

## Bio-Fertilizers an Eco-friendly Technology for Environmental Sustainability Present Status and Future Prospects in india

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### Introduction

The agriculture sector contributes a significant portion to the global GDP, but increasing population, estimated to reach 9.5 billion by 2050, along with challenges like declining fertile land, urbanization, climate change, and nutrient deficiencies, threatens future food security. Modern high-input farming systems rely heavily on synthetic fertilizers to meet crop nutrient demands, yet only 30–40% of these nutrients are utilized by plants, with the rest causing environmental pollution, eutrophication, and health hazards due to heavy metals and nitrates.

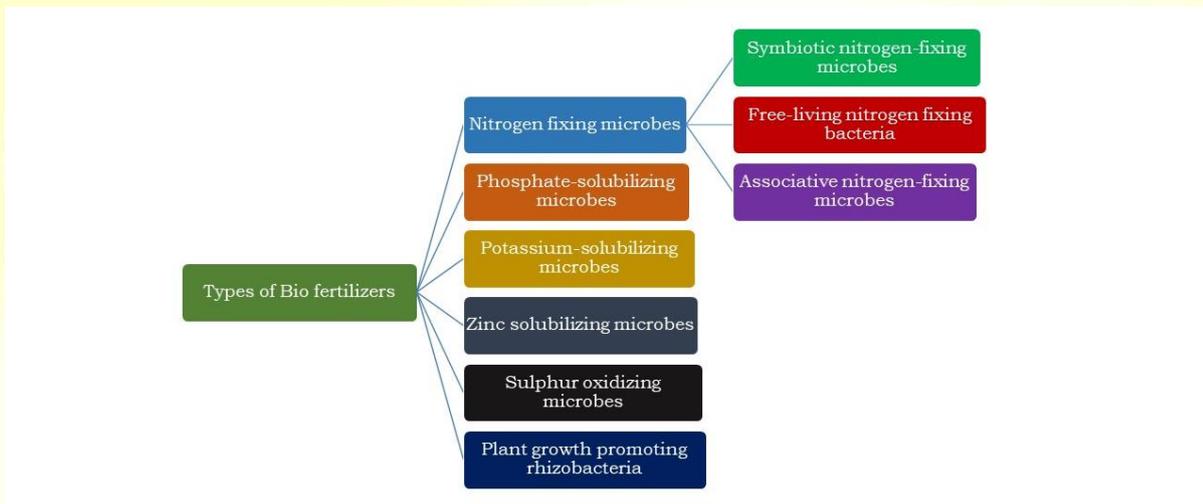
This calls for sustainable alternatives, and biofertilizers—beneficial microbes like *Rhizobium*, *Azotobacter*, and blue-green algae—have emerged as eco-friendly solutions. These microbes promote plant growth by nitrogen fixation, solubilizing phosphorus and potassium, producing phytohormones, enhancing nutrient availability, and improving resistance to stress. For example, *Rhizobium* benefits legumes, *Azotobacter* suits cereals like wheat and maize, while *Anabaena* with water fern can contribute up to 60 kg N/ha in rice fields. Though not a complete replacement for chemical fertilizers, biofertilizers play a vital role in integrated nutrient management (iNM), offering a cost-effective and environmentally sustainable way to enhance soil health and agricultural productivity.

### Types of bio fertilizers

Bio fertilizers are the formulation of living or latent cells of microbes, which provides additional advantage in nutrient uptake and plant performance in rhizosphere. The biofertilizer formulation technique is simple with low installation cost and the former can be composed of single or a mix of two or more diverse microbial strains including Acetobacter, Azotobacter, Bacillus, Pseudomonas, Rhizobium, PGPB or plant growth promoting bacteria and AM or arbuscular mycorrhiza (Basu et al., 2021; Fausi et al., 2021; Mohanty et al., 2021). Biofertilizers are subdivided into different groups (Fig. 1)

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**Fig.1. schematic presentation of types of biofertilizers.**

which are as follows:

