

# Role of bio-fertilizers towards sustainable agricultural development: A review

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## Abstract

As bioinoculants, numerous eco-friendly microorganisms with a wide range of products are regularly utilised to improve the soil's potential and provide the host plant with the nutrients it requires. The inorganic chemical-based fertilisers employed in the soil management practises are a serious threat to both human health and the environment. Biofertilizers are alternatives that are used in sustainable agriculture to increase soil fertility and crop productivity. The use of beneficial microorganisms as bio-fertilizers has become crucial in the agricultural sector due to its potential impact on food safety and sustainable crop production. The numerous bacteria used in bioinoculant formulations, the carrier materials used, and the applications of biofertilizers are the main subjects of this paper. In especially in appropriate farming, bio-fertilizers are essential for maintaining soil fertility over the long term and crop production sustainability.

**Keywords:** Biofertilizers, Soil Fertility, Microorganisms, Sustainable Farming, Crop Production

## 1 INTRODUCTION

Sustainability of agriculture is important, for the production of large quantities and good quality crops without damaging the soil so it does not affect the crops grown afterward. It is a challenging issue to provide food for the whole world population, another challenging issue faced by the agricultural field is providing fodder to livestock and providing raw material for industry. The green revolution initially increased the production of crops but it degraded the

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quality of soil by increasing its pH, disturbing the microflora of the soil, the pesticides and insecticides polluted waterbodies and killed earthworms which were useful in maintaining the soil quality, it increased the susceptibility to abiotic stress and thus famine can be seen. The GRT became dangerous to sustainability (Getahun Hassen November 2021). After the researcher noticed this problem, many sustainable concepts were put out. Their main goals were to abolish chemical fertilizers as they produced greenhouse gas NO<sub>2</sub>, degraded soil quality, and many antagonists' effects on the soil. The current agricultural field is facing many abnormalities because of global warming like the unpredictable rainfall phenomenon, which is causing damage to the crops.

Sustainable agricultural production mainly focuses on maintaining soil health, abolishing chemical fertilizers, pesticides and insecticides as it also affects human health, crops produced must be stress-free, and the natural flora and fauna of the soil.

Utilizing bioinoculants is one of the sustainable agricultural production methods. Bioinoculants are nutrient-rich microorganisms that are utilised to increase the soil's potential and host plants' nutrient needs. Bioinoculants are the biofertilizers and bio controllers. The living things can be fungi, protists, or bacteria. These microbes help in maintaining the elemental cycle like carbon, nitrogen, and even phosphorous. It helps to increase the potential of soil without damaging it.

## **2 TYPES OF INOCULANTS**

### **2.1 Bacterial inoculants**

Rhizobacteria are the commonly applied inoculants which includes nitrogen fixers, phosphate solubliser and other root associated beneficial bacteria. (S.L. Sivapriya et al., 2017) These bacteria are commonly said as plant growth promoting rhizobacteria (PGPR) as they enhance the availability of the macronutrients. (Jupinder Kaur, 2022)

### **2.2 Nitrogen fixing bacteria**

Rhizobium is that the most typically applied rhizobacteria. They're nitrogen fixing bacteria which forms symbiotic associations with the basis nodules of legume plants. This enhances the nitrogen availability and increases the cultivation of soy beans, chickpeas and lots of other leguminous crops.(Jupinder Kaur, 2022) Azospirillum has been proven to be beneficial in certain cases for nitrogen fixation and plant nutrition as in for non leguminous crops.( Aremu BR et al., 2017)

### 2.3 Phosphate solubilising bacteria

The phosphate solubilising bacteria (PSB) namely *Agrobacterium radiobacter* has gained enough attention because it enriches the phosphorous nutrition. (Bahadur I et al., 2016)

### 2.4 Fungal inoculants

The most common symbiotic relationships between fungi and host plant roots are brought up as Mycorrhiza association. (Ma Y, 2019) This symbiotic relationship is sort of seen altogether land plants, which provides survival advantages to both the host plant and fungi. The fungi increase the root absorptive area of the host plant for successively the plant provides 5<sup>to</sup> 30% of its energy production. (Malusa E et al., 2012) Arbuscular mycorrhizae and Ectomycorrhizae are the two most typical mycorrhizae. Ectomycorrhiza associations are most commonly found in woody species.

