimal yield   | (84)       |

clay, or chalk, with particle sizes between 50-100 µm. While finer particles adhere better to surfaces, they also increase the user's inhalation risk and potential drift during application. Key factors in controlling dust formulations include particle size (0.5-50 µm), bulk density (0.5-0.6 g/cm<sup>3</sup>) and flowability. Smaller particles accumulate on the target during application, but larger surfaces may not retain them effectively. Consequently, desiccants to prevent caking and stickers to enhance adhesion are commonly utilized.

#### Granules:

Granules are primarily produced through wet granulation processes. To achieve the desired particle size, a mixture of a powder carrier and a microbiological solution sometimes modified with an adhesive, is combined and the resulting matrix is sheared or extruded through a sieve. After this, the granules are air-dried in conventional ovens or fluidized bed dryers, with the option of granulating before drying in fluidized beds by spraying biocontrol chemicals into the moving mass of carriers while hot air dries the agglomerates. An alternative method known as dry granulation, or briquetting, involves using roller compactors to integrate microorganisms into a powder mixture and press them (18). According to McQuilken *et al.*, (19), granules can be classified into two categories based on particle size: coarse particles (100-1000 µm) and micro granules (100-600 µm). For effective release of the active ingredient, granules should disintegrate in the soil while remaining free-flowing, non-dusty and non-caking. Primarily used for soil treatment, granular formulations are generally safer as they pose no inhalation risks. They focus on achieving longer shelf life and storage stability. Although granular formulations are effective, their application can be limited due to the deactivation of the active ingredient by ultraviolet radiation. To mitigate this, certain UV protectants such as folic acid, uric acid and various dyes can be incorporated into the formulation or applied as coatings to counteract UV inactivation of microorganisms. Granular inoculants are less dusty and easier to handle, store and apply (20-22).

#### Liquid-Based Bioformulations:

Liquid bioformulations consist of microbial cultures treated with water, oil, or polymers (i.e., additives) that enhance cell suspensions' viscosity, stability and dispersion capacity (23-25). Pindi (26) defines such preparations as those that meet the requirements for preserving organisms and delivering them to target regions to enhance their biological activity, or as a consortium of microorganisms supplied with a suitable medium to maintain viability over a specific period, thereby improving the biological activity at the target site. Liquid biofertilizers contain beneficial microbes that fix, solubilize, or mobilize plant nutrients through biological activity (27). A key advantage of liquid inoculants is their compatibility with advanced sowing equipment and farming systems. These formulations can be produced through straightforward fermentation, aseptically packed directly from the fermenter, treated with suitable osmoprotectants and stored for extended periods without losing viability (28).

In a previous study Rai *et al.* (29) describe the sequential process of developing liquid bioformulations, which begins with identifying potential microorganisms, then screening and characterizing their metabolites and bioactive compounds. Once an aqueous medium such as mineral oils, water, or organic oils is selected, various surfactants and additives are incorporated to enhance stability. Stability assessments across different crops are conducted to ensure efficacy (30). Liquid formulations available in the market include Suspension Concentrates (SCs), Oil Miscible Flowable Concentrates (OF), Ultralow Volume (ULV) Suspensions (SU) and Oil Dispersions (OD). In these formulations, microbial organisms exist in a dormant cyst form, which reactivates upon application in the field, thereby extending the shelf life of liquid bioformulations to over a year (31). The primary benefits of liquid inoculants include (a) ease of handling, (b) the ability to incorporate a variety of nutrients such as cell protectants and additives that promote cell/spore/cyst formation, enhancing their efficacy, (c) superior protection against environmental stresses and (d) higher field efficacy compared to peat-based inoculants (32).



