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Microbial fertilizers: Their potential impact on environment sustainability and ecosystem services

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Abstract
Microbial fertilizers have been used by farmers in agriculture for more than a century starting from rhizobia inoculants to a more recent Plant Growth Promoting rhizobacteria (PGPR) for increasing the nitrogen fixation and synthesis of plant hormones. Biofertilizers consists of microorganism that improves the nutrient content of the soil and enhances their availability to the crops. Pant nutrients represent the most crucial component of sustainable agriculture. At present, soil management approaches are largely dependent on chemical-based inorganic fertilizers which have posed a serious threat to human health and the ecosystem. Even though the usage of various chemical fertilizers and pesticides has provided high yield and consequently, population growth over the last century. The environment can no longer tolerate these practices as it has sustained significant damage such as loss of soil quality, atmospheric pollution, high eutrophication, and groundwater contamination. Thus maintaining food security along with environmental sustainability has become a massive challenge. However, the adoption of bio fertilizers represents a dependable and eco-friendly solution. Biofertilizers being organic in nature holds a vast expectation in fulfilling nutrient requirements, soil and water quality thus providing a low-cost approach compared to synthetic fertilizers in managing crop yield without causing much harm to the environment. Biofertilizers are becoming an irreplaceable component in Integrated Nutrient Management in order to achieve sustainability in agriculture. This review paper brings to light the potential effects of biofertilizers on ecosystem services and environmental sustainability.

Keywords: Microbial fertilizers, rhizobia inoculants, PGPR, ecosystem, eutrophication, pesticides

Introduction
Soil nutrients are critical for continuous growth and efficient production of crops thereby meeting the demands of the ever-increasing population with healthy food. India needs 280 MT of food grains to meet the requirement of the population (Singh *et al*., 2016) [59]. Fertilizers are the main source of nutrients and hence agriculture is completely relied on fertilizers for increasing the yield of the crops. Fertilizers include synthetic, organic, or biofertilizers and their characteristics are different and each may possess a different ability to improve the yield of the crops (Chen, 2016) [68]. Chemical fertilizers are widely used to fulfill the nutrient requirement of the crops as they provide rapid results by providing nutrients over a short period of time. The nutrients are provided in high concentration which results in accelerated growth but causes a massive toll on the environment polluting both land and water by the deposition of harmful chemicals (Mahdi *et al.*, 2010) [41]. This practice became much prominent after the green revolution in India. Green revolution bought about a great change in the Indian agriculture scenario with the usage of high-yielding varieties, nutrient management (synthetic and organic fertilizers), and agriculture chemicals including pesticides, fungicides, and herbicides. These practices made India sufficient during the time of post-independence (Dotaniya *et al*., 2014) [17]. Extensive usage of chemical fertilizers adversely affected the soil health reducing soil productivity. Increased ratios of fertilizers application i.e. 61:7:19:2:1 and 61:4:18:7:1 were practiced in Punjab and Haryana instead of the appropriate ratio of 4:2:1 (NAAS, 2014). This leads to a decrease in the yield and multi-nutrient deficiency in crops.
Biofertilizers are considered to be among the most promising means to improve the productivity of the crop in an environment-friendly manner. Microbes present in biofertilizers consist of growth-promoting characteristics which play a pivotal role in optimizing various

activities like organic matter decomposition, plant nutrient accessibility such as that of magnesium, iron, potassium, nitrogen, phosphorous in plants (Lalitha, 2017) [38]. Biofertilizers are turning out to be an irreplaceable component in Integrated Nutrient Management thereby achieving sustainability in agriculture. Biofertilizers are utilized as a cost-efficient means of improving the crop yield with minimized use of synthetic fertilizers and utilizing the already present nutrients of soil into available forms (Gentili *et al.,* 2006) [26]. Biofertilizers are basically preparation made of living cells or microbial strains that aids the plant in the uptake of nutrients in the rhizospheric region when they are provided to the plants by means of seed or soil treatment (Youssef *et al.,* 2014) [69]. Various researches have been conducted on the potential effects of biofertilizers on soil health and the ecosystem.