- **Nitrogen-fixing microbes**

Biological nitrogen fixation (BNF) is the process by which diazotrophic microbes convert atmospheric nitrogen ( $N_2$ ) into ammonia, supporting crop growth sustainably without the environmental damage caused by chemical fertilizers (e.g., nitrogen oxide emissions, eutrophication, and soil acidification). BNF is mainly performed by certain bacteria and archaea, including groups like green sulphur bacteria, firmibacteria, actinomycetes, cyanobacteria, and proteobacteria. Methanogens are the only nitrogen-fixing archaea. These microbes have varied physiologies—some are aerobic (*Azotobacter*), anaerobic (*Clostridium*), facultatively anaerobic (*Klebsiella*), phototrophic (*Anabaena*, *Rhodobacter*), or chemolithotrophic (*Leptospirillum ferrooxidans*). Diazotrophs inhabit soil and water and can contribute between 20–300 kg N/ha/year. They form associations with grasses, legumes (via root nodules), woody plants, termites, and cyanobacteria. The enzyme **nitrogenase** plays a central role in converting nitrogen gas to ammonia during fixation.

### **.1. Symbiotic nitrogen-fixing microbes**

Symbiotic nitrogen-fixing microbes, especially those belonging to the *Rhizobium* group (including *Mesorhizobium*, *Azorhizobium*, *Allorhizobium*, *Sinorhizobium*, and *Rhizobium* spp.), establish symbiotic associations with the roots of leguminous plants, forming specialized structures called root nodules. These nodules host nitrogenase, an oxygen-sensitive enzyme responsible for converting atmospheric nitrogen ( $N_2$ ) into ammonia ( $NH_3$ ), thereby enhancing plant nitrogen availability (Sindhu & Dadarwal, 1997; Marchal & Vanderleyden, 2000). The presence of

leghemoglobin in nodules helps maintain low oxygen levels required for nitrogenase activity. This biological nitrogen fixation significantly reduces the need for synthetic nitrogen fertilizers in legumes, compared to non-leguminous crops (Goyal et al., 2021). Inoculation with efficient *Rhizobium* strains has demonstrated substantial increases in plant biomass and grain yield across various legume crops (Sindhu et al., 1992; Thies et al., 1991; Goel et al., 2001). Additionally, residual soil nitrogen after legume harvest often equals 30–80 kg N/ha, further improving soil fertility (Sindhu et al., 1992). Apart from *Rhizobium*, *Azolla-Anabaena azollae* symbiosis is another highly effective nitrogen-fixing system. *Anabaena*, a blue-green alga found in rice fields, can fix 40–60 kg N/ha and enrich the soil with organic matter, making it an important biofertilizer in lowland paddy cultivation (Kannaiyan, 1993). Despite proven benefits, field-level inconsistencies in *Rhizobium* performance have also been reported (Miller & May, 1991), indicating the need for strain specificity and favorable environmental conditions.

## **.2. Free-living nitrogen fixing bacteria**

*Azotobacter* is one of the most prominent free-living diazotrophic bacteria commonly found in the rhizosphere of non-leguminous crops like wheat, rice, cotton, and vegetables (Sindhu & Lakshminarayana, 1982; Jain et al., 2021). Species such as *A. chroococcum*, *A. vinelandii*, and others can fix about 2–18 mg of nitrogen per gram of carbon used in culture (Moraditochae et al., 2014). Some strains also exhibit biocontrol activity and secrete bioactive compounds like phytohormones, enhancing root growth and nutrient uptake (Mahanty et al., 2016). *A. vinelandii* produces azotobactin siderophores under iron-deficient conditions (Noar & Bruno-Bárcena, 2018). Wang et al. (2018) observed a 158% increase in nitrogenase activity when *A. chroococcum* was supplemented with a carbon source. These properties make *Azotobacter* an effective and eco-friendly biofertilizer in sustainable agriculture.

## **.3. Associative nitrogen-fixing microbes**

*Azospirillum* is a widely used biofertilizer in wetland agriculture across many countries such as Italy, USA, Brazil, India, and Pakistan (Okon & Labandera-Gonzalez, 1994; Bashan & De-Bashan, 2010). It associates with plant roots and enhances growth through the production of phytohormones like IAA, gibberellins, and cytokinins. Among the 17 identified species, *Azospirillum brasilense* and *A. lipoferum* are the most studied (Rodrigues et al., 2015). It can fix 20–40 kg nitrogen per hectare annually in non-leguminous crops. *Azospirillum* improves root morphology and helps plants tolerate stress by modulating osmotic balance and cell wall flexibility

(Groppa et al., 2012). Bacilio et al. (2004) found that *A. lipoferum* improved plant growth under salt stress. Co-inoculation with *Rhizobium* and *Azospirillum* significantly boosts nodulation, biomass, and nitrogen fixation compared to *Rhizobium* alone (Molla et al., 2001; Remans et al., 2008). This makes *Azospirillum* a valuable biofertilizer in sustainable and stress-resilient agriculture.

