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# Why Do We Fail to Harness the Benefits of Bio-Fertilizer Use in The Agricultural Production System

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**ABSTRACT**

Bio-fertilizers and beneficial microbes have been used in crop cultivation systems for the last 6 to 7 decades to minimise the use of inorganic farm inputs in agriculture, but have not transformed agriculture holistically into an organic-based crop cultivation system. The obvious reasons seem to be associated with the developments and transfer of this bio-fertilizer use technology to geographically different areas, where these microbes may be alien, facing soil ecological, soil environmental, and soil biological challenges. In this paper, all such challenges are discussed, which may be useful for further refinement and proper use of this bio-fertilizer and beneficial microbes' use technology, to have an organic crop cultivation system for healthy food on this globe.

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## Introduction

Challenges for agricultural crop cultivation may be numerous, but the major challenge is to produce food without the use of inorganic farm inputs, ensuring that the food is safe and pesticide-residue free for public consumption, and promoting better nourishment and health. Nature has its own system of recycling the natural waste through the use of pertinent microbes in that ecosystem, to support the natural vegetation and plant growth. Research scientists have developed such microbes into microbial formulations to be used as various farming-related inputs or in activities such as nitrogen fixation, phosphorus solubilization, potassium mineralization, soil remediation, plant growth-promoting rhizobia, or biocontrol agents [1]. These microbial use technologies have been promoted over the last 6 to 7 decades, but have not had much impact, and therefore could not replace inorganic farm inputs in agricultural crop cultivation. It is therefore obvious to know the reasons for the non-performance of these microbial technologies in a larger scenario when compared to the use of these microbes in their natural ecosystems.

Several reasons, as discussed below, appear to be playing a role in the non-performance of these microbial formulations as bio-fertilizers, which need attention for their refinement and use.

## Bio-Fertilizers are Crop-Specific

Various types of beneficial microbial inoculants are used in agricultural production systems as bio-fertilizers [2]. The beneficial microbes, which are used as nitrogen-fixing bio-fertilizers, are crop-specific and are tailored to enhance nutrient uptake and growth for particular crops. For example, *Rhizobium* is used for leguminous crops like pulses, while *Azotobacter* and *Azospirillum* are suitable for cereals, vegetables, and oilseeds. Phosphate-solubilizing biofertilizers can be used to solubilize fixed phosphorus in the soil to make it available to the plants. Bio-fertilizers work through various mechanisms like nitrogen fixation, phosphate solubilization, demineralization, and plant growth hormone production. Furthermore, the nitrogen fixer, particularly *Rhizobium* on legumes, is legume crop-specific [3]. Both legumes and rhizobia bacteria are phylogenetically diverse, and no single rhizobial strain can form a symbiotic relationship with all legumes, and vice versa. Specificity occurs at both species and genotypic levels in this symbiotic association [4,5]. For example, Soybean root nodulates with *Bradyrhizobium japonicum*, Alfalfa root nodules with *Sinorhizobium melilotii*, while Peas root commonly nodulates with *Rhizobium leguminosarum* bv. viceae. A *Rhizobium* strain specific for chickpea does not fix nitrogen in cowpea or vice versa. Another nitrogen fixer, like *Azotobacter* or *Azospirillium*, is also crop specific, in the sense that a crop-specific strain fixes more nitrogen in its host crop than the other crop from the same family [6]. Therefore, one species/strain of bio-fertilizer microbial inoculant can't be guaranteed as equally effective in other crops or species of crops. Therefore, it is always advisable to use the crop-specific strains of bio-fertilizers rather than a general strain. The general crop specificity of bio-fertilizer is given in Table 1.

**Table 1: Crop-Specific Biofertilizer Microbes**

| Sr.No | Biofertilizer MIcrobe | Crop Type | Efficient for the Crop Plant |
|---|---|---|---|
| 1 | *Rhizobium* strains | Legume and Oilseed | Pulses, soybeans, Groundnut |
| 2 | *Azotobacter* and *Azospirillum* | Cereals, Oilseeds, Vegetables | Wheat, Rice, Maize, Sorghum, Cotton, Mustard |
| 3 | Azolla | Cereal | Lowland Rice |
| 4 | BGA | Cereal | Transplanted Rice |
| 5 | *Acetobacter* | Perennial grass | Sugarcane |
| 6 | Mycorrhiza | Fruit Crops and Ornamentals | Mango, Palm |

Besides these, several other microbes play their role in phosphorus solubilization, potassium mobilization, and production of plant growth-promoting hormones. *Bacillus* and *Aspergillus* species can solubilize phosphate, while Bacillus can also mobilize potassium.

