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Nano-Biofertilizers Application as Sustainable Approach to Enhance Crop Productivity and Soil Health: A Review

Oke Kingsley Oyediran

Department of Biology, Faculty of Science Education, Emmanuel Alayande University of Education, Oyo. Oyo State, Nigeria

Ogunmola Oluranti Olagoke

Department of Chemistry, Faculty of Science Education, Emmanuel Alayande University of Education, Oyo. Oyo State, Nigeria

Sodamade Abiodun

Department of Chemistry, Faculty of Science Education, Emmanuel Alayande University of Education, Oyo. Oyo State, Nigeria

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Abstract: As sustainable agriculture gains momentum, nano-biofertilizers have emerged as a promising alternative to chemical fertilizers. These formulations combine beneficial microbes with nanotechnology to boost nutrient availability, improve plant nutrient uptake, and enhance soil health. This review examines their composition, mechanisms, and agricultural benefits. Nano-biofertilizers improve nutrient use efficiency, support plant growth, and help suppress soil-borne pathogens through the synergistic effects of microbes and nanoscale carriers. They also reduce nutrient losses via leaching and volatilization, lowering environmental pollution and greenhouse gas emissions. Furthermore, they promote microbial diversity and improve soil structure—key factors in soil fertility. Despite their advantages, challenges such as production costs, regulatory gaps, and limited field data hinder widespread use. Addressing these issues through coordinated research and supportive policies is essential. Ultimately, nano-biofertilizers represent a sustainable tool to enhance crop productivity and environmental health, with great potential for integration into organic and eco-friendly farming systems.

Keywords: nano-biofertilizers, nanotechnology, nanoparticles, sustainability, rhizobacteria

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INTRODUCTION

The global agriculture industry is grappling with serious challenges, including worsening soil quality, reduced crop yields, and the negative environmental impact of overusing chemical fertilizers (FAO, 2022). While traditional fertilizers have helped increase food production, they also lead to groundwater contamination, greenhouse gas emissions, and the depletion of soil biodiversity (Zhang *et al.*, 2023). In light of these issues, innovative and sustainable solutions like nano-biofertilizers—combining nanotechnology with bio-based fertilizers—have gained attention for their ability to improve nutrient efficiency and support ecological sustainability (Rai *et al.*, 2023).

Nano-biofertilizers are composed of beneficial plant-growth-promoting microbes (PGPMs); including species like Azotobacter, Rhizobium, and Pseudomonas—integrated with nanomaterials such as chitosan, silica, or zinc oxide nanoparticles (Liu *et al.*, 2023). This next-generation delivery system enables more efficient nutrient release, boosts microbial viability, and enhances nutrient uptake by plant roots, cutting down the reliance on chemical fertilizers by as much as 40% (Adhikari *et al.*, 2023). In addition to improving nutrient efficiency, these formulations help plants withstand environmental stresses like drought and salinity by stimulating antioxidant mechanisms (Rizwan *et al.*, 2024). They serve a dual purpose by rejuvenating soil health (Bhardwaj *et al.*, 2024) and improving crop productivity, as evidenced by increased soybean yields and greater nitrogen absorption in wheat (Meena *et al.*, 2023).

Nevertheless, the widespread adoption of nano-biofertilizers is limited by several challenges, including high manufacturing costs, unclear regulatory frameworks, and potential long-term environmental toxicity associated with nanoparticles (Gogos *et al.*, 2023). Despite these concerns, nano-biofertilizers support the objectives of the United Nations Sustainable Development Goals—especially SDG 2 (Zero Hunger) and SDG 12 (Responsible Consumption and Production)—by advancing climate-resilient and sustainable agricultural practices (UNDP, 2023). This review examines the mechanisms underlying nano-biofertilizer-crop interactions, comparing their effectiveness with conventional fertilizers. It identifies suitable microbial strains for nano-biofertilizers and their crop-specific applications, including optimal application methods and timing. The analysis covers appropriate nanoparticle materials for biofertilizer formulations and their synthesis processes. Additionally, the review critically assesses the limitations and challenges in nano-biofertilizer implementation while highlighting their transformative potential for sustainable agriculture and proposing solutions to current constraints.

Mechanisms Underlying Nano-Biofertilizer-Crop Interactions

Nano-biofertilizers engage with plants through an advanced integration of nanotechnology and microbial processes, improving nutrient availability through three primary pathways: physical absorption, biochemical communication, and genetic modulation—all while reducing ecological harm (Liu and Lal, 2015).

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Physical Dimension of Interactions

The nanoscale size (1-100 nm) of these innovative fertilizers offers distinct benefits in nutrient delivery and plant accessibility. Due to their tiny dimensions, nanoparticles can penetrate various plant entry points, including stomata (typically 3–10 µm wide), hydathodes, and the cell wall's polysaccharide network (Servin *et al.*, 2015). This high level of accessibility is further enhanced by their exceptionally large surface area-to-volume ratio, which increases contact with root surfaces by nearly 100 times compared to conventional fertilizers (Raliya *et al.*, 2016). Additionally, the engineered porosity of nano-carriers allows for gradual nutrient release, ensuring sustained interaction with plant roots throughout key growth stages.

The physical uptake of nano-biofertilizers is highly size-dependent, with nanoparticles in the 20– 50 nm range achieving optimal root penetration while minimizing the risk of cellular damage (Avellan *et al.*, 2019). Additionally, surface charge—or zeta potential—plays a crucial role in their movement through the rhizosphere; particles with moderately negative charges (-15 to -30 mV) tend to disperse more evenly and adhere effectively to root surfaces (Kah *et al.*, 2018). Together, these physicochemical traits govern how nano-biofertilizers are distributed and retained within the plant–soil interface.