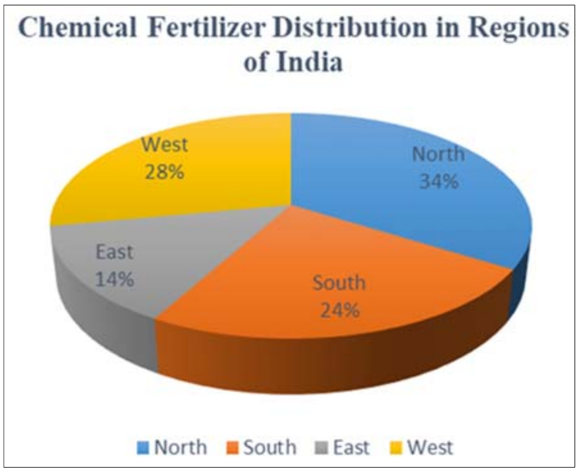

Fig 1: Chemical fertilizer usage in India according to the region (NAAS, 2014)

Need for Sustainable Agriculture

The global population at present is attaining unprecedented growth which in turn is taking a massive toll on the environment with over usage of natural resources (Robertson, 2012) [52]. According to the study conducted by the Food and Agriculture Organization (FAO) of the United Nation, The population is going to reach an unexpected size in the coming years, this being mostly due to the high fertility rate of children in both developed and developing nation and the high life expectancy seen in developed countries (FAO, 2018) [62]. Increasing the production to fulfill the requirements of the global population has become one of the major concerns of coming years. Agriculture being the primary sector that plays a pivotal role in meeting these needs has already taken up one-third of the global land area available for cultivation. Acquiring more land for intensive cultivation has got severe negative impacts on the environment. (Garcia *et al*., 2017) [44]. In order to increase crop productivity, large-scale usage of chemical fertilizers mainly nitrogen, potassium, and phosphate fertilizers have severely degraded the soil quality leading to soil acidification, Eutrophication, and air pollution along with several other problems (Gouda *et al*., 2018) [28]. Soil degradation and environmental pollution have become a rising concern since they directly influence crop production and ecological balance. The concept of sustainable agriculture is a form of farming that focuses on growing long-term crops and livestock while having minimal environmental impacts. This form of agriculture aims to strike a good balance between the need for food production and environmental protection of the ecological system (Hazell *et al*., 2008) [21]. In addition to food production, there are other major priorities related to sustainable agriculture, including water conservation, minimizing the utilization of fertilizers and pesticides, and promotion of biodiversity in crops grown and the environment. Sustainable farming also focuses on economic stability and helping farmers develop their techniques and standard of living (Horrigan *et al*., 2002) [33]. Crops produced need to be engineered with properties like disease resistance, drought tolerance, salt tolerance, heavy metal stress tolerance, and greater nutritional value to achieve a sustainable agricultural dream (Amanda *et al.,* 2014). There are several farming strategies adopted in sustainable agriculture, biofertilizer usage being one of the major practices. The usage of eco-friendly fertilizers namely biofertilizers which are mostly based on plant probiotic bacteria is a solution to most of the negative effects caused by chemical fertilizers (Menendez *et al*., 2017) [44]. This heterogeneous group of bacteria has got a lot of positive effects on the plant, biofertilizers there are bacteria which are capable of nitrogen fixation, and there are others which are capable of solubilizing both Phosphorous and Potassium to make it available for plants (Saharan *et al.,* 2011) [54]. They are also having the ability to synthesize siderophores and phytohormones which are some of the plant growth-promoting mechanism of plant probiotic bacteria (Etesami *et al*., 2018) [21].

