THE BENEFIT OF RHIZOBACTERIA AS BIOFERTILIZER FOR PLANT GROWTH

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CERTIFICATION

This is to certify that AJOSE OYINDAMOLA DEBORAH, with matriculation number 16010101016 carried out this project under the close supervision of Dr. Abiala. It was done based on the analysis of drought tolerant bacteria in Nigeria.

DR ABIALA, M.A. DATE

PROJECT SUPERVISOR

HOD DATE

DEDICATION

I, dedicates this work to God Almighty, the giver of strength, wisdom and knowledge who saw me through the completion of this great work. Also, to my parents and siblings for their enormous supports financially and morally and to my guardian Mr. Gboyega Ojo who encouraged me to be strong despite several challenges, these joint efforts helped in the completion of this work.

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ABSTRACT

The expansion in human population has raised a threatening remark to the food security of each individuals as the land for agriculture is restricted and, in any event, it gets diminished over time. For that reason, it is necessary that agricultural production ought to be improved essentially within the next few decades to meet the immense request of food by the arising populace. Without dwelling on the reliance of chemical fertilizers for crop production because of its adverse effect in the ecosystem and human health, microbial fertilizers were introduced to be a sustainable alternative to chemical fertilizer by being eco-friendly and enhancement in food safety and crop production. Microbial fertilizer is significant group of microbial inoculants that exist in rhizosphere and are able to occupy the root of the plants and enhance their development. This is accomplished through nitrogen fixation, phosphate solubilization, production of phytohormones, increase uptake of plant nutrients, and also protects against pathogens and ability to withstand environmental stress. As a result of this, I uncover in this write up on the significance of microbial fertilizers and how beneficial they are to plants and the future prospects of microbial fertilizer.

Keywords: Bio fertilizer. Chemical fertilizer. Ecosystem. Crop production. Sustainable agriculture

Abbreviations used: BNF (biological nitrogen fixation)

PGPM (plant growth promoting microorganism),

PGPB (plant growth promoting bacteria),

IAA (indole acetic acid)

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CHAPTER ONE

1.0 INTRODUCTION

In the past decades, there has been an irrefutable significant increase in agricultural production due to the rise in population growth and, hence, there is need for food (Hassen *et al*., 2016). A fundamental transition in modern agriculture, was characterized by chemical fertilizers being introduced. Hillel (2008) said, the invention of these fertilizers, this has led to a significant increase in crop yields, along with the advancement of crop varieties and methods to resist diseases and pests, as well as to prevent soil erosion. However, the abuse of chemical fertilizer has shown to be unsustainable and has contributed adversely to the ecological balance and pollution of the ecosystem. Thus, the need to minimize their usage is therefore clear (Szilagyi-zecchin *et al.*, 2016).

In order to increase the soil fertility and crop production, bio fertilizers have been identified as a sustainable alternative to chemical fertilizer for a successful farming. According to Kumawat *et al* (2017). Biofertilizer has a significant role, particularly in the current context of increased cost of chemical fertilizer and their adverse effect on soil health and ecosystem as a whole.

Biofertilizers are products containing one or more species of microorganisms which via biological processes such as Nitrogen fixation, phosphate solubilization, excretion of plant hormones or cellulose, and bio-degradation in soil, compost and other environments, are able to mobilize nutritionally important elements from non-usable to usable forms. Bio fertilizer are natural fertilizers which contains living microbial inoculants such as; bacteria, algae, fungi. They can either be combined or present singly, biofertilizers are not source of nutrient, but elevates the availability of nutrients which is easily accessed by the plants (Stamenkovic *et al*., 2018). They help to improve soil fertility by fixing atmospheric nitrogen and solubilizing, phosphate solubilization, Phyto hormones and helps in growing plant growth promoting substances in the soil (Mazid and Khan 2015). Biofertilizers have been elevated to reap the naturally available biological system of nutrient mobilization which aids in increments soil richness and at last, crop yield (Pandey and Singh 2012).