#### ■ **Phosphate-solubilizing/mobilizing microbes**

Phosphorus is an essential macronutrient for plant growth, but its bioavailability in soil is very limited despite total phosphorus content ranging from 400–1200 mg/kg of soil (Bamagoos et al., 2021). Most phosphorus exists in insoluble forms like tricalcium and dicalcium phosphate, which are not readily available to plants (Miller et al., 2010; Wang et al., 2017). Phosphate-solubilizing bacteria (PSB) help convert these insoluble forms into plant-available inorganic orthophosphates through solubilization and mineralization processes (Oteino et al., 2015; Tandon et al., 2020). PSBs secrete organic acids such as citric and gluconic acid to solubilize mineral phosphates, and enzymes like phytases and nucleases to mineralize organic phosphate sources (Novo et al., 2018; Ku et al., 2018). In addition, PSBs promote plant growth by producing phytohormones like indole acetic acid (iAA) and siderophores (Hariprasad & Niranjana, 2009). Notably, rhizobacteria that produce higher levels of iAA (>20 µg/mL) show enhanced phosphate-solubilizing capacity, especially when L-tryptophan is added to the growth medium (Alemneh et al., 2021).

#### ■ **Potassium-solubilizing microbes**

Potassium is ranked at third position as crucial plant nutrient after nitrogen and phosphorus (Ding et al., 2021; Patel et al., 2021). Potassium is available in plentiful amount in the soil but only a small fraction (1–2%) of it is available to plants. Hence, a system of continuous replenishment of potassium in soil solution is needed for its adequate availability to crop plants (Park et al., 2009; Meena et al., 2014; Parmar and Sindhu, 2019). Like other nutrients, potassium also influences growth and development of plants, and if it is not supplied in required amount, plant growth will be slow with poorly developed roots and low yield (Williams and Pittman, 2010). Potassium also affects important physiological processes such as starch production, root growth and stomatal movement (Gallegos-Cedillo et al., 2016). In deficiency of potassium, root growth becomes slow and gets poorly developed, seeds will be of small size and disease susceptibility will be more leading to reduction in crop yield (Troufflard et al., 2010).

- **Zinc solubilizing microbes**

Among micronutrients, zinc deficiency is the most widespread nutrient deficiency (Hafeez et al., 2013). Deficiency of zinc (Zn) imparts negative effects not only to plants but also to human health. Deficiency of zinc is ranked at 5th position in terms of human-related death in under developed countries. Zinc is involved in the synthesis of chlorophyll, enzymes, proteins and metabolic reactions (Ali et al., 2008). Plants suffering from zinc deficiency produce symptoms like chlorosis, low membrane integrity and leaf size, retarded shoot growth, reduced grain yield, pollen formation, root development, water uptake and transport and increased vulnerability to heat, light and fungal infections (Tavallali et al., 2010; Kamran et al., 2017). In wheat, Zn deficiency causes stunted growth and yellowing of leaves. Hence, it becomes utmost important to address zinc deficiency as a top priority concern among other micronutrients (Hussain et al., 2018; Kumar et al., 2019).

- **Sulphur oxidizing microbes**

Macronutrient sulphur is needed in high amount by plants as it is a constituent of macromolecules like amino acids (cysteine, cystine and methionine) and also involved in regulation of various enzymes like superoxide dismutase, ascorbate peroxidase, monodehydro-ascorbate reductase, dehydro-ascorbate reductase and glutathione reductase. Sulphur deficiency causes chlorosis and low lipid content along with lower plant growth and yield (Saha et al., 2018). Soil is composed of organic as well as inorganic sulphur and the process of conversion of organic sulphur into plant utilizable inorganic sulphur (i.e.,  $\text{SO}_4^{2-}$ ) form is carried out by sulphur-oxidizing bacteria (SOB) including Xanthobacter, Alcaligenes, Bacillus, Pseudomonas, Thiobacillus sp., Thiobacillusthioparous and T. thiooxidans (Kertesz and Mirleau, 2004; Riaz et al., 2020). Sulphur-oxidizing microorganisms also exhibited other plant growth-promoting activities.