**Parasitism of Bio-Fertilizer Microbes in Alien Soils**

Parasitism is a known phenomenon in the soil microbial world [7]. Alien soils are those soils where the bio-fertilizer inoculant is applied but have not been previously tested for their efficacy and compatibility with the native soil microflora. It is known that certain microbial species parasitize other microbial species for their food requirement and survival. In the known ecological soil environment, different microbial species may live symbiotically or commensally, but when these are placed in an alien or different ecological soil environment, the interaction of these microbial species with the native soil microbes may be different or is not known. This is the case with the beneficial microbial species also. When these beneficial microbial species are added to alien soils, they may succumb to parasitism by native soil microbial strains. This parasitism can significantly reduce the effectiveness of bio-fertilizer inoculants. If the beneficial microorganism is attacked by a parasite, it may not be able to colonize the plant roots, fix nitrogen, or perform other beneficial functions as effectively as it would do in the absence of the parasite.

Understanding parasitism is crucial for selecting and developing effective bio-fertilizer strains for alien soils. Scientists need to identify bio-fertilizer strains that are less susceptible to parasitism or find ways to manage parasites to ensure the biofertilizer's success across different regions and areas in the country. For example, *Mesorhizobium ciceri* is a prevalent species as a bio-nitrogen fixer in chickpea around the world, except in China, where *M. muleiense* is the main species associated with chickpea. Different chickpea rhizobial inoculations of *M. muleiense* and *M. ciceri* affected the rhizosphere microbial composition in different sampling soils. Through high-throughput sequencing and statistical analysis, it was found that *Pseudomonas* was the key microorganism influencing the competitive nodulation of different chickpea rhizobia in Xinjiang rhizosphere soils, indicating that the bacterium *Pseudomonas* was a parasite of nitrogen-fixing rhizobia in this region [8].

Some fungi like *Acremonium sclerotigenum* have been shown to parasitize *Azotobacter* cells, potentially impacting their nitrogen fixation and survival. A strain of *Bdellovibrio bacteriovorus* isolated from soil as a parasite of *Escherichia coli* was shown to produce plaques on *Azotobacter* chroococcum but not on *Azotobacter vinelandii* or *Rhizobium species*, indicating the specificity in parasitism. Sullivan & Casida; Postgate reported that deliberate enrichment of garden soil led to the isolation of *Achromobacter* capable of lysing various *Azotobacteriaceae* on agar plates [9,10]. Two strains out of seven of *Pseudomonas aeruginosa* showed some degree of lytic action, indicating their parasitic nature. Campbell & Hoffer reported the special medium for the growth of *Rhizobium leguminosarum* and its bacteriophage and observed the bacteriophage interaction with the *Rhizobium* cell [11].

Further research into these biofertilizer parasitisms is needed to optimize biofertilizers use in sustainable agriculture.

**Ecology vs Bio-Fertilizer Microbes' Efficiency**

Ecology is the study of how living organisms interact with each other and their environment. It encompasses the relationships between organisms, their populations, communities, and the larger ecosystems they inhabit [12]. Ecology examines the relationships between living organisms (biotic factors) and their non-living surroundings (abiotic factors). Organismal ecology focuses on how individual organisms adapt to their environment. In this case, the biofertilizer microbes to their soil environments [13]. Soil environmental factors significantly impact biofertilizer efficiency. Key factors include soil moisture, temperature, pH, organic matter, and the presence of other microorganisms. These factors can affect biofertilizer survival, colonization, and nutrient solubilization or fixation, thus influencing crop yields [14].

Adequate moisture is essential for the survival and activity of biofertilizers, as many beneficial microbes depend on it for their metabolic processes and movement in the soil [15]. Similarly, soil temperature influences microbial growth, activity, and biomass [16]. Extreme temperatures, whether too hot or too cold, can inhibit or even kill biofertilizer microorganisms. Soil pH also affects nutrient availability and microorganism survival. Biofertilizers have specific pH optima for their activity, and deviations from these can decrease their effectiveness. Organic matter serves as a food source for microorganisms and improves soil structure, which enhances nutrient availability and microbial activity [17]. A healthy soil with enough organic matter is more favorable for biofertilizer performance.