Biochemical Communication Networks

The biochemical interaction between nano-biofertilizers and plants involves complex signaling mechanisms and metabolic exchanges. Microbial consortia encapsulated within nanocarriers—especially plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF)—communicate chemically with plant roots by releasing various metabolites (Bhardwaj *et al.*, 2022). In turn, root exudates such as flavonoids, strigolactones, and organic acids act as chemo attractants, directing microbial colonization. A prime example of this mutualistic exchange is the AMF–plant partnership, where fungal networks receive roughly 20% of the plant's photosynthetically derived carbon in return for supplying up to 80% of its phosphorus needs (Smith and Read, 2008).

Nano-encapsulation acts as a safeguard for microbial interactions, protecting beneficial microbes from environmental stressors such as UV radiation, extreme pH levels, and dehydration (Dimkpa *et al.*, 2020). The encapsulating material also regulates the timing and release of microbial signaling molecules, allowing for more synchronized and effective communication with host plants. This enhanced control promotes more efficient symbiotic relationships, increasing root colonization rates by 30–50% compared to non-encapsulated microbial inoculants (Bashan *et al.*, 2014).

Molecular Genetic Regulation

At the molecular scale, nano-biofertilizers have a pronounced impact on plant gene activity and protein production. Transcriptomic studies show that nano-formulated *Azospirillum brasilense* can boost the expression of critical nitrogen assimilation genes—such as nitrate reductase and glutamine synthetase—by two to three times in maize roots (Prasad *et al.*, 2017). Likewise, nano-encapsulated Pseudomonas fluorescens stimulates plant immune responses by activating the

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jasmonic acid signaling pathway, thereby improving resistance to soilborne pathogens (Dimkpa *et al.*, 2020).

Nano-biofertilizers also influence nutrient transporter gene systems, as evidenced by nano-iron formulations triggering a 40% higher expression of iron-regulated transporter (IRT) genes in soybean plants compared to traditional iron supplements (Wu *et al.*, 2019). These genetic effects are often tissue-specific, with especially strong transcriptional responses observed in the root apical meristems following nano-biofertilizer treatment (Subramanian *et al.*, 2015).

The interaction between nano-biofertilizers and plants occurs in three distinct stages. In the initial phase (0-48 hours), microbes attach to the plant roots and move towards the root exudates. In the next stage (3-14 days), microbial populations proliferate rapidly, forming symbiotic structures such as arbuscules and biofilms. In the final phase (>14 days), the symbiotic relationship reaches its peak, optimizing nutrient exchange and stimulating plant growth, aligning with key crop development stages (Avellan *et al.*, 2019). This gradual process ensures that the benefits are delivered at the right time throughout the plant's growth cycle.

Comparative Analysis of Conventional Fertilizers and Nano-Biofertilizers

Although traditional fertilizers increase crop yields, they also contribute to environmental issues such as soil degradation, water contamination, and greenhouse gas emissions (Savci, 2012). In contrast, nano-biofertilizers—combining nanotechnology with beneficial microbes—offer improved nutrient utilization, reduced ecological impact, and enhanced soil quality (Liu and Lal, 2015). This analysis evaluates and compares both approaches in terms of nutrient delivery efficiency, environmental impact, cost-effectiveness, and long-term sustainability.

Efficiency and Nutrient Delivery

Traditional chemical fertilizers such as urea, diammonium phosphate (DAP), and potassium chloride supply essential nutrients like nitrogen, phosphorus, and potassium in readily soluble forms for immediate plant uptake. However, a large proportion of these nutrients is lost through leaching, volatilization, and runoff, which significantly reduces their overall effectiveness. Research indicates that these fertilizers have low nutrient use efficiency, with much of the input lost to environmental processes (Chien *et al.*, 2009). Nitrogen fertilizers, in particular, are inefficient—only about 30–50% of the nitrogen from urea is absorbed by crops, while the rest escapes into the environment, leading to both financial waste and ecological damage (Zhang *et al.*, 2015). These shortcomings underscore the urgent need for more efficient nutrient delivery technologies.

Nano-biofertilizers integrate nanotechnology with beneficial microbial inoculants to enhance nutrient delivery in farming systems. These advanced solutions pair engineered nanoparticles—such as nano-urea or nano-zeolite—with microbes like rhizobia and phospho-bacteria to enable coordinated and efficient nutrient release (DeRosa *et al.*, 2010). The nano-encapsulation process allows for controlled, slow nutrient delivery, while microbial processes improve nutrient bioavailability, significantly cutting down nutrient losses. Studies show that this combined system

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can raise nitrogen use efficiency to as high as 80%, effectively doubling the performance of conventional fertilizers (Subramanian *et al.*, 2015). This cutting-edge method supports long-term soil health and reduces environmental harm by enabling highly targeted nutrient management at the molecular level.

Environmental Impact

Excessive use of chemical fertilizers results in a range of environmental issues, such as soil acidification, nutrient runoff that pollutes waterways, and the emission of nitrous oxide (N₂O), a potent greenhouse gas (Tilman *et al.*, 2002). Over time, this practice also deteriorates soil quality by reducing beneficial microbial communities and depleting organic matter (Geisseler and Scow, 2014). These negative outcomes threaten both agricultural sustainability and ecological stability, underscoring the need for more responsible nutrient management strategies. The cumulative impact of conventional fertilization practices emphasizes the importance of adopting precision-based approaches and environmentally friendly alternatives.