According to Umesha *et al*., (2010) stated that of recent, biofertilizers are gaining vast recognition due to the advantages attached to the application of biofertilizer such as; maintenance of soil health, reduction of environmental pollution by using of the chemicals in agriculture (Muraleedharan, Seshadri, & Perumal, 2010)., increased in crop yield solely, increase in essential nutrients for plant growth and development. For optimal plant growth, nutrients are required in sufficient and balanced quantities but from the soil only a small portion of nutrients are released every year through biological or chemical processes. Biofertilizer aims at supplementing the nutrients which already exist in the soil, asides nutrient supplements, biofertilizers have other numerous benefits. For example; control of pathogens, improvement in soil health, soil properties which results in a profuse yield rates. Here are few micro-organisms that are commonly used as biofertilizers including nitrogen fixing bacteria (Azotobacter, Rhizobium); nitrogen fixing cyanobacteria (Anabena), phosphate solubilizing bacteria (Pseudomas sp.) and AM fungi.

Also, phytohormone (auxin)-producing bacteria and cellulolytic microorganism are being used also as biofertilizer formulation. These microbial formulations used helps in enhancing certain microbial process which aids in the increment and availability of nutrients in a form which can be easily assimilated by plant. Biofertilizers are low-cost, they are renewable source of plant nutrients.

CHAPTER TWO

2.0 PAST: ADVERSE EFFECT OF CHEMICAL FERTILIZERS TO THE ECOSYSTEM

The chemical fertilizer raises the growth and vigor of a plant, and also meets the world's food security but the outcome of the plants grown do not possess good plant characters such as, good root system, shoot system, nutritional characters and also insufficient time to rise and mature properly. Plants which are chemically produced, will assemble in the human body by producing toxic chemicals which are threatening to human's health. The deleterious effects of the chemical fertilizer will commence by manufacturing these chemicals which by products and products are some toxic chemicals or gases for example: NH_4, CO_2, CH_4 etc. which will cause air pollution. It can also cause water pollution when untreated wastes from factories/industries are been disposed into nearby water bodies, whereby waste accumulated in the water bodies can lead to a devastating effect i.e., water eutrophication. And when added in the soil, continuous usage degrades the soil health and quality which causes soil pollution. It is therefore high time to realize that this input into crop production is depleting our biodiversity and climate (Chandini *et al.,* 2019). Here I will be discussing about the various hazards which occurs due to the excessive usage of chemical fertilizers which is been used for enhancement crop production.

2.1 EFFECT ON THE QUALITY OF THE SOIL ECOSYSTEM

Soil is a natural resource, and a medium for the growth of plants. The soil is a habitat of soil flora and soil fauna it is a nutrient recycling system, and provides many other ecosystem services. The misuse of chemical fertilizers can pilot towards soil acidity level in the soil and cryptobiotic soil crust thereby reducing organic matter content, humus content, beneficial organisms, stunting plant growth, can change the soil pH, increase soil pathogens, and even contribute to the release of greenhouse gases (Chandini *et al.*, 2019). The depletion of humus in the soil, leads to its in-ability to store nutrients.