The presence and abundance of other microorganisms in the soil can influence the success of biofertilizers. Some microorganisms may compete for resources or even inhibit the growth of biofertilizer strains. Proper soil preparation and suitable application methods are vital for biofertilizer success. Factors like soil compaction and aeration can impact microbial activity. Biofertilizers work best when the soil contains adequate levels of other essential nutrients. For example, nitrogen-fixing bacteria need molybdenum and other micronutrients to operate at their best.

The soil ecology plays a crucial role in the effectiveness of *Rhizobium* biofertilizers. Factors like soil pH, temperature, moisture, and nutrient levels significantly impact *Rhizobium*'s ability to colonize plant roots, form nodules, and fix nitrogen. Most *Rhizobium* species thrive in neutral to slightly alkaline soils. Acidic soils can hinder *Rhizobium* survival, movement, and the chemical signalling necessary for nodulation. Soil temperature affects nodule formation. Cold soils can slow down *Rhizobium* activity and nodule development early in the season. Adequate soil moisture is essential for *Rhizobium*'s movement and survival, while good aeration ensures oxygen availability for nitrogen fixation. While *Rhizobium* helps fix nitrogen, high levels of available nitrogen (from fertilizers or manure) can suppress nodulation as plants prefer to uptake readily available nitrogen. Soil organic matter provides a habitat and energy source for *Rhizobium*, contributing to their survival and activity. Phosphorus and other nutrients are crucial for both *Rhizobium* and plant growth. Deficiencies in these nutrients can hinder nodulation and nitrogen fixation. Thus, understanding and managing soil conditions is crucial for maximizing the benefits of *Rhizobium* biofertilizers. Factors like pH, temperature, moisture, and nutrient availability directly influence *Rhizobium*'s ability to colonize roots, form nodules, and fix nitrogen, ultimately impacting plant growth and soil health. Wang et.al reported that soil property impacts the efficacy of rhizobial inoculation and rhizosphere processes [18]. In their studies, the soil pH determined the effectiveness of rhizobial inoculation. Low soil P availability suppressed soybean nodulation and N fixation. Inoculation enhanced the secretion of carboxylates

and P uptake in calcareous soil. The beneficial effects of rhizobial inoculation were pronounced in calcareous soil.
*Azotobacter*, another nitrogen-fixing bacterium, thrives in specific soil conditions [19]. *Azotobacter* prefers a neutral to slightly alkaline pH (6.5-7.5). Acidic soils (below pH 6) can inhibit or even prevent its growth. The optimal temperature range for *Azotobacter* is 20-30°C. While it can survive at higher temperatures (up to 45-48°C) by forming cysts, its activity is significantly reduced. As an obligate aerobe, *Azotobacter* requires oxygen for nitrogen fixation. It has adaptations like high respiration rates to utilize oxygen efficiently. Adequate soil moisture is necessary for *Azotobacter* activity. However, excessive waterlogging can create anaerobic conditions, hindering its growth. High salt concentrations can inhibit *Azotobacter*'s growth and nitrogen fixation, though some species like *A. salinestris* exhibit tolerance to moderate salt levels. Decomposing organic matter can provide a carbon source for *Azotobacter*, potentially enhancing its growth and nitrogen fixation. However, excessive carbon input can also inhibit nitrogen fixation if it leads to nitrogen deficiency. Soil moisture levels can influence the activity of *Azotobacter.* Dry conditions may favour the survival of *Azotobacter* cyst, while wetter conditions might favour the activity of the parasite.