Nano-biofertilizers present a sustainable and environmentally friendly alternative to traditional fertilizers by greatly minimizing nutrient loss through leaching and encouraging the growth of beneficial soil microbes (Gogos *et al.*, 2012). Their advanced formulations improve nitrogen use efficiency, leading to a reduction in greenhouse gas emissions (Mishra *et al.*, 2017). These cutting-edge solutions support natural symbiotic relationships between plants and microbes, enhancing crop yields while reducing dependence on synthetic inputs (Bhardwaj *et al.*, 2014). By integrating nanotechnology with biological functions, nano-biofertilizers offer a balanced approach to tackling both environmental impact and productivity challenges in agriculture. Their dual benefits help safeguard ecosystems while sustaining soil health and agricultural output.

Cost-Effectiveness and Long-Term Sustainability

While conventional fertilizers may seem economically advantageous at first, they come with considerable long-term costs. These include expenses related to restoring degraded soils and treating water systems contaminated by fertilizer runoff (Sutton *et al.*, 2013). To compensate for inefficient nutrient uptake, farmers often apply fertilizers in excess, inadvertently increasing their overall spending. As a result, the initial cost savings are frequently negated by escalating environmental and financial burdens, leading to a pattern of rising expenses and declining efficiency that threatens the sustainability of agricultural systems. This reveals the hidden economic drawbacks behind the perceived affordability of traditional fertilizer use.

While nano-biofertilizers require greater upfront investment, their slow-release mechanisms significantly decrease application needs, resulting in cost efficiency over time (Naderi and Danesh-Shahraki, 2013). By sustainably improving soil health and nutrient availability, these advanced fertilizers prove economically advantageous for prolonged agricultural use (Prasad *et al.*, 2017). The initial price premium is offset by reduced labor costs, lower input requirements, and lasting soil productivity benefits, making them a financially sound choice for forward-thinking farmers focused on both profitability and environmental stewardship. Their value proposition improves with continued use as soil quality enhances.

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Microbes Suitable for Nano-Biofertilizers with Crop-Specific Applications, Methods, and Timing

Nano-biofertilizers combine plant-growth-promoting microorganisms with nanomaterials to improve nutrient availability, enhance microbial resilience, and increase agricultural yields. Their efficacy depends on selecting compatible microbial strains, tailored crop-specific formulations, and precise application timing aligned with benefits and key growth phases. Table 1 provides a comprehensive overview of recommended microbial-nanomaterial combinations for different crops, detailing their agronomic benefits and application protocols (including timing and methods).

Nitrogen-fixers

i. *Rhizobium species*: Modern application methods leverage nanotechnology to enhance *Rhizobium* delivery and effectiveness of particularly valuable pulse crops including soybean (*Glycine max*), chickpea (*Cicer arietinum*), pea (*Pisum sativum*), lentils (*Lens culinaris*), and forage crops like alfalfa (*Medicago sativa*). Seed coating with nano-encapsulated Rhizobium (using chitosan or alginate nanoparticles) ensures optimal bacterial survival and early nodule formation. Alternatively, soil application of nano-formulated Rhizobium inoculants (applied at 2-5 kg/ha) provides sustained nitrogen fixation throughout the growing season. For maximum effectiveness, application timing should follow two critical phases: pre-sowing seed treatment to establish early symbiosis, and early vegetative stage application (10-15 days after sowing) to support peak nitrogen demand during rapid plant growth. These precision application methods significantly improve nitrogen use efficiency while reducing dependence on synthetic fertilizers.

ii. *Azotobacter species*: *Azotobacter* species serve as effective free-living nitrogen fixers that convert atmospheric nitrogen while simultaneously producing growth-promoting phytohormones like indole-3-acetic acid (IAA) and gibberellins. These beneficial bacteria are particularly suitable for cereal crops including wheat, rice, maize, and sugarcane. Modern nano-formulations enable efficient application through foliar sprays (using 10⁸ CFU/mL suspensions at tillering stage) or soil drenching (500 mL/ha mixed with irrigation water) (Prasad *et al.*, 2017). Optimal application timing occurs at two critical growth phases: during early seedling development (10-20 days after sowing) to enhance root establishment, and pre-flowering stage (45-60 days after sowing) to maximize grain yield potential. The nano-encapsulation technology protects bacterial viability while ensuring controlled release of both fixed nitrogen and phytohormones, significantly improving crop productivity and reducing synthetic fertilizer dependence. This dual-action mechanism makes Azotobacter-based nano-biofertilizers particularly valuable for sustainable cereal production systems.

iii. Azospirillum species: *Azospirillum species* function as efficient plant growth promoters through associative nitrogen fixation and the production of phytohormones. These beneficial bacteria are particularly effective for cereal crops such as maize, sorghum, millet, and barley. Advanced nano-formulations allow for optimized delivery via seed priming (soaking seeds in a 10⁷ CFU/mL nano-*Azospirillum* suspension for 6–8 hours) or fertigation (applying 2 L/ha through drip irrigation) (Bhardwaj *et al.*, 2014). For best results, application should occur at two key stages: during sowing

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to ensure early root colonization, and at the tillering phase (25–30 days after sowing) to stimulate robust shoot development. The nano-encapsulation enhances bacterial survival and nutrient release, improving crop performance while reducing reliance on synthetic fertilizers. This sustainable approach enhances yield potential in grain production systems.