## Newer Bioformulations:

### Wettable Powders:

Wettable powders (WPs) represent one of the oldest types of formulations, consisting of 50-80% technical powder, 15-45% filler and 1-10% dispersant, alongside 3-5% surfactant by weight (33). Bejarano and Puopolo (18) explain that microorganisms can be blended with finely ground carriers and adjuvants to create a homogeneous mixture, or granules can be crushed into a fine powder. This process can be achieved using milling machines, either mechanically or manually, while alternative methods like spray drying and lyophilization are also employed. The ideal form for maximizing the shelf life and efficacy of the active material in powder formulations is spore-based, particularly from gram-positive microorganisms that produce spores resistant to heat and desiccation, facilitating the creation of stable, dry powder products (34). These formulations are particularly appealing due to their ease of miscibility with water, allowing for straightforward application (35). In a recent study, a WP formulation containing 60% freeze-dried *Bacillus cereus*, 28.9% diatomite as a carrier and 4% sodium lignin sulfonate as a dispersant was shown to effectively biocontrol postharvest diseases compared to chemical alternatives. This formulation also included 6% alkyl naphthalene sulfonate as a wetting agent, 1%  $K_2HPO_4$  as a stabilizer and 0.1%  $\beta$ -cyclodextrins as a UV protectant (36).

### Water-Dispersible Granules:

Water dispersible granules (WDGs), also known as dry flowables, consist of fine particles of active ingredients and can contain up to 90% active components by weight. Unlike wettable powders or suspension concentrates, WDG formulations generate less dust, facilitating safer measurement and mixing, thereby reducing inhalation risks for applicators. The development of WDG formulations for *Bacillus thuringiensis* (Bt), particularly *Bt* var. *israelensis* and *B. sphaericus*, focuses on mosquito management (36). Additionally, WDG formulations of the antagonist fungus *Ampelomyces quisqualis* have been reported for biocontrol of powdery mildew (37). These granules are effective against nematodes and have a long shelf life. For instance, water-soluble granules containing *Bacillus megaterium* have been formulated for biological control of rice sheath blight, demonstrating excellent water solubility and suitable viscosity for spray application (38). Tadros (39) found that the WDG formulation is comparable to the commercial *Btk* formulation Delfin, showing promise for controlling older larvae, as opposed to the conventional focus on early instar larvae. Furthermore, this formulation maintains viability and effectiveness even after 24 months at room temperature and meets the physicochemical standards set by the Collaborative International Pesticides Analytical Council Limited (CIPAC).

### Suspension Concentrates:

Suspension concentrates (SCs) are created by blending solid active ingredients that are poorly soluble in water and resistant to hydrolysis (39). This formulation is dust-free, easy to measure and simple to pour for spraying. SCs require dilution in water before application and incorporating

surfactants and other additives can improve their solubility and storage stability. Farmers prefer SCs over wettable powders due to their lower dustiness and easier handling (35). For example, *Pseudomonas fluorescens* can be cultivated in coconut water with glycerol or polyvinylpyrrolidone (PVP) to create effective suspensions (41).

### Oil-Miscible Flowable Concentrate (OMFC):

Oil-miscible flowable concentrates (OFs) are stable liquid suspensions that can be dispersed in organic solvents before application (17). They are water-dispersible, enhancing safety and ease of use while minimizing dust issues. OFs promote excellent seed retention and facilitate straightforward cleaning of seed treatment equipment. However, extreme temperature fluctuations may compromise their storage stability, which can affect viscosity and efficacy. High concentrations of active ingredients might lead to seed stickiness and poor flow properties, potentially resulting in uneven distribution and reduced effectiveness of seed treatments (29). Overall, OMFCs provide a user-friendly and effective solution for seed protection, contributing to enhanced agricultural yields.

### Oil Dispersion Formulations

Oil Dispersion (OD) formulations involve the stable suspension of active ingredients in a non-aqueous phase, typically an oil. The oil can range from vegetable oils to methylated seed oils and may include paraffinic or aromatic solvents. Plant-based oils are often favoured due to their retention, spread and penetration benefits. OD formulations are particularly useful for delivering water-sensitive chemicals and utilize adjuvant fluids instead of water, potentially enhancing pest control effectiveness (29).