The effectiveness of *Azospirillum* as a biofertilizer is significantly influenced by the soil properties [20]. *Azospirillum* thrives in a range of soil conditions, but its nitrogen fixation activity and overall growth are optimized under specific environmental parameters. *Azospirillum* species generally prefer slightly acidic to neutral pH conditions, with some species exhibiting tolerance to more acidic conditions, while very acidic soils (pH < 4.0) can reduce nitrogen-fixing activity. *Azospirillum* can also thrive and fix nitrogen even in acid-sulfate saline soils with low pH. Soil redox potential is an important factor for the growth and activity of *Azospirillum*. *Azospirillum* bacteria are microaerophilic, meaning they prefer environments with low oxygen levels. Their nitrogen-fixing activity is closely linked to soil redox potential, with optimal activity observed between -50 to -150mV. Waterlogging can negatively impact *Azospirillum* activity by decreasing redox potential. The presence of organic matter in the soil, such as rice straw, can enhance *Azospirillum* populations and nitrogen fixation activity, especially under submerged conditions. Azospirillum thrives in temperate to tropical environments. The availability of carbon sources and other nutrients can influence *Azospirillum* growth and activity. *Azospirillum* forms symbiotic relationships with plants, and the host plant's ability to provide a suitable rhizosphere environment (low oxygen pressure, readily available photosynthates) is crucial for nitrogen fixation. Thus, understanding the soil environment and its impact on *Azospirillum* is crucial for maximizing the benefits of this biofertilizer.

*Acetobacter* is another nitrogen-fixing biofertilizer for several crops like sugarcane, rice, and coffee, etc, is also affected by various soil conditions, including moisture, temperature, pH, and nutrient availability [21-23]. Optimal soil conditions are essential for the survival, activity, and effectiveness of Acetobacter in nitrogen fixation and promoting plant growth. Acetobacter thrives in moist soil, but excessive moisture, such as in waterlogged conditions, can be detrimental, reducing its effectiveness and potentially harming soil health. Extreme temperatures, both high and low, can negatively impact *Acetobacter*'s performance and survival. The ideal pH range for *Acetobacter* is generally neutral to slightly alkaline, but it can vary depending on the specific species or strain. Adequate levels of essential nutrients, particularly nitrogen, phosphorus, and potassium, are crucial for *Acetobacter*'s growth and nitrogen fixation activity. Good soil aeration is important for *Acetobacter*'s respiration and overall activity. Higher levels of soil organic matter can support a more diverse microbial community, potentially benefiting *Acetobacter*. Different soil types can have varying water retention, aeration, and nutrient availability, influencing *Acetobacter*'s effectiveness.

Thus, ecological parameters and the soil environment, which vary at different geographical locations, need to be considered when using biofertilizer inoculants to derive their full benefits.

**Sustainability of Bio-Fertilizer Microbes in An Alien Soil Environment**
The sustainability of biofertilizer in an alien soil environment is an important issue [24]. In the R&D program, these bio-fertilizers are developed and tested in particular soil environments at specific locations, trials, or sometimes in multi-location trials. The yield potentials of these bio-fertilizers in multi-location trials are never the same and are observed to be variable due to different factors like soil ecology, soil environment, soil biology, soil properties, soil nutrient richness, and soil microbiology. Thus, one strain of bio-fertilizer can work differently in different soil environments and different ecological locations. This means that the bio-fertilizer strains developed for a particular location may sometimes be more competitive in that location than the alien strain formulated for some other soil and ecological environment.

Location-specific or ecological environment-specific bio-fertilizer strains are not yet available for the farmers. Thus, the sustainability and efficacy of bio-fertilizer microbes in an alien environment are uncertain, and this poses a significant challenge in bio-fertilizer development technology.

**Biofertilizer's Activity Depends on The Root-Microbe Recognition**
Plant root-beneficial microbe recognition is crucial for the effectiveness of bio-fertilizers, as it allows plants to select and interact with beneficial microorganisms in the soil, leading to improved nutrient uptake and overall plant health [25]. This recognition process involves plant roots releasing signals, like root exudates, that attract specific microbes, like *Rhizobium* and mycorrhizal fungi, which then form symbiotic relationships with the plant. Plant roots release a variety of organic compounds, including sugars, amino acids, and other signalling molecules, into the surrounding soil. These exudates act as chemical signals, influencing the composition and activity of the microbial community in the rhizosphere. Plants can selectively allow specific microbes based on their needs and the environment for this association. For example, leguminous plants will release signals that attract *Rhizobium* bacteria or other rhizobia for their non-legume interaction, which are essential for nitrogen fixation [26]. Similarly, mycorrhizal fungi, particularly arbuscular mycorrhizal (AM) fungi, are attracted to root exudates of certain plants to form symbiotic relationships with plant roots. These fungi enhance nutrient and water uptake, especially phosphorus, and improve plant resistance to stress. Once attracted to the roots, *Rhizobium* bacteria can penetrate the root hairs and form nodules, which are specialized structures where nitrogen fixation occurs. The plant provides the bacteria with carbon and energy, and in return, the bacteria convert atmospheric nitrogen into a usable form for the plant. AM fungi colonize the root cells and extend their hyphae into the surrounding soil. These hyphae act as an extension of the plant's root system, increasing the surface area for nutrient and water absorption.