Phosphate-Solubilizing Microbes

Nano-formulated phosphate-solubilizing biofertilizers convert insoluble phosphorus into plantavailable forms through organic acid secretion. Applied via soil incorporation or root treatment, they enhance phosphorus uptake in crops like potato and tomato while reducing fertilizer dependency. (Sharma *et al.*, 2013)

i. Bacillus species: This beneficial bacterium acts as an efficient phosphorus solubilizer by producing gluconic and citric acids that release bound phosphates from soil minerals. Particularly valuable for phosphorus-sensitive crops like potatoes, tomatoes, and soybeans, its nano-formulated version offers superior delivery methods. Farmers can apply it through soil incorporation (5 kg/ha nano-encapsulated bacteria blended with organic compost) or as a protective root dip for young seedlings (Sharma *et al.*, 2013). Strategic application timing maximizes benefits: initial incorporation during planting establishes phosphorus reserves, while a flowering stage boost (50-60 days after sowing) fuels critical fruit formation. The nanoparticle coating protects bacterial cells, extends shelf life, and enables gradual release of both bacteria and solubilized phosphorus, dramatically improving nutrient use efficiency. This technology offers a sustainable solution for phosphorus management, especially in alkaline soils where phosphorus availability is typically limited.

ii. Pseudomonas species: These versatile bacteria functions as a dual-action biofertilizer, solubilizing insoluble phosphates while producing iron-chelating siderophores to improve micronutrient availability. Particularly effective for staple crops including rice, wheat, and cotton, nano-formulated P. fluorescens can be applied through foliar sprays (10⁸ CFU/mL concentration during tillering) or via precision drip irrigation (1 L/ha in divided applications) (Raliya *et al.*, 2015). Optimal application occurs during two critical growth phases: early vegetative stage (15-20 days after sowing) to stimulate root development and at panicle initiation (60 days after sowing for rice) to support optimal grain formation. The nano-encapsulation technology enhances bacterial viability during application and extends its phosphate-solubilizing activity in the rhizosphere. This approach significantly improves phosphorus and iron uptake efficiency while reducing chemical fertilizer requirements, particularly in iron-deficient or phosphorus-fixing soils. The siderophore production additionally provides natural protection against soil-borne pathogens, making it a valuable component of integrated crop management systems for cereal and fiber crops.

Potassium-Solubilizing Microbes

Nano-formulated potassium-solubilizing microbes (KSMs) enhance K availability by breaking down insoluble minerals. Applied via seed treatment or soil application, they boost uptake in crops while reducing chemical fertilizer needs (Meena *et al.*, 2015). *Bacillus edaphicus* solubilizes potassium (K) via oxalic/citric acids, aiding wheat and maize growth (Meena *et al.*, 2015). *Pseudomonas putida* enhances K uptake in cereals/legumes through gluconic acid and

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pathogen suppression (Saha *et al.*, 2016). *Paenibacillus mucilaginosus* breaks down K-minerals (feldspar/mica) for sugarcane/potato (Liu *et al.*, 2012). *Arthrobacter* spp. mobilizes K via chelation, benefiting rice/soybean (Zhang *et al.*, 2013). *Enterobacter hormaechei* combines K solubilization with N-fixation, ideal for vegetable soils (Prajapati *et al.*, 2013). These biofertilizers sustainably replace chemical inputs while boosting soil fertility. *Bacillus mucilaginous* particularly acts as an effective nano-biofertilizer by solubilizing potassium (K) from minerals like feldspar and mica through organic acid secretion. It benefits potassium-demanding crops such as banana, sugarcane, and potato. Application methods include soil treatment (5 kg/ha of nano-encapsulated *Bacillus* mixed with farmyard manure) or fertigation via irrigation water. For optimal results, apply a basal dose before planting to ensure K availability, followed by a second dose during the tuber/bulking stage (60–70 days after sowing in potatoes) to boost yield. This approach enhances nutrient efficiency while reducing dependency on chemical fertilizers (Sindhu *et al.*, 2016).

Plant Growth-Promoting Rhizobacteria

Growth-Promoting Rhizobacteria (PGPR) serve as nano-biofertilizers, enhancing plant growth by improving nutrient availability, fixing nitrogen, and producing growth-stimulating hormones. Their nano-encapsulation increases efficiency, ensuring targeted delivery and sustained microbial activity in the rhizosphere. The application of PGPR as nano-fertilizers Plant represents a cutting-edge approach to sustainable agriculture. *Pseudomonas fluorescens* serves as an effective nano-biofertilizer by producing siderophores, antibiotics like 2,4-DAPG, and indole-3-acetic acid (IAA), which collectively enhance nutrient availability while suppressing pathogenic fungi such as *Fusarium* (Kloepper *et al.*, 1980). *Bacillus subtilis*, when nano-encapsulated, demonstrates enhanced delivery of its antibiotic surfactin and improved induction of systemic resistance (ISR) in crops like tomato and chili, significantly reducing damping-off disease incidence (Olanrewaju *et al.*, 2017).

Azospirillum brasilense functions as a dual-action nano-fertilizer by fixing atmospheric nitrogen and synthesizing auxins that stimulate root development in cereal crops including wheat and maize (Bashan and de-Bashan, 2010). The nitrogen-fixing symbiont *Rhizobium leguminosarum* shows particular promise when applied as nano-formulations, enhancing nodulation efficiency in leguminous crops such as peas and soybeans while improving overall soil fertility (Oldroyd *et al.*, 2011). *Serratia marcescens* contributes to plant growth through multiple mechanisms including phosphate solubilization and production of ACC deaminase, which mitigates ethylene-mediated stress responses in important crops like rice and tomato (Glick, 2014).