Emulsions, formed by mixing immiscible liquids with emulsifying agents, can be categorized as oil-in-water (O/W) or water-in-oil (W/O), the latter also known as invert emulsions. Typically, these formulations contain microorganism concentrations between 10% and 40% (33). In an OD formulation, the active ingredient is ground within an oil phase rather than a water phase, as seen in suspension concentrates (SC). To create these suspensions, oil-compatible dispersants, thickeners and emulsifiers are employed (42). Invert emulsions require specialized emulsifiers to incorporate water-soluble bioagents into an oil carrier with a high oil content. Since oil evaporates more slowly than water, droplets in invert emulsions experience less shrinkage, allowing more particles to reach target areas. This characteristic helps reduce runoff and enhance resistance to rain, minimizing drift (43).

Research indicates that 15 emulsifiers were tested at 1% and 2% concentrations to blend the oil and aqueous phases. Tween 20 effectively formed a thick interfacial layer between the phases. The aqueous and oil phases were mixed separately for a water-in-oil formulation before combining in a 50:50 ratio. Oil-based products are preferred for foliar applications and have been shown to enhance the efficacy of entomopathogens (45). A study by Peeran and Nagendran (46) demonstrated that a water-in-oil emulsion of *Pseudomonas fluorescens* (FP7) effectively reduced disease incidence pre- and post-harvest by bolstering plant defences. The development of cost-effective, accessible resources is

vital for formulating biocontrol agents, with fungal formulations in oils showing superior infectivity compared to water suspensions, as illustrated by the successful management of powdery mildew using *Verticillium lecanii* combined with arachnid oil (47).

### **Encapsulated Bioformulations**

John and Tyagi (48) highlight that traditional solid and liquid formulations often struggle with microbial viability during storage and field application due to a lack of understanding regarding optimal carriers. Encapsulation offers a promising solution by immobilizing microorganisms, extending their shelf life and enhancing field efficacy. This method is increasingly adopted to promote agricultural sustainability as the demand for bioinoculants rises. Conventional liquid or powder inoculants frequently fail to protect microbial strains against abiotic stresses such as temperature, humidity and UV radiation. Using polymers for encapsulation significantly improves microbial survival and resilience (49). Bacterial encapsulation ensures gradual release and protection of microbial cells in soil, with varying degradation rates of encapsulation matrices influencing the biological activity of soil microorganisms. These matrices can also support bacterial metabolic growth, facilitating plant development.

Encapsulation provides several advantages over traditional peat inoculants, including controlled bacterial release, protection from environmental stresses and reduced contamination risk during storage (50). The core principle of microbial immobilization involves trapping live microorganisms within a polymeric matrix while preserving their vitality. Following fermentation, the encapsulated products (bacteria-polymer) can be utilized in various industrial applications, including producing organic acids, enzymes and bioremediation. The straightforward production, storage and management of immobilized microbial cells make this method appealing.

Encapsulation typically involves three stages: incorporating the microorganism into a polymeric matrix, dispersion under agitation to form solid particles and subsequent polymerization and stabilization. This process can be categorized into macro-, micro- and nanoencapsulation, depending on particle size (ranging from nanometers to millimetres). Saxena (51) emphasizes that encapsulation is a leading method for manufacturing inoculants, offering significant advantages over other techniques. Encapsulated cells are safeguarded within nutrient-rich capsules, protecting them from mechanical and environmental stresses such as pH changes and predation. Benefits of encapsulation include non-toxicity, biodegradability, ease of handling, prevention of mechanical damage, gradual release for effective root colonization and cost-effectiveness (52).

### **Alginate-Based Formulations**

Alginate is a widely used material for encapsulating microorganisms due to its versatility in various applications, including the immobilization of cellular organisms and enzymes, the deployment of biological control agents and mycoherbicides and the enhancement of recombinant plasmid stability in host cells (53). Creating alginate beads containing bacteria is straightforward, requiring minimal

chemicals and equipment, contributing to its popularity in research (54). Key advantages of alginate formulations include their nontoxic nature, natural biodegradability, cost-effectiveness and the ability to control the gradual release of microorganisms into the soil, governed by the polymer's structure (55).

However, the use of macro-alginate beads as agricultural inoculants presents challenges. First, additional treatment during sowing is necessary, which may deter time-constrained growers in developed countries and discourage farmers in developing nations due to limited agricultural education and skepticism toward new technologies. Second, the bacteria released from these inoculants must navigate the soil to reach plants. When beads are mixed with seeds and sown, they can disperse several centimetres away from the seeds, making it difficult for bacteria to migrate through the soil, where they face competition from native microflora and potential mobility obstacles due to insufficient moisture (56). The effectiveness of alginate as an immobilizing agent relies on its ability to form stable gels set at room temperature with minimal conditions (57).