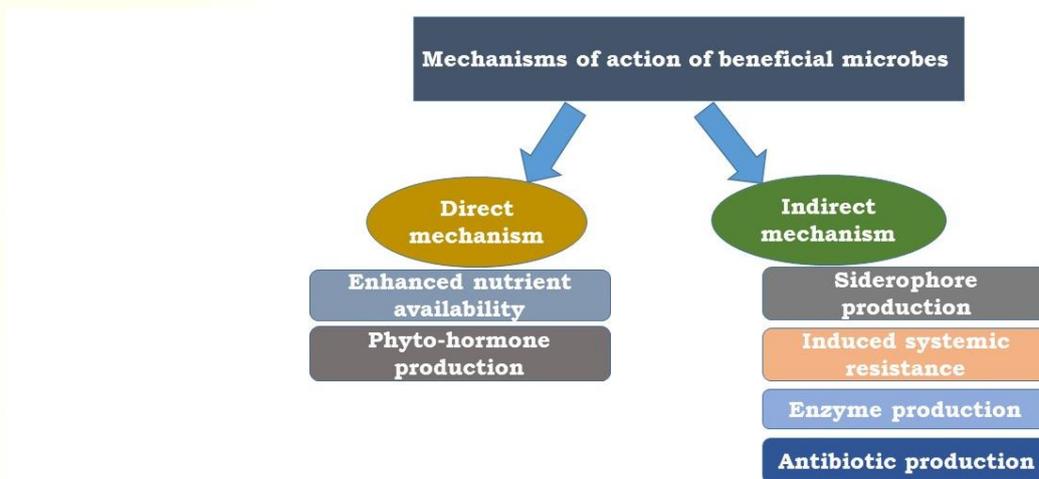
- **Plant growth promoting rhizobacteria**

PGPR includes bacteria, which are free living in nature and obtained from the rhizosphere having the capability to produce and secrete metabolites, which promote plant growth after colonizing their roots (Beneduzi et al., 2012). Upon inoculation, PGPR help the plant to withstand drought stress (Timmusk et al., 2014; Niu et al., 2018; Ilyas et al., 2020), salinity (Mayak et al., 2004; Bharti et al., 2013) and biotic stress (de Vasconcellos and Cardoso, 2009; Verma et al., 2016). Inoculation of PGPRs has been reported to enhance seed germination, soil fertility and plant growth via the production of auxins, ethylene, gibberellins etc. (Jang et al., 2017; Tahir et al., 2017).

Members from various genera like *Agrobacterium*, *Arthrobacter*, *Alcaligenes*, *Azotobacter*, *Acinetobacter*, *Actinoplanes*, *Bacillus*, *Frankia*, *Pseudomonas*, *Rhizobium*, *Micrococcus*, *Streptomyces*, *Xanthomonas*, *Enterobacter*, *Cellulomonas*, *Serratia*, *Flavobacterium*, *Thiobacillus* etc. are included in PGPR (Glick and Gamalaro, 2021; Kumar et al., 2021; Santoyo et al., 2021b).

### Mechanisms of action of beneficial microbes

Microbes, due to their phylogenetic diversity and functional versatility, interact with plants through various relationships like symbiosis, parasitism, commensalism, amensalism, and neutralism (Glick & Gamalero, 2021). These plant-associated microbes, collectively known as the plant microbiome, depend on plant photosynthesis for growth and in return promote plant health and development (Wang et al., 2008; Lebeis et al., 2012; Klaus & Bulgarelli, 2015; Zhang et al., 2021). Beneficial microbes in the plant microbiome enhance soil quality, increase nutrient availability, bolster resistance against pathogens, and produce growth-promoting hormones (Chaparro et al., 2012; Wasai & Minamisawa, 2018). Although the soil microbiome consists of diverse organisms—bacteria, fungi, algae, protozoa, archaea, and viruses—bacteria play a key role in promoting sustainable crop productivity (Mueller & Sachs, 2015; Haney et al., 2015). Microbes in the rhizosphere improve plant growth directly by nutrient solubilization and hormone production (Malik & Sindhu, 2011) and indirectly by suppressing pathogens, reducing abiotic stress, and remediating pollutants (Santoyo et al., 2021; Sehrawat et al., 2021). These microbial functions are being increasingly explored for enhancing crop production in an eco-friendly and sustainable manner.