Plant roots also interact with other beneficial microbes, such as phosphate-solubilizing bacteria, which can convert insoluble phosphorus into a soluble form that plants can absorb. By selectively recruiting beneficial microbes, plants can optimize their nutrient acquisition, reducing their reliance on chemical fertilizers. Furthermore, the symbiotic relationships with microbes can enhance plant resistance to various abiotic stresses, such as drought, salinity, and nutrient deficiencies, as well as biotic stresses like pathogens. Interactions between plants and microbes contribute to the overall health of the soil ecosystem, promoting soil aggregation, nutrient cycling, and microbial diversity. Bio-fertilizers, which are microbial inoculants, rely on the ability of these beneficial microbes to recognize and interact with their host plant roots, forming symbiotic relationships that enhance plant growth and nutrient uptake. Microbes, particularly those involved in nitrogen fixation like *Rhizobium* and mycorrhizal fungi, exhibit a degree of host specificity, meaning certain microbial strains are more effective with specific plant species or even varieties. Plants and microbes communicate through chemical signals. For instance, *Rhizobium* bacteria recognize and respond to flavonoids released by legume roots, triggering nodule formation. Similarly, mycorrhizal fungi are attracted to and recognize plant root exudates, leading to fungal colonization and nutrient exchange [27]. The recognition process leads to the formation of symbiotic structures like root nodules in legumes (where *Rhizobium* bacteria fix nitrogen) and mycorrhizae (where fungal hyphae colonize plant roots and enhance nutrient uptake). *Rhizobium* species are well-known for forming symbiotic relationships with legumes, while mycorrhizal fungi, such as arbuscular mycorrhizal fungi (AMF), form associations with a wide range of plant species, enhancing their access to phosphorus and other nutrients.

Microbes in the rhizosphere can also promote plant growth by producing plant hormones, solubilizing nutrients, or protecting plants from pathogens.

Understanding these interactions is crucial for developing effective bio-fertilizers [28]. The ability of microbes to recognize and interact with plant roots is fundamental to the functioning of bio-fertilizers in a given soil ecosystem and the overall sustainability of agricultural systems.

## Survival of Bio-Fertilizer Microbes in Different Geographical Soils

The concept of "geo-specificity" is an important consideration in the formulation of biofertilizer inoculants [29]. It highlights that rhizobia from different geographical regions can have varying compatibility with the same legume crop. This suggests that factors beyond species-level specificity, such as environmental conditions, can influence the *rhizobium*-legume interaction. Bio-fertilizers, which are microbial inoculants, face challenges in surviving and functioning effectively across diverse geographical soils due to variations in soil properties like pH, temperature, nutrient availability, and the presence of native microorganisms. Optimizing bio-fertilizer performance requires an understanding of these soil environmental factors and selecting strains that are well-suited to specific soil conditions/environments.

Biofertilizers require specific nutrients for their growth and activity. For example, nitrogen-fixing bacteria need molybdenum, while phosphate-solubilizing bacteria need phosphorus. Adequate moisture is crucial for microbial activity. Desiccation can lead to cell death, while excessive moisture can create anaerobic conditions that are unfavourable for some microorganisms. Sandy soils have poor water retention and aeration, while compacted soils can restrict root growth and nutrient exchange. Native soil microbes can compete with or antagonize introduced bio-fertilizer microbial strains. Some can be beneficial, while others may be detrimental. Bio-fertilizers need to tolerate various stresses like drought, salinity, and heavy metal toxicity, which vary across geographical locations.