### **Nano Formulations**

Recently, nanobiotechnology has emerged as a crucial tool in agriculture, aimed at enhancing growth and productivity by developing ultra-small particles with unique physicochemical properties and a high surface area-to-volume ratio. This multidisciplinary approach is considered one of the most revolutionary technologies of our time (58). Nanotechnology-based formulations are promising for improving pesticide performance and safety compared to traditional methods (59). Characterized by gradual release profiles, nanoformulations effectively prevent pathogen attacks and diseases, particularly during the early growth stages of crops, thereby maintaining pathogen populations below threshold levels (60-62).

Nano fungicide formulations enhance the solubility and targeted release of active chemicals, improving the bioavailability of agrochemicals with low water solubility (62). Furthermore, nanotechnology offers environmentally friendly pest control options with minimal ecological impact. Nano-biopesticides are characterized by small size, high surface area, durability and low toxicity, showing promise in revolutionizing global agriculture, particularly in food protection. These formulations can also mitigate the toxicity of chemical fertilizers and play a key role in developing intelligent nano-systems that address agricultural challenges related to environmental impact and nutrient management (64).

### **Hydrogel-Based Bioformulations**

Hydrogels (HGs) are functional polymers capable of absorbing and retaining large amounts of water, making them useful in various fields, including agriculture. Hydrogels can reduce water usage in agricultural applications, lower plant mortality, enhance nutrient retention and promote plant growth. Biopolymers are commonly used for hydrogel fabrication, offering advantages over synthetic polymers regarding safety and environmental impact (65).

For effective cell immobilization, hydrogels must be biocompatible, allowing for the transport of nutrients and oxygen and maintaining stability under varying conditions

(66). Next-generation bioformulations must consider formula design, storage stability and dynamic release profiles. Techniques developed for hydrogels can also be adapted for traditional formulations like wettable powders. Assessing the viability and stability of encapsulated biocontrol agents in hydrogel beads offers advantages, such as ease of adaptation for other microorganisms. However, adjustments for factors like incubation duration and pH may be necessary (67). Hydrogels can effectively immobilize *Rhizobium*, a vital nitrogen-fixing bacterium, preserving its catalytic activity while preventing free movement. These polymer networks, characterized by hydrophilic functional groups, are widely applied across medicine and agriculture for their exceptional water absorption capabilities (68). Research by Kadry and El-Gawad (69) focused on chemically treating rice straw to create novel hydrogels, including cellulose and cellulose/acrylic acid variants, characterized by improved swelling ratios and water retention capabilities. These newly developed hydrogels serve as effective soil additives.

### Comparison of Conventional and Newer Bioformulations

According to (70), undiluted and dry dust products are less favoured in applications than sprays due to handling difficulties and lower efficiency. In contrast, water-dispersible granules (WDG) dissolve quickly in water, minimizing dust and offering environmental advantages. However, they typically contain a higher concentration of dispersing agents than water-soluble granules (WG) (7). Liquid formulations facilitate rapid interaction between microorganisms and target plants, providing therapeutic effects. However, these formulations are susceptible to contamination and pose challenges for long-term storage (71). Inversion emulsion formulations are beneficial as their droplets shrink less than water droplets, allowing more particles to reach their targets, which reduces runoff and enhances rain resistance while minimizing drift (43).

Traditional solid and liquid formulations often face challenges regarding microbial viability during storage and field application, mainly due to insufficient understanding of optimal carriers. Microencapsulation is emerging as a solution to extend shelf life and enhance the controlled release of microorganisms, improving application efficacy (48). Advances in inoculant technology focus on enhancing quality, increasing shelf life and developing innovative formulations suitable for challenging environments. Alginate-based granular formulations present an alternative to peat and lignite-based inoculants, highlighting the demand for novel solid carrier formulations (72).