### 2.5 Arbuscular mycorrhiza (AM)

Arbuscular mycorrhiza for its ability to access and supply the host plant phosphorous nutrition has grabbed attention as a potential agriculture amendment. Inoculation mixture of Arbuscular fungi and Rhizobacteria has reduced fertilization greenhouse system. Tomato yields that got from 100% fertility were attained at 70% fertility. (Malusa E et al., 2012) Nutrient pollution are often overcome by this 30% reduction in fertilizer application and moreover it also aid within the prolong finite mineral resources such as phosphorous. (Malusa E et al., 2012)

### 2.6 Fungal partners

Fungal inoculation when paired with other amendments can further improvise the nutritional conditions. The most common household amendment for private gardens, agriculture and nurseries is Arbuscular mycorrhizal inoculation combined with compost. Higher yield and maturation in upland paddy is that the result of Arbuscular mycorrhizal inoculation paired with plant growth promoting bacteria. (P.B.B.N. Charyulu 2019 ) It's been observed that the pairing can even promote microbial functions in soils that are affected by mining. Likewise, Maize growth has been improvised by an amendment of Arbuscular mycorrhiza and Biochar. (Malusa E et al., 2012)

### 3 PREPARATION OF BIOINOCULANTS

#### 3.1 Preparation of Solid Bioinoculant

A solid carrier and the beneficial strain are combined to create a solid bioinoculant. Using this, strain can be moved from the lab to the fields. For this preparation, peat is primarily employed in powder and granule form. The bacteria that create microcolonies are given a safe and nourishing home by peat. The peat is blended with the appropriate strain after being dried and put through sieves measuring 250  $\mu$ m. For the growth of bacteria, peat is incubated at a particular temperature. Inoculants for ectomycorrhizal and arbuscular mycorrhizal fungi are primarily made from peat. Because ectomycorrhiza grows quickly and produces spores on glucose media, it is frequently utilised for inoculation in the field. By producing fulvic acid, this preparation accelerates the chelating process (Ceglie et al. 2015). With the use of technology, infected peat is applied to the surface of the seeds before planting. Peat's composition and quality fluctuation is its biggest disadvantage. It's possible for peat to release harmful substances when being sterilised. Due to granules' advantages over peat, they have recently been employed in place of the latter. Granules are less messy, easier to handle, transport, and store than powders (Twinkle Chaudhary 2020). Granular inoculants have a big benefit in stressful environments, such as those with high soil acidity, low soil moisture, and wet soil, since they have a high capacity for nitrogen fixation and nodule development. It promotes bioinoculant survival and is non-toxic and environmentally safe. The advantage of employing charcoal is that, because of its low water content, it may be stored without having to be sterilized. It offers an effective assurance of plant health for crop fields and is devoid of waxy materials and blending issues. (Mandeep Dixit 2020 )

#### 3.2 Preparation of Liquid Bioinoculants

the liquid state the term "preparation" refers to a microbial mixture containing those advantageous microorganisms that can biologically mobilise, fix, or solubilize crucial plant nutrients (Bahadur et al. 2016). In the liquid preparation, bacteria that mobilise potash, phosphate, nitrogen, and other elements are utilised. Due to its advantages over traditional carrier-based formulation, the liquid form of the preparation is the most cutting-edge and promising technology (Surendra and Baby 2016). The carrier-based formulation has a limited shelf life and cannot maintain throughout the crop cycle. This issue is solved by the liquid preparation, which gives the facility the ability to extend the chosen strain's survival rate over the course of the crop cycle. The preparation's endurance to temperature and stress, which carrier-based bioinoculants are