Choosing biofertilizer strains that are well-adapted to the specific soil conditions of a particular region is crucial. *Rhizobium*, *Azotobacter*, *Azospirillum*, and *cyanobacteria* fix atmospheric nitrogen, making it available to plants. Their survival is affected by soil pH, nutrient availability, and moisture. Bacillus, *Pseudomonas*, and fungi like *Aspergillus* solubilize insoluble phosphate, making it available to plants. Their survival and activity are influenced by soil pH, phosphorus availability, and the presence of other microorganisms. Some bacteria and fungi can mobilize potassium from insoluble forms. Their survival is affected by soil pH, potassium availability, and the presence of other microorganisms. These fungi form symbiotic relationships with plant roots, enhancing nutrient and water uptake. Their survival is influenced by soil pH, moisture, and the presence of host plants.

By understanding the factors influencing bio-fertilizer survival in different soil types and by employing appropriate strategies to enhance their performance, farmers can harness the benefits of bio-fertilizers for sustainable crop production.

## Soil Character and Bio-Fertilizer Efficiency

The effectiveness of bio-fertilizers is intertwined with various soil properties like soil texture, pH, organic matter content, water content, and aeration, and these all play crucial roles in determining bio-fertilizer efficacy [30]. Bio-fertilizers can be more effective in certain climates, but not in others. For optimal *Rhizobium* efficiency, soils should have a near-neutral to slightly alkaline pH, adequate moisture and aeration, and moderate nitrogen levels. Excessive nitrogen can inhibit nodulation. Acidic soils (low pH) can negatively impact *Rhizobium*, affecting their survival, movement, and the chemical signalling necessary for nodulation [31]. In very acidic soils, aluminium toxicity can also become a problem, further inhibiting nodulation. High nitrate levels can delay or reduce nodulation and even inhibit nitrogenase activity in existing nodules. Waterlogged conditions can reduce oxygen availability, which is also essential for *Rhizobium* activity. Drought can also limit nodulation or cause existing nodules to abort due to plant stress. Low soil temperature or cold soils slow down *Rhizobium* activity and nodule development. Phosphorus and other micronutrients are also important for both *Rhizobium* and the host plant. While *Rhizobium* can tolerate some salinity, very high salinity can be detrimental. The presence of other microorganisms in the soil can also influence *Rhizobium* populations and their effectiveness. *Azotobacter*, a nitrogen-fixing bacterium, thrives in specific soil conditions for optimal efficiency. Calcium and Molybdenum are important for *Azotobacter*'s growth and nitrogen fixation. While *Azotobacter* fixes atmospheric nitrogen, excessive nitrogen in the soil can inhibit its activity. The general characteristics of the soil, such as its texture and organic matter content, can influence *Azotobacter*'s activity. Well-drained soils with adequate organic matter are generally more suitable. By understanding and managing these soil characteristics, farmers and researchers can optimize the conditions for *Azotobacter* to thrive, leading to improved nitrogen fixation and potentially enhanced crop yields. *Azospirillum*, which fixes nitrogen and promotes plant growth, thrives in specific soil conditions. It can tolerate a range of moisture levels; moderate moisture is optimal for its activity. They can be negatively affected by prolonged waterlogging or extreme dryness. The presence of organic matter in the soil is beneficial as it provides a food source for *Azospirillum* and contributes to a healthy soil environment. The excessively high salt concentrations can inhibit its growth and activity. *Azospirillum* prefers well-aerated soils, although it can also tolerate some degree of anaerobic conditions. By providing these favourable soil conditions, farmers can enhance the efficiency of *Azospirillum* and maximize their benefits for plant growth and nitrogen fixation. *Acetobacter*, a genus of bacteria known for its role in nitrogen fixation, thrives in specific soil conditions. This can be provided by decaying organic matter in the soil. *Acetobacter* are aerobic bacteria, meaning they require oxygen for their metabolic processes. Therefore, well-aerated soils with good drainage are more suitable for their growth and activity. The optimal temperature for *Acetobacter* growth varies depending on the specific strain, but generally falls within the range of 25 to 30 °C, with an upper limit around 35 °C. *Acetobacter* also utilizes nitrogen, which can be obtained from various sources in the soil, including organic matter and nitrogen-fixing activity. For optimal Blue-Green Algae (BGA) efficiency in soil, particularly in paddy fields, the ideal soil characteristics include a slightly alkaline pH (around 7.5), with a pH range typically between 7.3 and 8.62 being suitable. Moderate electrical conductivity, which indicates the presence of dissolved salts, is also preferable for BGA growth. A decent amount of organic carbon (around 0.5-1%) in the soil is essential as it provides nutrients and energy for BGA. BGA can fix atmospheric nitrogen, but they also require a certain level of available nitrogen and phosphorus for their growth and activity. Good soil structure, including adequate porosity and water-holding capacity, is crucial for BGA to flourish and perform its functions effectively. Infiltration rate, which refers to how quickly water enters the soil, is also a key factor. Besides these soil characteristics, factors like light availability and temperature also play a role in BGA's efficiency. They need sufficient sunlight for photosynthesis, and their growth is influenced by temperature.