### Shelf Life

The shelf life of microbial formulations is crucial for successful commercialization. The inoculant industry faces challenges in delivering user-friendly, affordable formulations for farmers, ensuring long shelf life and viability in rhizosphere environments (1). Ideally, biocontrol products should have a shelf life of 8-12 months for industrial applications. Research should prioritize developing superior strains and organic formulations with long shelf lives and low contamination risks (76). Adequate

storage of bioformulations requires specialized knowledge and equipment, which many farmers and producers may lack. Proper production, packaging, storage and transportation processes are essential to maintain shelf life. Air drying and lyophilization have mitigated storage issues (77). Chakraborty (78) notes that the shelf life of formulations varies based on bacterial type and carrier substances. Factors influencing bacterial survival include cultivation methods, harvesting timing, physiological state, dehydration rate and technology used (79). Standard methods for assessing viable cell counts include plate counts and most probable number counts, while cell physiology can be evaluated using biochemical assays, immunological tests and optical techniques (18).

### Effects of Newer Bioformulations

Nano-fertilizers have demonstrated significant advantages over traditional fertilizers, including selectivity, reduced toxicity, and controlled nutrient release. Various methods, such as absorption, ligand-mediated attachment, encapsulation and entrapment, enable the loading of nutrients onto nanoparticles (59, 80, 81). In advanced "smart" farming, nano-formulations integrate nanoparticles with biofertilizers to create nano-biofertilizers (NBFs), enhancing plant growth and resilience against external stressors. Microbial production facilitates the incorporation of metallic nanoparticles, polysaccharides and chitosan into formulations, such as a nano-bio formulation of etofenprox, to improve insecticide effectiveness (82).

Next-generation bioformulations necessitate a thorough understanding of component design, storage stability, dynamic release and particle size interactions, applicable to innovative (e.g., microencapsulation) and traditional formulations (e.g., wettable powders and dispersible granules). Evaluating the viability, storage stability, *in vitro* release and particle size of encapsulated biocontrol fungi in hydrogel beads presents advantages and challenges (67). The bioactive antifungal *Chaetomium cupreum* has shown improved efficacy in nanoformulations combined with tebuconazole, outperforming spore and traditional bioformulations in reducing leaf spot disease in rice field tests (83).

Suspension concentrates (SCs), being water-based, offer numerous benefits, including improved safety and user convenience, while addressing concerns related to dust and flammable liquids. They are particularly suitable for active ingredients with low water solubility, where smaller particle sizes enhance dispersibility and built-in adjuvants facilitate bio-enhancement (41).

Key benefits of bio formulations include:

- **Nutrient Acquisition:** Plants' enhanced uptake of essential nutrients like nitrogen, phosphorus and potassium.
- **Biocontrol:** Microorganisms or metabolites that inhibit pathogen growth or induce systemic resistance in plants.
- **Stress Management:** Improved plant tolerance to environmental stresses such as drought and salinity.



●**Eco-Friendliness:** Lower environmental impact due to their natural and biodegradable origins.

●**Growth Promotion:** Enhancement of plant growth.

**Phytoremediation:** Ability to rehabilitate contaminated environments.

### Current Scenario/Market Trends

In India, around 151 biofertilizer manufacturing units are managed by both government and non-government organizations (93).

A significant obstacle to adoption is the absence of standardized terminology for formulated products. Both "biofertilizers" and "bioinoculants" employ live microorganisms and their metabolites to boost plant growth and nutrient uptake. Developed nations commonly use biofertilizers and bioinsecticides to enhance crop yields. A survey reveals that the biostimulant industry is primarily concentrated in Europe, with an anticipated annual growth rate of 12%, expected to reach \$2.241 billion by 2018 (94). The United States currently possesses the largest biopesticide market, followed by Europe. According to a 2011 report by Industrial Equipment News, the biopesticide market was experiencing an annual average growth rate of 15.0%, with sales in the Asia-Pacific region projected to hit \$362 million by 2012.