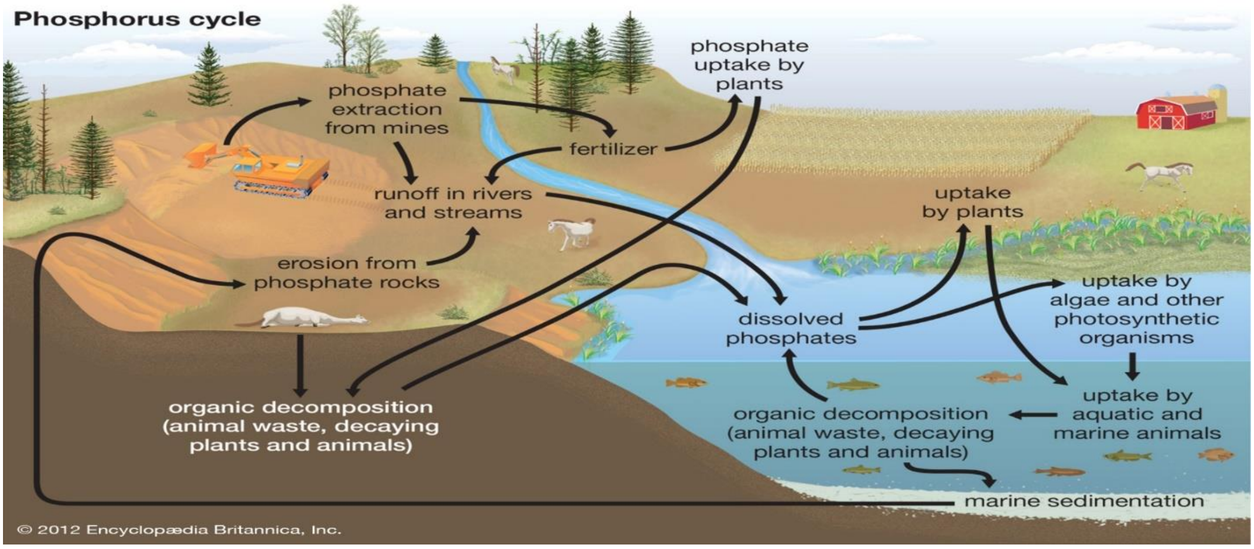
Continuous application of chemical fertilizers may lead to the development of heavy metals which are toxiferous such as arsenic, cadmium, and uranium in the soil. These toxic heavy metals may not only pollute the soil, but also get piled-up in food grains, vegetables and fruits. For example, fertilizers like like Triple superphosphate has minor components like cadmium and arsenic that assemble in plant and through food chains. it gets access to human that may cause adverse health problems. The adverse effect of chemical fertilizers on soil are prominent and irremediable (Chandini *et al.*, 2019). Fertilizers more than the recommended quantity, causes the formation, accumulation, and concentration of fertilizer mineral salts, which contributes to long term compaction layer and soil degradation.

2.2 EFFECT OF CHEMICAL FERTILIZERS ON WATER POLLUTION

Because the efficiency of the chemical fertilizer's nutrient usage is much less, they are therefore applied in higher quantity than required for more efficiency, when applied in an unfavorable environmental condition, these leaves the environment by different ways (Chandini *et al.*, 2019). These can be either by leaching, drainage, surface flow, run off etc.

For instance, Mineral N is likely to be oxidized to nitrate in most cultured upland soils because of microbial activity. As a result of this, moderately high parts of the applied N may practically be leached or taken out from the root zone into the surface and groundwater (Cooke, 1982). Major deleterious effect of the intensive use of fertilizers mainly Nitrogen and phosphorus is Water eutrophication. According to Encyclopedia Britannica (2019)., Eutrophication is the gradual increase in the concentration of phosphorus, nitrogen, and other plant nutrients in an aging aquatic ecosystem such as a lake. If The sum of organic matter which can be split into nutrients increases, the productivity and he fertility of an ecosystem like this is naturally growing. This substance mainly reaches the environment through runoff from land that carries debris and products of terrestrial organisms' reproduction and death. Water blooms, or great concentrations of algae and microscopic organisms, often develop on the surface, preventing the light penetration and oxygen absorption necessary for underwater life. Eutrophic waters are often gloomy and this supports minimal large animals, such as fish and birds, than non-eutrophic waters (Encyclopedia Britannica, 2012).

FIG 2.2.1: ILLUSTRATION OF WATER EUTROPHICATION (Adapted from Encyclopedia Britannica, 2012)



Source: Encyclopedia Britannica. Inc.

2.3 EFFECTS OF CHEMICAL FERTILIZERS ON AIR POLLUTION

According to Savci (2012)., diverse harmful greenhouse gases are created by high application rate of chemical fertilizers to increase production of crops, depletion of the protective ozone layer and exposing humans to harmful ultraviolet rays. The greenhouse gases like CO_2, CH_4 and N_2O are produced during the manufacture of nitrogenous fertilizer. The effects can be combined into an equivalent amount of CO_2. Nitrogen fertilizer can therefore be converted by soil bacteria into nitrous oxide, a greenhouse gas. Nitrogen fertilizer whose excess use results in an emission of nitrogen oxides (NO, N_2O, NO_2) is responsible for severe air pollution (Cooper *et al.,* 2017). The major concern regarding the emission of nitrous oxides has to do with the effect of global warming and the role of nitrous oxides in ozone depletion, which leads to holes in the atmosphere, exposing humans and animals to excessive ultraviolet radiation (Rutting *et al.,* 2018).