These nano-encapsulated PGPR strains offer significant advantages over conventional fertilizers by improving nutrient use efficiency, reducing dependency on chemical inputs, and enhancing plant resilience to biotic and abiotic stresses. Their targeted delivery through nano-formulations ensures prolonged microbial viability and controlled release of growth-promoting compounds, making them particularly valuable for precision agriculture applications. The integration of PGPR-based nano-fertilizers into agricultural systems represents a sustainable strategy to enhance crop productivity while minimizing environmental impact.

Bacillus subtilis functions as an effective nano-biofertilizer by producing antibiotics like surfactin and activating systemic resistance in plants. It is particularly beneficial for crops such as tomato,

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chili, and brinjal. Application methods include seed treatment with nano-coated formulations or soil drenching at 2 L/ha during transplanting. For optimal results, apply during the nursery stage to prevent damping-off disease and again at the fruit-setting phase to boost yield. This approach enhances plant immunity, reduces pathogen pressure, and improves productivity while minimizing chemical inputs. The nano-encapsulation ensures controlled release and prolonged microbial activity (Olanrewaju *et al.*, 2017).

Arbuscular Mycorrhizal Fungi (AMF)

Arbuscular Mycorrhizal Fungi (AMF) demonstrate remarkable potential as nano-biofertilizers, enhancing plant growth through improved nutrient acquisition and stress tolerance. *Rhizophagus irregularis* significantly boosts phosphorus uptake in wheat and maize while increasing drought resistance through extensive hyphal networks (Smith and Read, 2008). *Funneliformis mosseae* excels in nutrient-deficient soils, particularly benefiting tomatoes and cucumbers (Bücking *et al.*, 2012). *Glomus aggregatum* enhances soil structure and water retention, proving especially valuable for fruit trees like apple and citrus (Wu *et al.*, 2013). *Gigaspora margarita* aids plants in contaminated soils by improving heavy metal tolerance and zinc absorption (Audet and Charest, 2007), while *Claroideoglomus etunicatum* optimizes symbiosis in acidic soils, benefiting coffee and tea crops (Jansa *et al.*, 2014). These nano-formulated AMF species offer sustainable solutions by reducing chemical fertilizer needs, improving stress resilience, and promoting soil health. Their nano-encapsulation ensures targeted delivery and prolonged effectiveness, making them invaluable for modern agriculture.

The mycorrhizal fungus *Glomus intraradices* functions as an efficient nano-biofertilizer, significantly improving phosphorus acquisition and drought resistance in plants. This beneficial microorganism shows particular effectiveness in maize, soybean, and onion production systems. Farmers can apply it through two primary methods: coating seeds with nano-encapsulated fungal spores (Rouhani *et al.*, 2012) or incorporating it into soil at a rate of 10 kg per hectare during planting. Maximum benefits are achieved through dual-phase application - initially before planting to establish fungal-root associations, followed by a second application at the 30-day vegetative stage to optimize nutrient assimilation. This nano-formulation enhances agricultural sustainability by improving crop stress tolerance while decreasing reliance on synthetic fertilizers.

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Table 1: showing microbial-nanomaterial combinations for different crops with their agronomic benefits and application timing and methods.

Сгор	Common Name	Botanical Name	Bio- inoculant	Nano Composit e	Method of Applicatio n	Time of Applicatio n	Benefit	Ref
Legumes	Chicken pea	Cicer aestivum	Rhizobium japoniscum	FeO, ZnO nano-Ps	Seed application	Pre-sowing	Fixes atmospheri c nitrogen, improves plant growth, and enhances nodulation.	Morovat <i>et al.</i> (2019)
	Soybeans	Glycine max	Rhizobium japoniscum	FeO nano-Ps	Soil application	Pre-sowing	Enhances nitrogen fixation and plant growth.	Yusefi- Tanha <i>et</i> <i>al.</i> (2020)
	Peanut	Arachis hypogea	Bradyrhizo bisp.	nano- K(nK)	Soil application	Pre-sowing	Fixes atmospheri c nitrogen, improves plant growth, and enhances nodulation.	El- Akhal <i>et</i> <i>al.</i> (2013).
	Pomegran ate	Punica granatum	Bacillus subtilis, Pseudomon as fluorescens	FeO nanoPs	Soil application	Pre-sowing	Promotes plant growth, enhances nutrient uptake, and suppresses diseases.	Kumar <i>et al.</i> (2018).
	Alfaalfa	Medicago sativa	Sinorhizobi um meliloti	Copper nano-Ps	Soil application	Pre-sowing	Fixes atmospheri c nitrogen, improves plant growth, and enhances nodulation.	Jones <i>et</i> <i>al.</i> (2007).

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	Cowpea Pigeon pea	Virgna Unguiculat a Cajanus cajan	Burkholder ia seminalis Rhizobium tropici	nanohydr oxyapatite (nHA) particles CuO- nano-Ps	Seed application Soil application	Pre-sowing Pre-sowing	Fixes atmospheri c nitrogen, improves plant growth, and enhances nodulation Enhances nitrogen fixation and plant growth.	Kaur <i>et</i> <i>al</i> (2022) Feng <i>et</i> <i>al.</i> (2019)
Cereals	Wheat	Triticum aestivum	Azospirillu m brasilense	ZnO- zinc nitrate	Seed application	30-60 days after sowing	Promotes plant growth by producing phytohorm ones and enhancing nutrient uptake.	Sheoran <i>et al.</i> (2021)
	Rice	Oryza sativa	Azospirillu m sp	nano- NPK	Seed application	At sowing or after sowing	Promotes plant growth by producing phytohorm ones and enhancing nutrient uptake.	Sadati Valojai <i>et al.</i> (2021)
	Maize	Zea mays	Azospirillu m brasilense	ZnO	Seed application	30-60 days after sowing	Promotes plant growth by producing phytohorm ones and enhancing nutrient uptake.	Bashan <i>et al.</i> (2014).
	Sorghum	Sorghum bicolor	Azotobacte r sp	nano- NPK	Foliar Spray	30-60 days after sowing	Fixes atmospheri c nitrogen and	Altabba a <i>et al.</i> (2023)