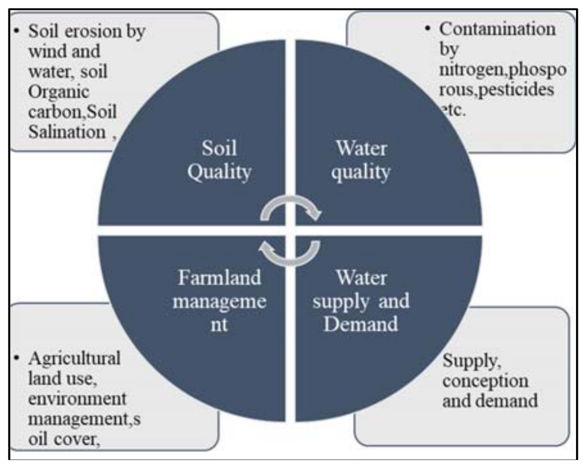

Fig 2: Need for Sustainable Agriculture (Sustainable Agriculture and Climate change)

Bio-Fertilizers and their Types

Biofertilizers are microorganism-containing, liquid, or carrier-based preparations inadequate numbers that support the growth and nutrition of the plants (Motsara *et al*., 1995) [47]. They are biologically active bacteria, fungi, or algae with the ability to provide nutrients to the plant (Rao *et al*., 2015) [51]. These microbial cells will cause no harm to the ecosystem. Biofertilizers are regarded as a supplement for chemical fertilizers as they can reduce the NPK requirement of crops from various chemical fertilizers (Kawalekar, 2013) [34]. They are known to be cheaper and cost-efficient compared to chemical fertilizers and free from harmful effects on soil health or the ecosystem (Sahoo *et al*., 2014) [54]. When chemical fertilizers are incorporated into the soil nearly 60-90% of applied fertilizers are lost due to leaching, nitrification, immobilization, denitrification, etc. Only 10-40% is absorbed by the plant (Bharadwaj *et al*., 2014). Whereas biofertilizers multiply rapidly when applied as soil or seed inoculant and participate in the fixation of nutrients in the soil increasing crop growth and yield (Herrmann *et al*., 2013) [32]. Depending on the mode of active role played in the crop growth they are classified into different types of biofertilizers.

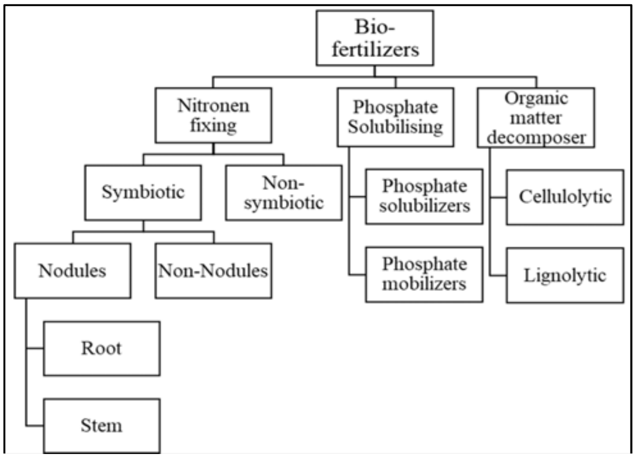

Fig 3: Bio-fertilizers classification (Debnath *et al*., 2019) [13]

Nitrogen Fixation

Nitrogen is an important component of all life forms and is the most vital resource for the growth and yield of the plant. Even though 78 percent of the nitrogen is present in the atmosphere, it remains inaccessible to plants (Stevenson *et al.,* 1999) [63]. Unfortunately, no plant species can fix atmospheric nitrogen into ammonia and spend it for its growth. However, nitrogen-fixing microorganisms convert the atmospheric nitrogen into forms that can be utilized by crops such as ammonia using an enzyme system known as nitrogenase (Doty, 2011) [18]. Some of the nitrogen-fixing bacteria are as follows

Rhizobium

Rhizobium is a nitrogen-fixing symbiotic strain of bacteria that belongs to the family *Rhizobiaceae*. They approximately fix about 50-100 kg ha^{-1} (Mahdi *et al*., 2010) [41]. This form of a symbiotic relationship between Rhizobium and leguminous crops is unique in nature. It is proposed that N2 fixation in root leguminous nodules by Rhizobium is on the order of 14 million tons on a global basis and is approximately 15 percent of industrial N fixation (Franche *et al*., 2009) [22]. The yield of several legumes can be greatly increased by using suitable cultures of Rhizobium. For productive nodulation per legume needs a particular Rhizobium species to create effective nodules (Zahran, 2001) [70]. Rhizobium can be used in both legumes and trees as they are crop specific inoculants for e.g. *Rhizobium japonicum* in soya bean, *Rhizobium lupini* in chickpea, *Rhizobium trifolii* in berseem, and *Rhizobium* spp. in cowpea.