not capable of, is another benefit. In contrast to carrier-based formulations, where bulk sterilisation is not practicable, adequate sterilisation can be carried out during this process to control contamination. By maintaining a high moisture capacity for longer periods of time, it improves the strain's viability and survival. It comprises a desired strain together with certain protectants to lengthen the shelf life during demanding conditions by up to 19–25 months (Chandra et al. 2018). *Azospirillum* is a microaerophilic, free-living, plant growth-promoting rhizobacterium that is mostly utilised to create liquid formulations. In addition to *Azospirillum*, microorganisms from the genera *Pseudomonas*, *Bacillus*, *Penicillium*, and *Aspergillus* that mobilise phosphate are also employed. By altering *Azospirillum* with Polyvinylpyrrolidone (PVP), glycerol, and trehalose in nitrogen-free bromothymol blue meat broth, it is claimed that  $10^8$  cells/ml can be stored for up to 8–10 months. PVP is said to protect microorganisms in hazardous and stressful conditions because of its high water-retentive ability. It is possible to use *B. megaterium* with glucose, PVP, and glycerol as a liquid formulation technique to store endospores ( $\log_{10}$  8.21), CFU/ml, for up to 4–6 months. When compared to preparations without PVP addition, those of *Azospirillum* exhibited a longer shelf life (Mandeep Dixit 2020). A higher microbial population, or  $10^8$  CFU/ml, was seen with PVP addition. This formulation contains a population of *Azospirillum* ( $1.66 \times 10^8$  CFU/ml) in trehalose (16 mM) and a phosphate-solubilizing strain ( $3.66 \times 10^8$  CFU/ml) in PVP (3%). Thus, it was determined that trehalose (16 mM) and PVP (3%) are both excellent bioinoculants that extend the shelf life of PSB (Phosphate Solubilizing Bacteria) and *Azospirillum* sp., respectively, up to 10 months with the greatest microbial population ( $10^8$  CFU/ml) at room temperature (Twinkle Chaudhary 2020).

### 3.3 Preparation of Metabolite Bioinoculants

The microbial metabolites found in metabolite preparations aid in delivering bioregulators, vital nutrients, and defence against phytopathogens. Strains from the genera *Mesorhizobium*, *Rhizobium*, *Pseudomonas*, *Trichoderma*, and various mycorrhizal fungi are employed for the synthesis of metabolites. Metabolite bioformulation has recently been created to address the shortcomings of cell-based formulation (Tewari and Arora 2016). According to reports, in stressful conditions, flavonoids associated with the rhizobial strain improve nitrogen fixation and nodulation. Leguminous host plants that are connected with rhizobial organisms release lipochitooligosaccharides. In rhizobial deficient fields, lipochitooligosaccharide is said to aid in symbiosis. Through the use of flavonoids and lipochitooligosaccharide promoter, Novozymes crop yield for both non-leguminous and leguminous crops have significantly boosted. Mycor-

rhizal fungi release Myc factors as well as Nod factors, which aid in symbiosis and activate the signal transduction pathway (Maillet et al. 2011). Exopolysaccharide (EPS) is a recently discovered plant growth-promoting bacterium that increases root colonisation, biofilm formation, and nodulation under toxic and stressful conditions. Rhizobium's exopolysaccharide release was essential for microbial defence. The addition of EPS to bioformulations helps to protect strains from osmotic shock, extremely high pH, radiation, desiccation, predators, and poisonous chemicals. Adding a precursor supplement, like tryptophan, has been shown to boost the production of IAA and increase plant biomass, grain output, and the growth of root hairs (Wang et al. 2019). Growth was accelerated by PGPR that had been modified to include l-methionine, which is a precursor to ethylene and serves as the main metabolite. Nutrients including molasses, amino acids, and effluent from starch production have reportedly been employed as helpful additions. These additions can prolong the useful strain's shelf life and increase its ability to survive in the challenging soil environment (Timmusk et al. 2014). In addition to releasing biosurfactants with antibacterial, emulsifying, wetting, antiviral, and insecticidal properties, it has been claimed that PSB (Phosphate Solubilizing Bacteria) also produces a chemical called a phospholipid. Liquid bioinoculants often contain biosurfactants that are sprayed over the plant's aerial portions. Antimicrobials like pyrrolnitrin, fanzines, omission A, and diacetyl phloroglucinol are produced by strains such as fluorescent *Pseudomonas*. Similar to this, *Bacillus* species are also investigated for their potential to produce antibiotics. Several secondary metabolites generated by *Bacillus* and *Pseudomonas* are said to have antiphytopathogenic properties (Arora and Mishra 2016).