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							promotes seedling developme nt	
	Finger Millet	Eleusine coracana	Bacillus tequilensis	Nano urea	Foliar Spray	30-60 days after sowing	Production of antifungal compounds	Kumar <i>et al.</i> (2023)
	Barley	Hordeum vulgare	Acinetobact er baumannii	Nano phosphate	Foliar Spray	30-60 days after sowing	Promotes growth via phytohorm ones production.	Ibrahim <i>et al.</i> (2022)
	Pearl Millet	Pennisetum glaucum	Paenibacill us dendritifor mis	Zn and Ag nano- Ps	Foliar Spray	30-60 days after sowing	Promotes plant growth and has biocontrol activities	Kumar <i>et al.</i> (2023)
	Oats	Avena sativa	Pseudomon as aeruginosa	Nano copper	Foliar Spray	30-60 days after sowing	Induces systemic resistance against diseases	Yasari <i>et</i> <i>al.</i> (2022)
Vegetable s	Tomato	Solanum lycopersicu	Bacillus subtilis	Chitosan nano-Ps	Soil application	Early growth stage	Enhances nutrient uptake, and suppresses soil-borne pathogens.	Iqbal, M.A. (2020).
	Chili	Capsicum annuum	Bacillus amyloliquef aciens	Chitosan nano-Ps	Soil application	Early growth stage	Suppresses soil-borne pathogens and enhances plant growth.	Iqbal, M.A. (2020).
	Cucumber	Cucumis sativus	Bacillus cereus	Chitosan nano-Ps	Soil application	Early growth stage	Induces systemic resistance against diseases and promotes	Kakbra, Roshna. (2023).

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							plant	
							growth.	
							Fixes	Moham
							atmospheri	mad
			Rhizobium	Cu, Ni, Co Nano-			c nitrogen,	Javad
	Okra	Abelmosch	leguminosa		Soil	At sowing	improves	Javid-
		esculentus	rum	Ps	application		plant	Naderi
							growth, and	et al.
							enhances	(2023)
							nodulation.	
	Cabbage	Brassica oleracea	Bacillus subtilis	ZnO, FeO, MnO nano-Ps	Foliar spray	Vegetative stage	Promotes	Murguei
							plant	tio- Herrera
							growth, enhances	<i>et al.</i>
							nutrient	(2022)
							uptake, and	(2022)
							suppresses	
							diseases.	
							Enhances	Mary
	Fenugree k	Trigonella foenum	Pseudomon as gessardi,	Cerium oxide (CeO ₂)	Soil drenching	Germinatio n stage	plant	Isabella
							growth and	Sonali
							induces	(2022)
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Note: Ps = Particles

Nano-particle materials suitable for biofertilizer applications

Nanotechnology has emerged as a promising tool in agriculture, particularly in the development of biofertilizers. Nanoparticles (NPs) enhance nutrient delivery, improve soil health, and promote plant growth by increasing the efficiency of microbial inoculants.

Metal-Based Nanoparticles

i. Silver Nanoparticles: Silver nanoparticles (AgNPs) have demonstrated exceptional potential in legume production systems when combined with specific biofertilizers. Research on soybean (*Glycine max*) cultivation reveals that AgNPs at 20-30 ppm concentrations enhance the symbiotic efficiency of *Bradyrhizobium japonicum* by 25-30%, leading to significant improvements in nodulation and nitrogen fixation (Siddiqui *et al.*, 2021). In chickpea (*Cicer arietinum*) fields, AgNP-treated *Mesorhizobium ciceri* inoculants show 40% reduction in *Fusarium oxysporum* infections while boosting plant growth parameters (Abd-Alla *et al.*, 2020). The nanoparticles' antimicrobial action selectively protects rhizobial cells during critical early growth stages, particularly valuable in pulse crops grown in pathogen-rich tropical soils. Recent field trials in

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organic lentil (*Lens culinaris*) production demonstrate that optimized AgNP-biofertilizer combinations can achieve yields comparable to conventional systems while eliminating synthetic fungicides (Pérez-de-Luque *et al.*, 2022).

ii. Iron and Zinc Nanocomposites: Iron oxide nanoparticles (Fe₂O₃ NPs) have shown remarkable results in wheat (*Triticum aestivum*) production when integrated with phosphate-solubilizing *Bacillus megaterium*. Field studies in calcareous soils demonstrate 35% higher iron content in grains and 28% yield increases compared to traditional fertilization methods (Zhao *et al.*, 2021). For rice (*Oryza sativa*) cultivation in zinc-deficient paddies, ZnO nanoparticles (20-30 nm) combined with zinc-mobilizing *Pseudomonas brassicacearum* enhance grain zinc concentrations by 45-50%, addressing widespread micronutrient malnutrition (Zhang *et al.*, 2022). These nanobiofertilizer systems are particularly effective in alkaline soils where conventional micronutrient fertilizers show poor availability. Recent innovations include dual-loaded Fe-Zn nanocomposites that simultaneously address both micronutrient deficiencies in maize (*Zea mays*), showing 30% greater efficacy than single-element formulations (Liu *et al.*, 2023).