Table 1: Biological Nitrogen fixed by Rhizobium in different crops

Host Group	*Rhizobium* Species	Crops	N fix kg/ha
Pea Group	*Rhizobium leguminosarum*	Green pea, Lentil	62-132
Soybean Group	*R. japonicum*	Soybean	57-105
Lupini Group	*R. lupine orinthopus*	Lupinus	70-90
Alfalfa Group	*R. mellilotiMedicago Trigonell*	Melilotus	100-150
Beans Group	*R. phaseoli*	Phaseoli	80-110
Clover Group	*R. trifoli*	Trifolium	130
Cowpea Group	*R. species*	Moong, Red gram Groundnut, Cowpea	57-105
Cicer Group	*R. species*	Bengal Gram	75-117

Azospirillum

Azospirillum belongs to the family *Spirilaceae*. They are heterotrophic as well as associative in nature. It is known for the fixing of nitrogen with a capacity of 20-40kg ha^{-1}. *Azospirillum* produces substances that regulate growth and help protect against soil-borne diseases. This increases the leaf area index and eventually crop yields (Ahemad *et al.,* 2014) [1]. Some of the major species under this genus are *A. lipoferum* and *A. brasilense.* Other species like *A. amazonense*, *A. halopraeferens* are also used (Roychowdhury *et al*., 2014) [153]. *Azospirillium* forms an associative symbiosis with several plants, mainly with those plants that have a C_4 dicarboxylic pathway for photosynthesis (Gerk *et al*., 2000). They grow and fix nitrogen on the salts of an organic acid such as that of malic and aspartic acid (Arun, 2007) [4]. *Azospirillum* is more beneficial in maize, pearl millet, sugarcane, and sorghum. It is applied as a seed treatment or by dipping the roots of rice seedling in a suspension of 2% *Azospirillum* inoculant has improved the yield by 100kg ha^{-1} (Kennedy *et al*. 2004) [35]. The major drawback that limits the use of *Azospirillum* is the great uncertainty and unpredictability of its results (Broek *et al*., 2000) [10].

Azotobacter

Azotobacter belongs to the family *Azotobacteraeae*. They are a free-living organism and motile genus with a thick-walled cyst (Gurikar *et al*., 2016) [30]. They are aerobic and heterotrophic in nature. *Azobacter* mainly exists in neutral or alkaline soil and *A. chroococcum* is the most commonly appearing species in arable soil (Mishra *et al.,* 2013) [46]. *A. vinelandii, A. macrocytogenes, A. beijerinckii*, and *A. insignis* are some of the other identified species that are playing a pivotal role in Nitrogen dynamics. Poor organic material has an adverse effect on the *Azotobacter* population which hardly ever exceeds around 10^4–10^5 g^{-1} of soil (Dwivedi *et al*., 2015) [19]. The antagonistic relationships with several other soil micro-organisms often restrict the *Azotobacter* population and N fixation rate in soil (Dar, 2009) [12]. The presence of free-living nitrogen-fixing organisms is identified in the soil of crops such as sugarcane, maize, rice pearl millets (Mazid *et al.,* 2011) [43]. It has the ability to fix nitrogen in the atmosphere ranging 20-26kg ha^{-1} under favorable situations thereby increasing the crop yield to 40-50%. *Azotobacter* improves seed germination due to the synergetic response of NAA, B vitamins, and GA (Mahajan *et al.,* 2008) [40]. It improves the microbial soil properties and acts as a beneficial tool in sustainable agriculture.