Thus, bio-fertilizers' efficiency is directly linked to the existing soil characteristics and the overall soil health management practices employed.

**Cultural Practices and Bio-Fertilizers' Fate**

Cultural practices significantly influence the effectiveness of bio-fertilizers by impacting microbial activity, nutrient availability, and overall plant health. Practices like proper soil management, including organic matter addition and balanced nutrient application, create a favourable environment for bio-fertilizers to thrive [32]. Furthermore, selecting efficient microbial strains, optimizing application methods, and managing environmental factors like soil moisture and temperature play crucial roles in maximizing bio-fertilizer efficiency. Adding organic matter to the soil, such as compost or cover crops, improves soil structure, water retention, and provides a food source for beneficial microbes, enhancing bio-fertilizer activity. Balancing nutrient availability in the soil is crucial. While bio-fertilizers can supplement nutrients, excessive application of chemical fertilizers can inhibit their effectiveness. A balanced approach, considering both chemical and beneficial microbial inoculants, is ideal. Soil pH affects the activity of microorganisms [33]. Adjusting pH levels to the optimal range for specific bio-fertilizers can significantly improve their performance. Compacted soils restrict root growth and microbial activity. Practices like no-till farming can improve soil structure and enhance bio-fertilizer efficiency. Combining different crops can create a more diverse and stable rhizosphere environment, benefiting bio-fertilizer's activity. For example, certain legume-cereal intercrops can enhance nitrogen fixation. The type of irrigation water significantly influences the effectiveness of bio-fertilizers. Water quality, including factors like salinity and nutrient content, can either enhance or hinder the survival and activity of beneficial microbes in bio-fertilizers, ultimately affecting crop yield and nutrient uptake. High salinity levels in irrigation water can reduce microbial diversity and activity, thus decreasing bio-fertilizer efficacy.

The presence of specific nutrients in irrigation water can either complement or compete with the nutrients provided by biofertilizers, impacting their overall impact. Irrigation methods also influence the bio-fertilizer efficiency. Drip Irrigation and fertigation (applying fertilizer through irrigation water) can improve nutrient distribution and uptake, potentially enhancing bio-fertilizer efficiency. Sprinkler Irrigation can lead to salt deposits affecting plant health and potentially reducing bio-fertilizer effectiveness. Different bio-fertilizers (e.g., nitrogen-fixing bacteria, phosphate solubilizers) may have varying sensitivities to water quality parameters. The water and nutrient requirements of different crops can influence how irrigation water and bio-fertilizers interact to affect plant growth and yield. Irrigation water quality can alter the composition and abundance of microbial communities in the soil, affecting the overall function of bio-fertilizers. Bio-fertilizers can enhance nitrogen fixation, nitrification, and denitrification processes in the soil, but these processes are influenced by water quality and can be disrupted by salinity or other imbalances. Biofertilizers can improve nutrient uptake by plants, but this efficiency can be compromised by poor water quality or improper irrigation practices. Monitoring and managing irrigation water quality is crucial for maximizing bio-fertilizer efficiency. Choosing appropriate irrigation methods and scheduling can ensure adequate water availability for microbial activity and plant growth. Combining bio-fertilizers with chemical fertilizers or organic amendments can help optimize nutrient availability and improve crop productivity. Matching biofertilizer types to specific crop and soil conditions can improve their effectiveness. Thus, the influence of irrigation water on biofertilizer efficiency is multifaceted, requiring careful consideration of water quality, irrigation methods, and biofertilizer characteristics for optimal agricultural outcomes.