Carbon Nanostructures

Carbon nanotube (CNT)-based biofertilizer carriers have revolutionized tomato (*Solanum lycopersicum*) production in protected cultivation systems. When loaded with a consortium of *Azotobacter chroococcum* and *Pseudomonas fluorescens*, CNT matrices maintain microbial viability for 120 days under greenhouse conditions, compared to 45 days for conventional peat-based carriers (García *et al.*, 2021). In hydroponic lettuce (*Lactuca sativa*) systems, graphene oxide (GO) sheets functionalized with plant growth-promoting *Bacillus amyloliquefaciens* enhance nutrient uptake efficiency by 35-40% while reducing nitrate accumulation in leaves (Torres *et al.*, 2022). These carbon-based systems are particularly valuable for high-value vegetable crops where precise nutrient management is crucial for both yield and quality parameters.

Silica-Based Nanoparticles

Silica nanoparticle-enhanced biofertilizers show exceptional promise for crops grown under abiotic stress conditions. In drought-prone sorghum (Sorghum bicolor) fields, SiO₂ NP-coated *Azospirillum brasilense* inoculants maintain 75% colonization efficiency even at 40% field capacity, compared to 30% for uncoated cells (Nguyen *et al.*, 2023). For rice cultivation in saline coastal areas, silica-encapsulated *Halomonas venusta* biofertilizers demonstrate 50% higher survival rates and maintain nitrogenase activity at 8 dS/m salinity (Wang *et al.*, 2023). These systems are proving particularly valuable for climate-resilient agriculture, helping maintain productivity in increasingly marginal growing environments.

Chitosan Nano-carriers

Chitosan nanoparticle delivery systems have transformed biofertilizer application in perennial cropping systems. In citrus (*Citrus sinensis*) orchards, chitosan-encapsulated *Glomus intraradices* spores show 80% root colonization after 8 weeks compared to 35% for conventional applications (Silva *et al.*, 2023). For grapevine (*Vitis vinifera*) production, chitosan nano-formulations containing *Trichoderma harzianum* provide season-long protection against root pathogens while enhancing nutrient uptake efficiency (Martínez *et al.*, 2023). These controlled-release systems are

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particularly advantageous for perennial crops where repeated fertilizer applications are costly and labor-intensive.

Hybrid nano-composites

Hybrid nanocomposites, formed by combining organic and inorganic nanomaterials, offer enhanced functionality for biofertilizer development. These advanced materials integrate multiple nanoparticle types (carbon-based, metallic, or polymeric) to create superior formulations compared to single-component systems (Chakraborty *et al.*, 2022). Their improved biocompatibility and multifunctional properties make them particularly valuable for agricultural applications, including nutrient delivery, as well as medical and pharmaceutical uses (Vejan *et al.*, 2021). These hybrid systems demonstrate greater versatility and effectiveness in diverse biological applications.

Polymer-based nanoparticles

Polymer-based nanoparticles—including nanospheres, nano-capsules, and other nanostructures are increasingly utilized in agriculture, medicine, and pest management (Fortunati *et al.*, 2019). These systems encapsulate bioactive compounds (insecticides, fungicides) within biodegradable matrices such as chitosan, silica, or polyethylene glycol (PEG), enhancing stability and controlled release (Ashraf *et al.*, 2021). Nano-capsules, featuring a polymer-coated hollow core, efficiently deliver agrochemicals like tebuconazole and carbendazim as nano-fungicides (Rani *et al.*, 2022). Similarly, copolymer nano-capsules (e.g., poly (MMA-co-St)) loaded with nematicides demonstrate targeted pest control. Such nano-formulations ensure sustained release, maintaining effective concentrations throughout pest life cycles while minimizing environmental impact.

Nano-emulsions

Nano-emulsions (20-200 nm droplets) offer superior agrochemical delivery with up to10% surfactant content, making them more stable than microemulsions. These systems enhance pesticide solubility and bioavailability while enabling deep plant penetration and strong surface adhesion (Sheth *et al.*, 2020). They effectively deliver water-insoluble actives like β -cypermethrin through eco-friendly formulations using methyl laurate and mixed surfactants (Ravichandran *et al.*, 2022). Beyond crop protection, nano-emulsions serve food preservation needs (Mustafa *et al.*, 2020) and can encapsulate natural insecticides (Choi *et al.*, 2023). Their weed-control potential (Aswathanarayan *et al* 2019) and disease-fighting capabilities (Pavoni *et al.*, 2020) make them versatile agricultural tools. For example, 100nm nicotine nano-emulsions showed high efficacy against pests (LT50) (Dwivedi *et al.*, 2022).

Clay-Based Nanocarriers

Clay-based nanomaterials, particularly halloysite nanotubes, have emerged as efficient pesticide delivery systems due to their affordability, prolonged release properties, and enhanced plant compatibility. These natural carriers significantly reduce pesticide runoff into water systems (Jakhar *et al.*, 2022). The category includes various modified clays like anionic layered double hydroxides (LDH) and organo-clay hybrids, all exhibiting well-documented molecular interactions with agricultural chemicals (Saleem *et al.*, 2021). Their layered structures and charge

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characteristics enable precise encapsulation and controlled release of active ingredients, making them valuable for sustainable pest management while minimizing environmental impact.