**Fig 2. Diagrammatic presentation of Mechanisms of action of beneficial microbes**

#### A. Direct mechanisms involved in plant growth promotion

Beneficial bacterial inoculants provide nitrogen, phosphorous, potassium and other plant nutrients to the crop without any chemical input to soil leading to improvement of plant

growth and increase in crop yield (Singh and Gupta, 2018; Tiwari et al., 2018; Vimal et al., 2018; Basu et al., 2021). Moreover, production and excretion of different phytohormones i.e., iAA, gibberellins (GA) and cytokinins have been reported to increase the root surface area for more adsorption of plant nutrients from the soil (Jangu and Sindhu, 2011; Duca et al., 2014; Khan et al., 2016; 2020).

- i. **Enhanced Nutrient Availability:** Plants require around 16 essential micro- and macro-nutrients for proper growth, and their availability is influenced by soil type, climate, and crop variety. Soil microbes, especially in the rhizosphere, enhance nutrient availability through nitrogen fixation, solubilization of phosphate, potassium, and zinc, and production of phytohormones (Richardson et al., 2009; Sehrawat and Sindhu, 2019). Mycorrhizal fungi and PGPR play a critical role in nutrient mobilization and stress mitigation (Santoyo et al., 2021a). These microbes improve plant metabolism, alter root exudates, and enhance interaction with other soil organisms (Adesemoye and Kloepper, 2009). Inoculation with *Azotobacter chroococcum* has shown increased nitrogen and phosphorus uptake in maize, leading to improved growth and yield (Song et al., 2021).
- ii. **Phytohormone production:** Plants and certain beneficial bacteria synthesize phytohormones in minute concentrations that regulate key physiological processes such as root and shoot growth, flowering, senescence, seed development, cell division, gene expression, and stress responses (Jangu & Sindhu, 2011; Khan et al., 2020). These hormones enhance root surface area and root hair length, thereby improving nutrient and water uptake (Tsegaye et al., 2017). In stressful conditions, plant growth-promoting rhizobacteria (PGPR) either produce phytohormones or modulate their levels within the plant, aiding in defense, metabolism, and abiotic stress management (Malik & Sindhu, 2011; Khan et al., 2020). Major phytohormones include auxins, cytokinins, gibberellins, ethylene, and abscisic acid, while other hormones like jasmonates, brassinosteroids, and strigolactones are also involved in stress regulation (Cassán et al., 2014). Most PGPRs are known to produce auxins, cytokinins, and ethylene, but only a few strains can synthesize gibberellins (van Loon, 2007; Egamberdieva et al., 2017; Abd Allah et al., 2018).

## B. indirect mechanisms

Plant pathogens such as bacteria, fungi, and viruses significantly reduce global crop yields, causing annual losses of 20–40% in cereals and legumes (Oerke, 2006). Overuse of chemical pesticides for controlling these diseases has led to environmental pollution and health

risks. As an eco-friendly alternative, antagonistic microorganisms are being explored as biopesticides to boost crop production (Santoyo et al., 2012; Anand et al., 2020; Jiao et al., 2021; Wang et al., 2022). These microbes promote plant health and suppress pathogens through multiple mechanisms including production of siderophores, hydrolytic enzymes, antibiotics, volatile organic compounds (VOCs), hydrogen cyanide, and by inducing systemic resistance in plants (Sehrawat & Sindhu, 2019; Sharma et al., 2019; Khanna et al., 2021). These biocontrol mechanisms not only reduce disease incidence but also contribute to enhanced plant growth and yield.

**I. Siderophore production:** iron is an essential element for plant metabolism, especially in respiration and photosynthesis, but in aerobic soils, it primarily exists as  $Fe^{3+}$ , which is poorly available to plants due to its tendency to form insoluble hydroxides and oxyhydroxides (Zuo & Zhang, 2011; Pahari & Mishra, 2017). Microorganisms secrete low-molecular-weight compounds known as siderophores, which chelate  $Fe^{3+}$  and convert it into the more absorbable  $Fe^{2+}$  form, thereby enhancing iron uptake by plants (Kashyap et al., 2017; Rasouli-Sadaghiani et al., 2014). These siderophores, rich in electron-donating atoms like oxygen and nitrogen, also help in the acquisition of other micronutrients such as molybdenum and vanadium, especially in nitrogen-fixing microbes like *Azotobacter vinelandii* (McRose et al., 2017).