Fig 4: Biological Nitrogen Fixing Bacteria, slide share (Wagner S.C., 2012)

Cyanobacteria

Commonly known as blue-green algae, cyanobacteria are not completely eukaryotic algae. They are gram-negative prokaryotes, conduct oxygenic photosynthesis, and repair atmospheric N2 as well. They are omnipresent in reservoirs, lakes, bodies of water, rivers, and wetlands. They can also exist in extreme conditions such as hot springs, hyper-saline oceans, freezing temperatures, and arid deserts.

Phosphate Solubilisation

Phosphorus is a crucial element of plant nutrition, besides nitrogen (N). It plays a key role in nearly all major processes of plant metabolism including photosynthesis, energy transfer, macromolecular biosynthesis, signal transduction, and respiration (Khan *et al*., 2010) [36]. It is vastly present in soils in organic as well as inorganic forms. Plants are not being able to completely utilized phosphate because 90-99% phosphate present in forms that are insoluble, immobilized, and precipitated (Pandey *et al.,* 2007) [50]. Monobasic (H_2PO_4) and the diabasic (HPO_4^{2-}) ions are the only two forms by which the plant absorbs phosphates (Bhattacharyya *et al.,* 2012) [8]. Usage of inorganic fertilizers during crop production, more than 80% of the fertilizers are converted into unavailable form with the aid of aluminum (Al-P), iron (Fe-P), and calcium (Ca-P) (Dotaniya *et al*., 2013) [16]. In general, the Al-P and Fe-P represent 1–25 percent of total P in soil, mainly in the acidic type of soils. The Ca-P represents ~40 percent in neutral and calcareous soils. Ca-P is comparatively more common in soil than Al-P and Fe-P (Oburger *et al.,* 2011) [48]. Phosphate fertilizers are to be applied as a single dose known as basal dose at the time of sowing. P ion mobility is slow compared to other nutrients as plant roots nearly take a 2–4 mm distance (Lambers *et al*., 2019) [39]. Whereas plant roots can utilize other nutrients from a longer distance. Plant growth-promoting rhizobacteria found in the soil use various techniques to take advantage of unavailable sources of phosphorus which in turn help to make phosphorus available for plants to consume (Gupta *et al*., 2015) [29]. The key phosphate solubilization mechanisms used by plant growth-promoting rhizobacteria are (1) discharge of complexing or mineral dissolving compounds. (2) Emancipation of extracellular enzymes and (3) the discharge of phosphate during substrate degradation (Sharma *et al*., 2013) [56]. Some of the phosphate solubilizing bacteria are *Pseudomonas, Bacillus, Rhizobium, Agrobacterium, Micrococcus*, etc.

Potassium Solubilisation

Potassium is the third crucial macronutrient for plant growth after nitrogen and phosphorous. Soluble potassium concentration is highly scarce as 90% of potassium present in the soil is in the form of silicate minerals and insoluble rocks (Teotia *et al*., 2016) [64]. Plants are facing a lot of problems like underdeveloped roots, poor growth and yield due to the unavailability of potassium (Gopalakrishan *et al.,* 2015). Potassium solubilizing plant growth-promoting rhizobacteria such as *Acidothiobacillus ferrooxidans, Bacillus mucilaginosus, Bacillus edaphicus, Paenibacillus sp. and Pseudomonas* are capable of solubilizing potassium rock through the synthesis and secretion of organic acids (Meena *et al*., 2016) [16]. Utilization of these phosphate solubilizing rhizobacteria as biofertilizers will reduce the reliance on synthetic fertilizers for supplementing potassium and promote eco-friendly agriculture (Ahmad *et al*., 2016) [2].

Table 2: Biofertilizers with examples

S. N		GROUPS	Examples
A	**N_2 fixing Bio-fertilizers**		
	1.	Free-living	*Azotobacter, Clostridium, Anabaena*
	2.	Symbiotic	*Rhizobium, Anabaena azollae*
	3.	Associative Symbiotic	*Azospirillum*
B	**P Solubilizing Bio-fertilizers**		
	1.	Bacteria	*Bacillus subtilis, Pseudomonas striata*
	2.	Fungi	*Pencillium sp, Aspergillus awamori*
C	**P Mobilizing Bio-fertilizers**		
	1.	Arbuscular Mycorrhiza	*Glomus sp., Scutellospora sp.*
	2.	Ectomycorrhiza	*Laccaria sp., Pisolithus sp., Boletus sp.*
	3.	Ericoid Mycorrhiza	*Pezizella ericae*
D	**Bio-fertilizers for Micro-nutrients**		
	1.	Silicate and Zinc solubilizers	*Bacillus sp.*
E	**Plant Growth Promoting Rhizobacteria**		
	1.	Pseudomonas	*Pseudomonas fluorescence*