Processes of Nanomaterial synthesis

Nanomaterial synthesis requires precise control of temperature, pressure, and chemical composition to optimize particle properties. Researchers continuously develop innovative biological, physical, and chemical methods to produce nanoparticles with enhanced size, shape, and commercial viability (Rathinavel *et al.*, 2021) using the top-down and bottom-up approaches.

i. Top-Down Nanomaterial Synthesis

The top-down method involves reducing bulk materials to nanoscale dimensions through mechanical and vapor-phase techniques. Common processes include ball milling, chemical vapor deposition (CVD), and physical vapor deposition, which produce carbon nanotubes, graphene, and fullerenes (Rocha-Meneses *et al.*, 2022). CVD specifically enables gas-phase reactions to create carbon nanomaterials, coatings, and composites. However, these methods present challenges in achieving precise nano-structural control due to their complex reaction mechanisms (Abid *et al.*, 2019). While valuable for industrial applications like thin films and powders, the approach faces limitations in tailoring exact nanomaterial properties. The technique's effectiveness is counterbalanced by its sophisticated equipment requirements and process complexity.

ii. Bottom-Up Nanomaterial Synthesis

The bottom-up approach constructs nanoparticles from atomic/molecular components using biological, chemical, or physical assembly methods. Unlike top-down techniques, it utilizes self-assembling molecules to build nanostructures through processes like green synthesis and spin coating (Belusso *et al.*, 2019). This method proves particularly effective for producing quantum dots and colloidal nanoparticles via controlled epitaxial growth (Abid *et al.*, 2019). Its success relies on precursor materials with inherent self-organization capabilities that facilitate precise nanoscale architecture. The approach offers superior control over particle morphology compared to top-down methods, enabling tailored nanomaterial properties for advanced applications.

Process of Nano-Biofertilizers Fabrication

The production of nano-biofertilizers involves three critical phases: microbial cultivation, nanoparticle encapsulation, and product validation (Mansoor *et al.*, 2021). Unlike inorganic materials that can be mechanically reduced to nanoscale, living microorganisms (typically micrometer-sized) cannot be physically shrunk without losing viability (Jagtap *et al.*, 2021). Instead, biological synthesis approaches utilize microbial cells as nano-factories. For instance, Bacillus megaterium facilitates silver nanoparticle formation through extracellular processes, creating antimicrobial agents (Nayana *et al.*, 2020).

Fungal-mediated synthesis offers another green approach. Fungal biomass cultured in MGYP broth reacts with silver nitrate under dark conditions to produce Ag-nanoparticles, verified spectroscopically before lyophilization (Saha *et al.*, 2021). Similarly, bacterial supernatants enable

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scalable nanoparticle production by eliminating cell disruption and purification steps (Rahman *et al.*, 2020).

Advanced synthesis combines metallic solutions with bioorganic compounds like auxin. The process involves filtering bacterial supernatants, adjusting concentrations (200 µg/mL), and mixing with metal solutions (2mM for monometallic, 1mM for bimetallic compounds). Controlled reactions occur at pH 4.8 and 45°C for 5 hours without agitation (Akhtar *et al.*, 2022). Notably, *Paenibacillus polymyxa* cultures have successfully generated iron and manganese mono-/bimetallic nanoparticles when combined with auxin complexes (DeFrança Bettencourt *et al.*, 2022).

These biological fabrication methods demonstrate superior control over nanoparticle characteristics compared to physical top-down approaches, while maintaining microbial viability for agricultural applications. The resulting nano-biofertilizers combine the nutritional benefits of microorganisms with enhanced nanoparticle delivery systems.

Limitations and Challenges of Nano-biofertilizers application

The combination of nanotechnology and microbial biofertilizers has led to the development of nano-biofertilizers, offering significant potential for sustainable farming through improved nutrient absorption and minimized ecological impact (Rai *et al.*, 2018). Despite these advantages, multiple barriers impede their widespread implementation. A primary constraint involves the substantial manufacturing expenses required for nanoparticle synthesis and microbial encapsulation, rendering them less cost-effective than conventional alternatives (Liu and Lal, 2015). Another obstacle lies in preserving formulation integrity, as both nanoscale components and beneficial microorganisms tend to deteriorate during storage, diminishing their agricultural performance (Subramanian *et al.*, 2015).

Environmental safety represents another critical consideration for nano-biofertilizer deployment. Research demonstrates that certain metallic nanoparticles may persist in agricultural systems, potentially causing adverse effects on crops, soil biota, and surrounding environments (Rizwan *et al.*, 2017). Compounding these concerns is the absence of standardized global regulations, with governing bodies currently establishing protocols to evaluate potential risks associated with prolonged usage (Kah *et al.*, 2019).

Field implementation presents additional complications, as product effectiveness varies considerably depending on soil characteristics and regional climate patterns (Dimkpa and Bindraban, 2018). Although experimental data appears encouraging, the scarcity of commercial-scale testing creates uncertainty about practical applications. Furthermore, agricultural producers often hesitate to adopt these innovations due to insufficient technical knowledge and general apprehensions about nanotechnologies (Gogos *et al.*, 2012). Overcoming these limitations will demand continued scientific investigation, development of affordable manufacturing processes, and establishment of coherent safety regulations to facilitate responsible agricultural application.

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CONCLUSION

Nano-biofertilizers offer a promising, eco-friendly alternative to traditional fertilizers by increasing nutrient uptake efficiency, supporting healthier soils, and minimizing environmental contamination. Although barriers such as high production costs, limited large-scale adoption, and regulatory uncertainties still exist, advances in nanotechnology and biotechnology are steadily addressing these challenges. Continued research and innovation are expected to lower costs, improve formulations, and build farmer confidence. As these developments progress, nanobiofertilizers are likely to become a widely accepted input in sustainable agriculture, supporting global efforts to boost crop productivity while preserving ecological balance and reducing dependence on chemical fertilizers.

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