### 3.4 Preparation of Polymeric Bioinoculants

Most often, alginate beads are used in the production of polymeric bioinoculants. It is a naturally occurring polymeric substance made of the fungus *Macrocystispyrifera*'s d-mannuronic acid and l-glucuronic acid (brown algae). According to studies, *Sargassum sinicolais* was also utilised to make it (macroalga) (Yabur et al. 2007). It has recently been proposed that different bacterial strains, including *Azotobacter* and *Pseudomonas*, are utilised in this kind of formulation. Microbeads and macrobeads, with dimensions of 2-3 mm and 50-200 mm, respectively, are the two sizes of alginate beads (colony forming unit per gram). The stability of bacterial chemotaxis, plasmid growth, and mushroom development in the host cell are all enhanced by this mixture. The trapping technique for alginate matrixes uses AMF. Gel matrix has the ability to prolong the usable strain's shelf life in challenging biotic and abiotic conditions. In the modern encapsulation method, nutritional supplements that encourage healthy growth both under

anaerobic and aerobic conditions are frequently used (Schoebitz and Belchi 2016). First of all, during formulation, this method was applied to the *Pseudomonas fluorescens*, *A. Brasiliense*, and *Aspergillus* (filamentous fungal) strains (Singh et al. 2011). A paper claims that supplementing the strain with healthy nutrients like skim milk can boost its viability when glycerol is present. It was discovered that starch-filled encapsulated cells cannot achieve the same level of porosity as chitin-filled beads. Recent research has revealed that glycerol-alginate beads have a much better proportion of UV radiation survival (Zohar-Perez et al. 2002). After 10 weeks of storage, it has been suggested that soy oil and alginate can boost *Sinorhizobium meliloti*'s growth and cell viability to 10<sup>8</sup> CFU/ml (Malusa et al. 2012). Alginate beads are created through a laborious, multi-step process at ambient temperature. Alginate is reportedly harmless, biodegradable, and releases gradually in the field ( Rajesh Gera 2020).

### **3.5 CARRIER MATERIAL**

Carrier materials are the materials that are used as a carrier for soil inoculants. A carrier material should have highly absorptive, easy to process, nontoxic, easy to sterilize effectively, cost-effective, good adhesion to seeds, survival of inoculum bacteria in the carrier material, long storage time, and high buffering quality. Various types of carrier materials used are peat soil, lignite, vermiculite, Charcoal, press mud, farmyard manure, and soil mixture (solid carrier materials). Liquid carrier materials are nothing but nutrient medias used to grow the microbes. Adhesive material for biofertilizer to stick to the plant e.g., vegetable oil, glycerol (also has arresting agent).

### **3.6 Solid carrier material**

Peat is one of the most used carrier materials for soil inoculation. This material is mainly used to carry rhizobia inoculums. Peat is used as a solid bioinoculant. Peat has high water retention capacity, high organic content, and is easily available. Peat is not available in many parts of the world.

Lignite is used as a carrier material as it is rich in humic acid and can reduce the bioavailability of heavy metals. Lignite has less nutrient content than peat. In an experiment, it was seen that the dry weight of root and shoot was increased where lignite is used.( Dr.Aparna Gunjal Patil 2015)

Vermiculite is a mineral composed of magnesium; aluminium; silicate; which is used in solid bioinoculants of *Aspergillus* spp. It produces higher protease enzyme and Mycelium productivity in vermiculite is high compared to other carrier materials.

Charcoal has high water retention capacity, neutral pH, easy availability, and long-term viability of culture, but it was seen that it was not suitable for seed inoculation. (Effect of Liquid and Charcoal Based Consortium Biofertilizers Amended with Additives on Growth and Yield in Chickpea (*Cicer arietinum* L.) (Anjali, Sharma etc 2021)

Press mud is a solid residue, obtained from sugarcane juice before the crystallization of sugar. Press mud has 74-75% of organic matter. It is a renewable material while lignite is not.