Conversely, Latin America exhibited the slowest growth, with its market rising from \$70 million in 2005 to \$88 million in 2010, reflecting an average annual growth rate of 5.0%. The global biofertilizers market was valued at \$1.5 billion in 2022 and is expected to grow at a compound annual growth rate (CAGR) of 12.8% from 2023 to 2030. This growth highlights the potential for sustainable agriculture and food safety, driven by rising concerns about food safety. The European Union's "Common Agricultural Policy" encourages using bio-based products and organic farming by offering up to 30% of the budget in direct payments to farmers who implement sustainable practices. A supportive regulatory landscape, particularly in North America and Europe, is anticipated to propel market growth further (Source: <http://www.grandviewresearch.com/industry-analysis/biofertilizers-industry>).

### Challenges and future perspective:

The earth's temperature is rising due to greenhouse gas emissions, which also upset environmental stability and create several stressful situations that impact natural systems and agriculture (95). In response to these challenges, sustainable agricultural practices and other methods are employed to meet demand without adversely affecting natural ecosystems. Achieving this requires balancing three main interacting domains: the environment, society and economy. The importance of maintaining equilibrium among these three domains becomes evident in sustainable development approaches (96). Increased industrial growth has led to unprecedented levels of contamination. However, the gradual efficacy of beneficial microbes in agroecosystems indicates that achieving sustainable agriculture is feasible. The more significant challenge lies in developing advanced

bioformulation techniques that produce environmentally friendly and user-friendly products while delivering excellent field performance, thus replacing harmful chemicals. Moreover, businesses worldwide strive to reduce manufacturing costs, which is expected to incentivize farmers to opt for microbe-based products as viable alternatives for crop production (35).

Unforeseeable climatic conditions present a considerable challenge for bioformulation technologies. This challenge is particularly daunting for researchers in semi-arid regions and developing countries like India, as it heightens the uncertainty regarding performance. In low-input agriculture, farmers face heightened difficulties and lack the resources to retry if biofertilizers fail to yield desired results, given the high costs and technical expertise required. In semi-arid environments, introduced bacteria may struggle to thrive due to extreme environmental conditions such as drought, insufficient irrigation, high salinity and soil erosion (9). The inoculant industry faces the problem of creating a formulation that meets all criteria while still being suitable for field application. Transforming a promising microorganism into a commercial product capable of generating consistent results across diverse field settings is challenging (97). Hynes and Boyetchko (98) Research projects introduced in the field of formulation development, which combines art and science, have addressed several challenges hindering the advancement of simulation technologies. These challenges include the scarcity of existing formulation technology and the complexity of the registration process itself. The current outlook for the development and utilization of bioformulations is unsatisfactory.

Although the commercialization of biocontrol products is increasing globally, their penetration in the agricultural sector remains limited, particularly in the European Union, where registration procedures are complex and time-consuming. Biopesticide regulations vary nationwide, and registration processes often resemble those for chemical compounds. Global harmonization of biopesticide legislation could alleviate constraints and foster the commercialization of microbial-based insecticides worldwide. Various global bodies, such as the Organization for Economic Co-operation and Development, the International Organization for Biological Control and EPPO, have made significant efforts towards harmonization. However, their success has been limited(18). The future of bioformulations is bright. As people become more aware of the harmful impacts of chemical formulations on their health and the environment, they are more likely to accept them. Governments and regulatory bodies should enforce these formulations in countries where they are not widely used by farmers (84).

### Conclusion

Bioformulations represent a promising avenue for sustainable agriculture, enhancing soil health, crop productivity and environmental sustainability. They offer diverse solutions, from microbial inoculants to organic



amendments, which can reduce reliance on synthetic chemicals and promote ecological balance. However, standardization, scalability and regulatory hurdles hinder their widespread adoption. Overcoming these obstacles will require collaboration among researchers, policymakers and industry stakeholders to improve production processes and establish quality control. Despite these challenges, bioformulations hold transformative potential for resilient agricultural systems and mitigating environmental impacts. Embracing advanced formulation technologies will be crucial for realizing their role in sustainable agriculture and global food production.

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## Authors' contributions

All the authors have contributed to the writing and revising of the manuscript. All authors have revised and confirmed the results

## Compliance with ethical standards

**Conflict of interest:** Authors declare no conflicts of interest.

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