Hoeing, or weeding, can positively influence bio-fertilizer efficiency by reducing weed competition for nutrients and water, allowing plants to better utilize the benefits of bio-fertilizers. Hoeing can also improve soil aeration and water infiltration, further enhancing bio-fertilizer effectiveness. Weeds compete with crop plants for essential nutrients and water, diminishing the uptake of nutrients provided by bio-fertilizers. Hoeing removes these weeds, allowing the bio-fertilizer's benefits to be directed towards the intended crop. Hoeing loosens the soil, increasing porosity and improving air circulation. This enhanced aeration can benefit the microorganisms in bio-fertilizers, promoting their growth and activity. Hoeing can improve water infiltration into the soil, ensuring that the bio-fertilizers and the nutrients they release are readily available to plant roots. By reducing weed competition and improving soil conditions, hoeing can lead to greater nutrient availability for the crop, maximizing the impact of bio-fertilizers. A study [34] on fennel found that hand weeding (a form of hoeing) increased fruit yield by 63% and oil yield by 78% compared to an un-weeded control, when bio-fertilizers were also applied. Thus, hoeing can be a valuable tool for enhancing bio-fertilizer efficiency by minimizing weed competition, improving soil conditions, and increasing nutrient availability for crop plants.

**Inorganic Inputs and Bio-Fertilizers' Fate**

Excessive use of chemical fertilizers or pesticides can negatively impact biofertilizers by disrupting the soil microbial community or directly harming the beneficial microorganisms [35-38]. Inorganic inputs, like chemical fertilizers, can have a mixed influence on bio-fertilizer efficiency. While they can provide readily available nutrients that support plant growth, these can also negatively impact the bio-fertilizers. However, when used judiciously and in combination with bio-fertilizers, inorganic inputs can enhance overall nutrient availability and improve crop yields. Inorganic fertilizers can quickly supply essential nutrients like nitrogen, phosphorus, and potassium, which can be beneficial, especially in the early stages of plant growth. When bio-fertilizers and inorganic fertilizers are used together, they can create a synergistic effect, where the benefits of both are amplified. Studies have shown that combining bio-fertilizers with inorganic fertilizers can lead to higher crop yields compared to using either alone [39]. Inorganic inputs also have a negative impact. High concentrations of inorganic fertilizers can negatively impact the population and activity of beneficial soil microorganisms, reducing the effectiveness of bio-fertilizers. Overuse of inorganic fertilizers can lead to soil acidification, reduced organic matter, and imbalances in soil pH, negatively impacting long-term soil health and sustainability. Inorganic fertilizers can contribute to environmental problems like water pollution through runoff and leaching. A balanced approach that combines the immediate nutrient supply of inorganic fertilizers with the long-term benefits of bio-fertilizers and organic matter is crucial for sustainable agriculture. Integrated Nutrient Management (INM) strategies that utilize a combination of organic, inorganic, and bio-fertilizers can optimize nutrient use efficiency, reduce reliance on chemical fertilizers, and improve soil health. Regular soil testing and monitoring can help determine the appropriate type and amounts of inorganic fertilizers to use in conjunction with bio-fertilizers, minimizing negative impacts and maximizing benefits.

Soil herbicides can negatively impact bio-fertilizers' efficiency by inhibiting the growth and activity of beneficial soil microorganisms, which are crucial for nutrient cycling and plant growth. While some herbicides may have minimal short-term effects, others can significantly reduce soil enzyme activities, slow down nitrogen mineralization, and disrupt beneficial microbial relationships. This can ultimately lead to reduced nutrient availability for plants and decreased bio-fertilizers' effectiveness. Herbicides can directly inhibit or kill beneficial bacteria and fungi in the soil, including those involved in nitrogen fixation, phosphorus solubilization, and decomposition of organic matter. By harming these microorganisms, herbicides can disrupt the natural processes of nutrient cycling, such as nitrogen mineralization, which is essential for plant growth. The long-term use of herbicides can lead to a decline in overall soil health, impacting soil structure, water retention, and other crucial properties. Different herbicides have varying degrees of toxicity to soil microorganisms. The concentration of the herbicide also plays a significant role. Factors like soil type, moisture, and temperature can influence how herbicides affect soil microorganisms. The way herbicides are applied (e.g., soil drench, foliar spray) can affect their impact on soil microorganisms. Careful consideration of herbicide selection, application methods, and integrated weed management strategies is essential to minimize these negative effects and maximize the benefits of bio-fertilizers.

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