2.4 OTHER ADVERSE EFFECTS OF CHEMICAL FERTILIZER

(Adapted by Chandini *et al*., 2019)

i. Extreme utilization of chemical fertilizer to plants may make the leaves turn yellow or earthy colored, harming the plant and decreasing the harvest yield.

ii. Applying higher amount of N fertilizers in malt barley may cause an unwanted impact on the nature and quality of the beer.

iii. Abundance amassing of nitrate or nitrite in plant parts consumed by animals and humans is probably going to cause deleterious effect related with nitrate pollution of water sources.

iv. Over-fertilization effects reduce the biodiversity resulting from ammonia deposition in forests and waters.

v. Reduction in the Mycorrhizal root colonization and inhibition of symbiotic N fixation due to high N fixation by rhizobia.

vi. Via fixation, leaching or gas pollution, nutrients are easily lost from the soil and can lead to reduction in fertilizer efficiency.

CHAPTER THREE

3.0 PRESENT: SUSTAINABLE SUBSTITUTE FOR CHEMICAL FERTILIZER

The abuse of chemical fertilizers has demonstrated to be unsustainable and has added to the unsettling influence of ecological balance and further contamination of natural environment, so therefore, the need to minimize their usage is clear (Szilagyi-zecchin *et al.,* 2016). Plant growth promoting microorganisms (PGPM) are a significant gathering of microbial inoculants, which exist in rhizosphere and be able to occupy the base of the plants and improve their development. Their positive influence is accomplished through solubilization of phosphorus, nitrogen fixation, creation of plants nutrient and phytohormones, security from pathogens and recuperation from distressing natural conditions. This is the principal explanation behind the increase in the usage of numerous PGPMs which plans are regularly known as Microbial Fertilizer. Microbial fertilizer, is an attractive substitution for chemical fertilizer that are contaminating the ecosystem. They are utilized to expand the harvest yield in an eco-accommodating way while depending on feasible farming standards (Stamenkovic *et al.,* 2018)

3.1 BIO FERTILIZER (Adapted from Stamenkovic *et al.*, 2018)

Plant growth promoting bacteria PGPBs inhabits the Rhizosphere naturally, favorably affect the plant, improve its productivity and resistance to pathogens (Mrkovacki *et al.*, 2012). Some of the PGPBs, which in general can be divided into bacteria and fungi, includes the following strains: *Azo-spirillum, Azoto-bacter, Pseudomonas, Enterobacter, Bacillus, Paenibacillus, Gluconobacter, Penicillum, Trichoderma, and Streptomyces* (Nadeem *et al.,* 2015). Microorganisms such as *Rhizobium*, *klebsiella*, *Clostridium*, *Bacillus megaterium,* *Penicillum species, Trichoderma viride,* elevates plants growth while *Pseudomonas aureofaciens, Trichoderma, Streptomyces sp.,* may act as bio control agents against pests and plants diseases (Abhilash *et al.*,2016). Based on their interaction with the plant, PGPB can be divided into symbiotic or free-living bacteria (Zaidi *et al.,* 2015). It can be divided further into intracellular and extracellular. In intracellular, PGPB colonizes the root cells, penetrate the cell wall and also integrates with the plants thereby, forming new organ on the plant tissue-nodule, which provides an optimal condition for the microorganism while extracellular PGPB colonizes the rhizosphere space, the root surface or the intracellular space of the root cells (Kiprovski 2012; Owen *et al.,* 2015).

Generally, bacteria enhance plants growth majorly in three different ways (Glick 1995;2001). Which are: Synthesizing growth promoting hormones for the plants (Dobbelaere *et al.*, 2003), facilitating the uptake of nutrients from the soil (Cakmakci *et al.,* 2007) and preventing the plants from diseases (Saravanakumar *et al.,* 2008). However, the in-depth mechanism in PGPB is yet to be thoroughly investigated (Dey *et al.*, 2004). However, PGPB mechanism of action can directly affect the plant by producing substances which can control growth and improve plant yield by facilitating water uptake, nutrient uptake, and essential elements uptake, which is of good benefit to the plant (Owen *et al.*,2015). While (Rojas-Tapias *et al.,* 2012; Grobelak *et al.,* 2015}. Indicates that indirect mechanism, includes the inhibition of pathogens through the production of antibiotics and enzymes. PGPM boosts the availability of micro nutrients uptake of (Fe, Zn, Se)

Table 1.1: Types of bio fertilizers (Adapted from Kumawat *et al.,* 2017).