Farmyard manure is a decomposition mixture of animal dung and urine. It is also one of the renewable carrier materials, low-cost production, and easy to sterilize.

### 3.7 Liquid carrier material

Yeast extract mannitol broth: Yeast extract mannitol broth composition is: Yeast extract, Mannitol, Dipotassium phosphate, Magnesium sulphate, Sodium chloride, Calcium carbonate, Final pH (at 25°C)  $6.8 \pm 0.2$ . Yeast Mannitol Broth is used for the cultivation of the symbiotic nitrogen fixing organisms viz. *Rhizobium* species. Yeast extract serves as a good source of readily available amino acids, contain vitamin B complex and accessory growth factors for *Rhizobia*.

The strains used for liquid biofertilizer formulation were *Rhizobium*, *Azotobacter*, *Azospirillum* and PSB (*Bacillus megaterium*). Waksman medium No.77 broth, Dobereiner's malic acid broth with  $\text{NH}_4\text{Cl}$  (1g per litre), and Pikovskaya medium were used to culture *Azotobacter*, *Azospirillum* and PSB (*Bacillus megaterium*) respectively.

Nutrient broth: composition of nutrient broth is peptone, beef extract, sodium chloride and water. This broth is a basal media and used to grow broad spectrum of bacteria. Potato dextrose broth: the composition of potato dextrose broth is Potatoes, Dextrose and water. It is used to grow fungi especially (*Trichoderma*). (Dulce Nombre Rodríguez-Navarro etc (2008)

## 4 APPLICATION OF BIOINOCULANTS

### 4.1 Arbuscular Mycorrhizal Fungi as a Bioinoculant

Arbuscular mycorrhizal fungi are aseptate in nature and distinguished by the development of typical root cortical features including arbuscles and vesicles. The primary point of contact between the host and the fungus is through arbuscles (Schulz and Boyle 2005). Arbuscular mycorrhizal fungi use the vesicles, which are hyphal swellings seen in the root cortex, as a storage

organ. Arbuscular mycorrhizal fungi's hyphae build a link between the plant root and sizable portions of the soil, acting as a conduit for nutrients to reach the plants. They aid in increasing the amount of soil that plant roots are in touch with. The reduction of plant salt stress is greatly aided by the symbiotic relationship between fungi and plants. Fungi help enhance the health and quality of the soil (Padmavathi Tallapragada 2017). Under stressed circumstances, mycorrhizal plants take more phosphorus from the soil than nonmycorrhizal plants. Plants that are in symbiotic relationships with AM fungus are more able to withstand the stress of drought (Biermann and Linderman 1983). The hyphae of AM fungus penetrate the soil deeply and aid in the efficient uptake of water and nutrients. Arbuscular mycorrhizal extraradical hyphae generate specific hydrolytic enzymes that break down complex macromolecules like chitin, lignin, nucleic acid, and protein into simpler monomers. Thus, AM fungi contribute to the more effective absorption of the transformed monomers. AM fungi are unique in that they enhance a variety of physiological functions, such as the digestion of nutrients and water (Parniske 2008). They are effective at preserving the osmotic equilibrium in plants. Mycorrhizal plants have higher stomatal conductance than nonmycorrhizal plants, which increases their need for transpiration. In mycorrhizal plants, increasing turgor pressure mostly improves the water status. Arbuscular mycorrhizal fungi have a significant impact on the host plants' photosynthetic pigments, proline accumulation, nutrient uptake, and antioxidant enzymes. *Panicum turgidum* Forssk's salt tolerance is improved by arbuscular mycorrhizal fungus through altering the photosynthetic and antioxidant pathways. *Glomus mosseae* application benefited *Erythrina variegata* Linn. to overcome the four degrees of drought stress. In *Triticum aestivum* L. the colonisation of arbuscular mycorrhizal fungi improved grain yield, growth, and helped plants reduce water stress (Padmavathi Tallapragada 2017).