Plant Growth Promoting Rhizobacteria (PGPR)

PGPR is a group of bacteria that promotes plant growth and yield through various growth-enhancing substances and acts as biofertilizers (Singh, 2013) [58]. PGPR functions include the regulation of hormonal and nutritional equilibrium, the induction of resistance to plant pathogens, and the solubilization of nutrients for efficient plant uptake. In addition, PGPR displays synergistic and antagonistic interactions within the rhizosphere and beyond in bulk soil, which indirectly increases the rate of plant growth (Vegan *et al.,* 2016). There are several bacteria that act as PGPR which are capable of improving plant growth but the mechanism of PGPR for growth promotion and their role as a biofertilizer is different. "Plant Growth Promoting Rhizobacteria" refers to the bacteria that colonize the plant roots. Microbial colonies include bacteria, fungi, acticomycetes, protozoa, and algae, bacteria being the most abundant in the Rhizosphere. They supply the plant with compounds that bacteria synthesize or promote the absorption of certain plant nutrients. Their beneficial effects are not limited to root associations but also have useful effects on controlling many phytopathogenic microorganisms (Son *et al.,* 2014) [61]. Biofertilizer products are typically dependent on microorganisms that encourage plant growth (PGPM). The PGPM can be categorized into three major microorganism groups: arbuscular mycorrhizal fungi (AMF) rhizobacteria that encourage plant growth and rhizobia that fix nitrogen that is considered good to plant growth and nutrition (Avis *et al.,* 2008) [5]. PGPR with composts may increase the effects of growth-promoting and plant bio-control. Two PGPRs that have been identified as effective bio-control agents are *Bacillus* spp (Fuente *et al*., 2006) [14]. *Pseodomonas* spp, *Bacillus subtilis, Bacillus cereus*, and *Basillus amyloliquefacients* are some of the most productive species among these species of bacteria for controlling plant diseases using different mechanisms (Francis *et al.,* 2010) [23]. Sufficient concentrations of PGPR in biofertilizer play a positive role in making a convenient rhizosphere for plant growth and transforming nutritionally essential components through biological processes, such as improving the availability of N, P, K and inhibiting pathogen growth (Vessey, 2003) [66]. Improved availability of N, P, and K could boost soil fertility, enhance bio-control, and antagonistic isolates. If they serve as a nourishment and enrichment source for plants that would replenish or rebuild the nutrient cycle between the soil, plant roots, and microorganisms present, PGPR can be categorized as biofertilizers (Yang *et al.,* 2011) [68]. The concern regarding this matter is whether the "living" biofertilizers used can be self-sustaining or need to be constantly re-applied to the soil, and also whether extensive usage will destabilize the microorganism's interaction in the soil.