S/N	TYPES OF BIOFERTILIZERS	EXAMPLES
N_2 FIXING BIOFERTILIZERS		
1	Free living	*Azotobacter, Beijerinkia, Clostridium, Klebsiella, Anabaena, Nostoc*
2	Symbiotic	*Rhizobium, Frankia, Anabaena azollae*
3	Associative symbiotic	*Azospirillum*
P SOLUBILIZING BIO FERTILIZERS		
4	Bacteria	*Bacillus megaterium var. phosphaticum, Bacillus subtilis, Bacillus circulans, Pseudomonas striata*
5	Fungi	*Penicillium sp., Aspergillus awamori*
P MOBILIZING BACTERIA		
6	*Arbuscularmycorrhiza*	*Glomus sp., Gigaspora sp., Acaulospora sp., Scutellospora sp. and Sclerocystis sp.*

7	*Ectomycorrhiza*	*Laccaria sp., Pisolithus sp., Boletus sp., Amanita sp.*
8	*Ericoid mycorrhizae*	*Pezizella ericae*
9	*Orchid mycorrhiza*	*Rhizoctonia solani*
BIO FERTILIZERS FOR MICRO NUTRIENTS		
10	Silicate and zinc solubilizers	*Bacillus sp.*
PLANT GROWTH PROMOTING RHIZOBACTERIA		
11	*Pseudomonas*	*Pseudomonas fluorescence*

Source: Popular kheti. 5. 63-66.

3.2 MECHANISM OF ACTION OF MICROBIAL FERTILIZER

There are basically two types of mechanism of action of microbial fertilizer namely:

i. Direct mechanism

ii. Indirect mechanism

3.2.1 DIRECT MECHANISM OF ACTION

The main mechanisms PGPMs use to add to the increment of nutrients in the soil are nitrogen fixation and phosphate solubilization, along with solubilization of other minerals. After photosynthesis, nitrogen fixation is the most important biological process in nature, enabling the circulation of nitrogen in the biosphere (Wani *et al.,* 2016). Symbiotic bacteria from the group Rhizobium and Frankia, and non-symbiotic bacteria such as *Azospirlillum sp., Azotobacter sp. and Acetobacter sp*. have the ability to assimilate N_2 from the atmosphere and convert it into NH_3 as part of a mechanism well known as nitrogen fixation using an enzymatic complex known as nitrogenase (Hoffman *et al*., 2009; Smith *et al.,* 2013). These complex organic molecules are accessible for usage by plants. Nitrogen is one of the most vital nutrients for plant development and productivity. Although, the amount of Nitrogen present in the atmosphere is approximately 78%, which is inaccessible for utilization by the plant. In-order to utilize the atmospheric nitrogen, it has to be converted to ammonia first which is easily assimilated by the plant through the cycle of biological nitrogen fixation (BNF) according to Tairo and Ndakidemi (2013)