Siderophores are produced by various beneficial microbes including *Pseudomonas*, *Azotobacter*, *Bacillus*, *Rhizobium*, and *Streptomyces*, contributing to both plant growth and biocontrol (Sahu & Sindhu, 2011; Sultana et al., 2021). Specifically, fluorescent *Pseudomonas* species have been shown to enhance iron nutrition in graminaceous and dicot plants and reduce disease incidence by strengthening structural tissues such as the sclerenchymatous sheath in maize, leading to improved yield and overall plant health (Shirley et al., 2011).

**II. Enzyme production:** Microbial extracellular enzymes regulate metabolic activities by breaking down complex biomolecules, aiding in carbon cycling, bioremediation, and plant growth promotion (Burns et al., 2013). Plant Growth-Promoting Rhizobacteria (PGPR) like *Pseudomonas*, *Bacillus*, *Xanthomonas*, and *Agrobacterium* produce enzymes such as proteases and lipases (Ghodsalavi et al., 2013). Under abiotic stress, antioxidant enzymes including ascorbate peroxidase (APX), catalase (CAT), glutathione peroxidase (GPX), and superoxide dismutase (MnSOD) help in stress mitigation (Willekens et al., 1995; Bharti et al., 2016). Hydrogen peroxide also acts as a signaling molecule in stress and growth processes (Sofa et al.,

2015). While these enzymes support plant health, inconsistent results in field conditions are due to complex interactions between plants, microbes, and environmental factors.

**III. Antibiotic production:** Soil harbors a vast diversity of microbes—commensals, pathogens, and symbionts—creating intense competition for nutrients and space (Mendes et al., 2013). To survive, microbes evolve various strategies, with antibiotic production being a common and effective one (Sehrawat and Sindhu, 2019; Jiao et al., 2021). Antibiotics, which are low-molecular-weight toxic compounds, help microbes suppress competitors. They may be volatile (e.g., aldehydes, ketones) or non-volatile (e.g., phenylpyrroles, cyclic lipopeptides) (Gouda et al., 2017). Besides antimicrobial activity, antibiotics can exhibit antiviral, antioxidant, antitumor, and even plant growth-promoting properties when used in low concentrations (Kim, 2012).

**IV. induced systemic resistance:** Plants defend themselves against pathogens using mechanisms like induced Systemic Resistance (iSR) and Systemic Acquired Resistance (SAR), primarily activated through jasmonate and ethylene signaling pathways (Pangesti et al., 2016). iSR is triggered by microbial molecules such as flagellin, chitin, siderophores, and salicylic acid, with biocontrol agents (e.g., *Bacillus*, *Pseudomonas*, *Serratia*) using PAMPs, MAMPs, and elicitor molecules like VOCs and miRNAs to stimulate defense responses (Doornbos et al., 2012; Rodriguez et al., 2019). iSR can enhance resistance in non-host specific ways, as shown in tomato plants treated with *Bacillus amyloliquefaciens* (Beris et al., 2018) and *Pseudomonas aeruginosa* (Kousar et al., 2020). *Serratia marcescens* also induces resistance in cucumber against multiple pathogens via siderophore production (Press et al., 2001). These responses lead to callose and lignin deposition, stress gene expression, enzyme activation (e.g., chitinase, PPO), and phytoalexin production (Heil and Bostock, 2002). For instance, salicylic acid in poplar plants enhances catechin synthesis to suppress foliar pathogens (Ullah et al., 2019b).

## Conclusion

The excessive and indiscriminate use of chemical fertilizers, especially phosphorus, has led to nutrient buildup and degradation of soil health, threatening long-term agricultural sustainability. In this context, the development and promotion of efficient biofertilizers offer a viable solution to reduce dependency on synthetic inputs and mitigate environmental pollution. Achieving this requires collaborative, interdisciplinary research involving soil microbiologists, agronomists, plant breeders, and economists. While biofertilizers like *Azotobacter*, *Azospirillum*, phosphate-solubilizing bacteria, and arbuscular mycorrhizal fungi hold immense promise, their potential remains underutilized.

Strengthening awareness, enhancing production capabilities, supporting startups, and digitizing the supply chain are crucial for scaling biofertilizer adoption. Though initiatives began during the Seventh Five-Year Plan, the current pro-agriculture policy environment presents an ideal opportunity to consolidate efforts for sustainable, eco-friendly farming practices.

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