#### **4.2 Plant Growth-Promoting Rhizobacteria as a Bioinoculant**

Plant growth-promoting rhizobacteria (PGPR) are naturally occurring, soilborne, free-living bacteria that colonise the rhizosphere area of soil and aid in the stimulation of plant growth through both direct and indirect mechanisms. By directly influencing root and shoot growth, the early application of specific PGPR strains to different crop plants enhances biomass production (Kanchana et al. 2013). Fluorescent *Bacillus*, *Azotobacter*, *Pseudomonas*, and *Azospirillum* species. are a few of the PGPR that are frequently used to promote plant development. Particularly as a biofertilizer and biocontrol agent, PGPR play a crucial role in agricultural systems. Additionally, PGPR affects the physiology of the entire plant and changes how the roots operate.

It also enhances plant nutrition. By exhibiting some of the crucial characteristics, Plant growth-promoting rhizobacteria reduce the effects of salt stress. Under salinity, plants tend to release ethylene, which at larger concentrations inhibits root growth and ultimately has an impact on plant growth as a whole. One important enzyme, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, controls the amount of ethylene. Rhizobacteria that encourage plant growth produce ACC deaminase, which lessens the inhibitory effects of salt by lowering the ethylene level. Prolific root growth is therefore facilitated, which is advantageous for nutrient intake and maintenance of plant growth (Nadeem et al. 2010). PGPR provide a cheap and easy solution for radish plants that are sensitive to salt. Inoculated maize plants with ACC deaminase-producing rhizobacterial bacteria showed an increase in chlorophyll a and b at various salt levels. In both water-deficit and well-watered environments, co-inoculating rice plants with *Azospirillum* and AM fungus promoted greater plant development. There are numerous processes through which PGPR impacts plant growth, yield, and nutrient uptake. They create phytohormones, boost the availability of other nutrients, fix nitrogen, and prevent bacterial and fungal infections in addition to increasing the availability of nitrogen (Saharan and Nehra 2011).

#### **4.3 Bioinoculants' Effect on the Solubilization of Phosphate**

Phosphorus is one of the most crucial macronutrients for biological growth and development (Fernandez et al. 2007). In addition to boosting disease resistance in plants, phosphorus also promotes crop maturation. Most agricultural soils depend on the ongoing application of chemical fertilisers or wastewater treatment sludge for a sizeable amount of their stored phosphorus (De-Bashan and Bashan 2004). Plants obtain phosphorus from the soil. Soils rarely contain the required amount of phosphorus. Plants only take up a small portion of the phosphorus in soil; the majority remains immobilised. Phosphate-solubilizing bacteria, which are frequently found in soil, change phosphate from its insoluble form to its soluble form. The metabolism of complex chemicals depends on the diversity of microbes in soil (Fernandez et al. 2007). In order for plants to obtain soluble forms of phosphate, soil microorganisms play a significant role in the solubilization of insoluble forms of phosphate. Three *Pseudomonas fluorescens* strains (CB501, CD511, and CE509) were chosen among all of the examined bacteria based on their capacity to solubilize three different phosphates found in liquid medium: tricalcium phosphate ( $\text{Ca}_3(\text{PO}_4)_2$ ), aluminium phosphate ( $\text{AlPO}_4\text{H}_2\text{O}$ ), and iron phosphate ( $\text{FePO}_4\cdot 2\text{H}_2\text{O}$ ). *Aspergillus* species Under in vitro circumstances, PS 104 was discovered to be a good rock phosphate solubilizer (Park et al. 2009).

## 5 CONCLUSION

Bioinoculants are soil microbial species that help the plant grow, uptake nutrients present in the soil, and provide immunity to the plant up to a certain extent against pathogens. Bioinoculants are the sustainable future of agriculture. The chemical fertilizers are creating more abiotic stress as it is increasing the pH of the soil and plants are not able to uptake these fertilizers, and these NPK fertilizers are washed off to water bodies and causing biomagnification and eutrophication in the water bodies making them unfit. Whereas bioinoculants try to break down the chemical fertilizers so that the plants can absorb them, these inoculants balance all elemental cycles increasing soil fertility and maintaining soil health. In the future, it is hoped that biofertilizers are preferred over chemical ones.

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