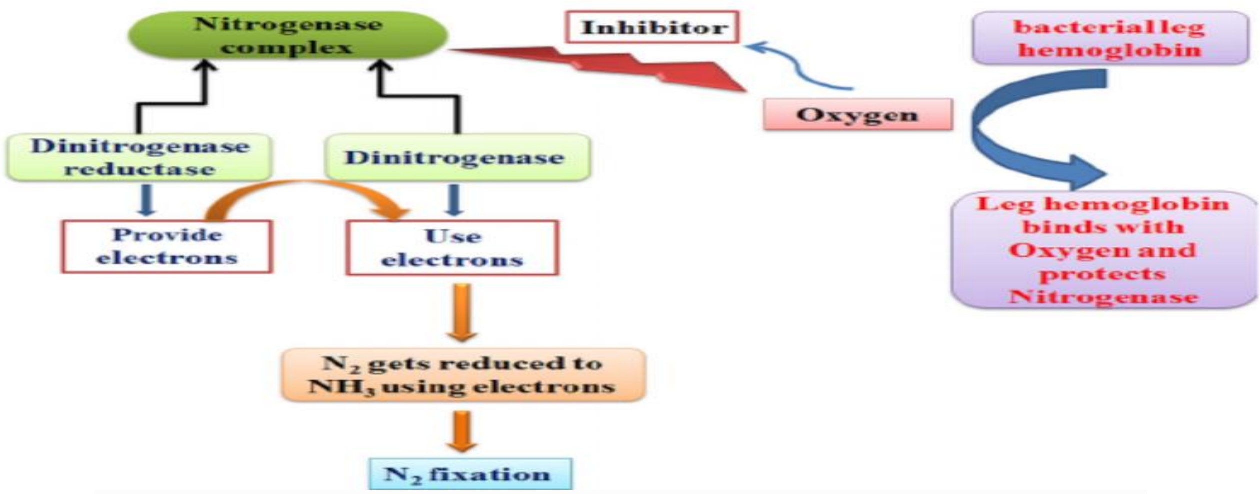
Nitrogen fixing bacteria are basically categorized as symbiotic and non-symbiotic. Symbiotic organisms which include the members of *Rhizobiaceae* that forms a symbiotic relationship with leguminous plants (Ahemad and Khan 2012). While, non-symbiotic organisms include the free-living and endophytic forms of microorganisms such as *Cyanobacteria, Azospirillum, Azotobacter* etc. (Bhattacharyya and Jha 2012).

Rhizobium

It is a symbiotic nitrogen fixing rhizobacteria which belongs to belong to the family *Rhizobiaceae* (α-proteobacteria) family and they infect as well build up a symbiotic relationship with the root nodules of a leguminous plant. This requires a complex interaction between the host and the symbiont which results to nodule formulation wherein the Rhizobia colonizes as an intracellular symbiont (Allito *et al.,* 2015*). Rhizobium, Bradyrhizobium, sinorrhizobium, Azorhizobium and Mesorhizobium which* are collectively called *Rhizobia*. Non symbiont rhizobacteria that fixes nitrogen to a non-leguminous plant are also called as diazotrophs and they can form a non-obligate interaction with the host plants (Verma *et al*. 2010). Nitrogen fixation is being carried out by the complex enzyme structure called nitrogenase, which consists of di-nitrogenase reductase which has iron (Fe) as a cofactor while di-nitrogenase has iron (Fe) and molybdenum (Mo) as its cofactor (Smith *et al*. 2013). Di-nitrogenase reductase provides electrons and di-nitrogenase which uses these electrons to reduce N_2 to NH_3 according to (Santi *et al*. 2013). Based on the variation in the co factor of di-nitrogenase, there are basically three different types of nitrogenase complexes such as Mo-nitrogenase, V-nitrogenase, and Fe-nitrogenase have been reported (Ahemad and Kibret 2014).

FIG 3.2.1: Molecular N2 fixation mechanism by plant growth-promoting rhizobacteria.

(Adapted from Mahanty et al.,)