Table 3: Effects of PGPR their host crop, mechanism and benefits

PGPR	Crops	Mechanism	Benefits	Reference
Azotobacter	Rice, wheat, maize, barley, oats, sunflowers, line, beetroot, tea, tobacco, coffee, and coconuts	Nitrogen fixation	Improves seed germination, Increase nutrient availability, Better crop response and high Crop growth rate.	(Wani *et al*., 2013) [67]
Azospirillum	sugarcane, maize, rice	Nitrogen fixation	promote plant growth, Abiotic stress tolerance, Osmotic adjustment, production of phytohormones	(Sahoo *et al.,* 2014) [55]
Frankia	*Alnus*	Nitrogen fixation	Increases growth and biomass Of *Alnus*	(Simonet *et al*.,1995) [57]
Pencillium spp,	wheat, sesame, pomegranate	Phosphorous solubilization	P uptake, and yield of several crops,	(Maity *et al.,* 2014) [42]
Bacillus	maize, pepper, potato Cucumber, alfalfa	P solubilization, Production of gibberellin, auxin, cytokinin, antibiotic, siderophore	Production of growth hormones, antibiotics, Siderophores	(Radhakrishan *et al.,*2017)
Beijerinckia	sugarcane	Nitrogen fixation	Significant plant growth effects	(Felipe *et al.,* 2016) [15]
Rhizobium	rice, Tomato, Pepper, Carrot, lettuce,	Nitrogen fixation IAA synthesis Siderophore prod.	Fixes atmospheric nitrogen in the soil for plant intake	(Garcia *et al.,* 2012) [44]
Mycobacterium	maize	Induction of plant stress resistance	Increase resistance to various stress	(Egamberdiyeva,2007)

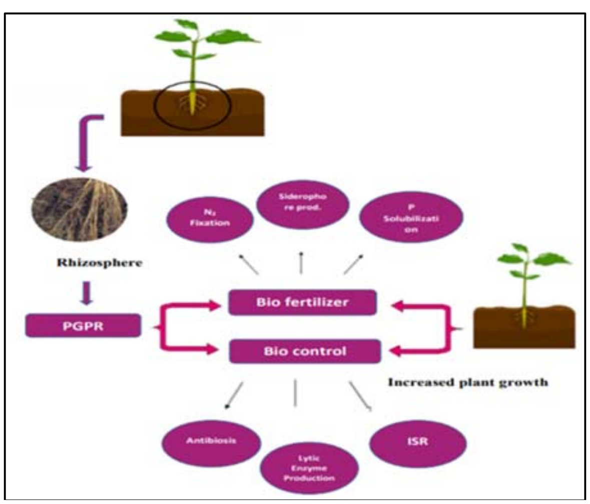

Fig 5: Important Mechanism for plant growth promotion

Constraints in the adoption of Bio-Fertilizers

- One of the main constraints of biofertilizers include the poor availability of a carrier; it affects the self-life
- Mutation in the microbes during fermentation
- Need for sophisticated equipment's skilled men for the application and storage of biofertilizers as adverse climatic conditions might affect the viability of biofertilizers
- The initial cost of investment is high compared to other fertilizers
- Unlike chemical fertilizers, bio-fertilizers takes time to show results but they improve the soil quality
- Lack of awareness among the farmers on the long term benefits of Bio-fertilizers
- Lack of marketing
- Poor storage led to the reduction of viable strains in biofertilizers that made farmers suspect the quality of bio fertilizers

Conclusion

Over usage of chemical fertilizers and inappropriate agronomical practices are taking a massive toll on the environment. Productivity is declining at an alarming rate. Agricultural crop production requires to be enhanced into new horizons without deteriorating the natural resources or environmental quality. The dependence on chemical fertilizers and pesticides are encouraging the industries to produce more life threatening chemicals which is both detrimental for human and soil health. Sustainable agriculture is one simple solution to counter the harmful effects of synthetic fertilizer. Biofertilizers are one of the key factors in sustainable agriculture that can assist in solving the problems of feeding an increased world population at a time when agriculture is going through various environmental stresses. Hence research should be focussed on new aspects of Biofertilizers. New alternatives should be identified for the utilization of bio inoculants for other crops of value such as flowers, vegetables, and fruits. Research on phosphate solubilization and nitrogen fixation by plant growth-promoting microorganism are in progress but little research is done on potassium solubilization which is the third crucial macronutrient for plant growth. Awareness is to be created among farmers on the positive effects of biofertilizers on crop yield and the environment. With the assistance of the government and non-government organizations (NGOs) and vast publicity through media, farmers are to be educated on the importance of sustainable agriculture. Extensive marketing efforts and identification of new microbial strains with regards to climate, crop, and environmental conditions enhance the soil as well as crop sustainability. Encouraging more investments for the development of new strains of biofertilizers, maintaining their quality, and improving shelf life is a major hurdle in its propagation and utilization.

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