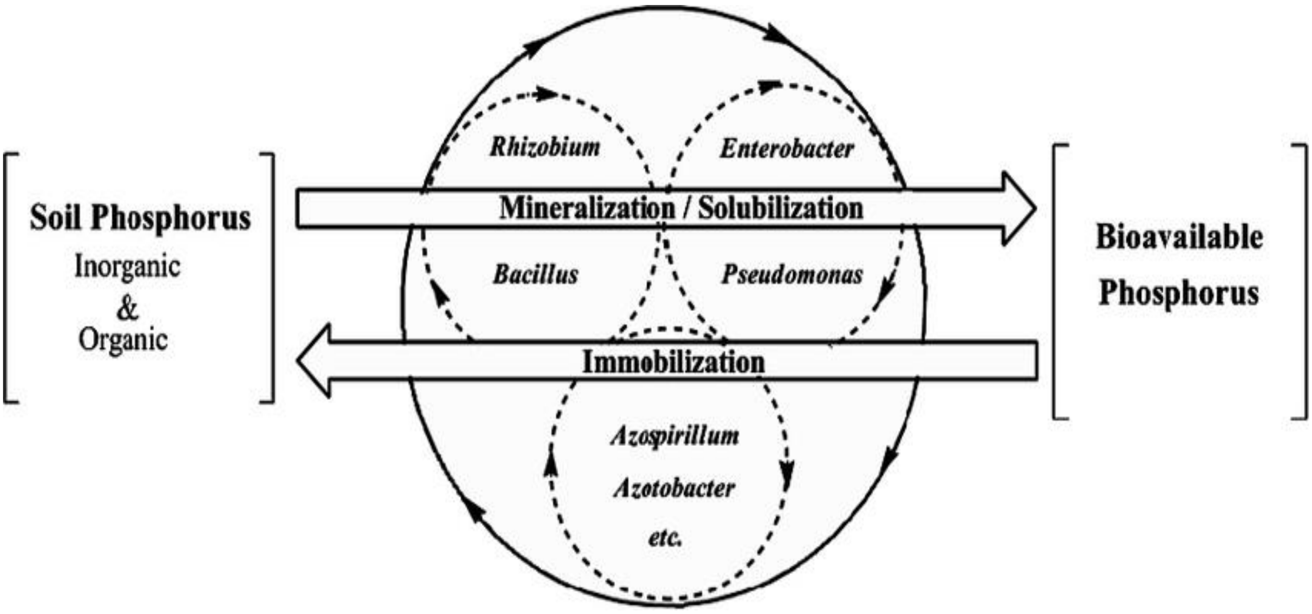
Source: Environment science pollution *Res* **24,** 3315–3335 (2017). https://doi.org/10.1007/s11356-016-8104-0

Azospirillum

These are free-living, aerobic, photoautotrophic non-symbiotic bacteria which belong to the family *Azoto-bacteriaceae*. They are commonly present in soils which are alkaline and neutral in nature. Azotobacter *chroococcum* is the most common species in arable soils (Moraditochaee *et al.* 2014). The other reported species include *Azoto bactervinelandii, Azotobacter beijerinckii, Azotobacter insignis,* and *Azotobacter macrocytogenes* (Mishra *et al.* 2013). It inhibits root pathogens but promote root growth and minerals uptake (Mathivanan *et al.* 2015).

Phosphorus in labile organic compounds, can be enzymatically mineralized by PSB like; Pseudomonas, Enterobacter, and Pantoea (Jorquera *et al*. 2009) which are available as inorganic P or it can be immobilized as part of the soil organic matter. It is a major nutrient found in the plant which undergoes various metabolic processes such as; cell division, cell development, signal transduction, macromolecular biosynthesis, energy transport, respiration and photosynthesis (Khan *et al.*, 2009b) The process of mineralization or immobilization has shown in Figure 3.2.1 below is carried out by microorganisms which is greatly influenced by soil moisture and temperature. Mineralization and immobilization are most rapid in warm, well-drained soils (Busman *et al*. 2002).

FIG 3.2.1: Schematic diagram of soil phosphorus mobilization and immobilization by bacteria (Adapted from Khan et al., 2009a).



Source: www. Researchgate.net

Some of the PGPM which includes the Pseudomonas sp., Bacillus sp., Buckholderia sp., Rhizobium sp., and Flavobacterium sp., have the ability to solubilize some insoluble phosphate compounds. PGPMs are also able to make phytohormones which stimulate plant growth; thus the mechanism of their activity is known as bio-stimulation. Some of the most important phytohormones are auxins, cytokinin, gibberellins and abscisic acid (Gopalakrishnan *et al.*, 2014). Auxins are plant hormones with a cardinal role to modulate the development of a plant. As much as 80% of the PGPMs can synthesize the indole acetic acid (IAA), which has an important role in the stimulation of cellular division and differentiation (Mrkovački *et al.*, 2012). IAA, induces the appearance of lateral roots amongst dicotyledons and adventive roots amongst monocotyledon. It also improves the secondary thickening of the walls, and an increase in xylem cells results in better minerals and water uptake (Hassen *et al.*, 2016).

Gibberellin promotes cell elongation and division. However, the mechanism through which plant grows through gibberellin is yet to be known (Vijayabharathi *et al.*, 2016). Lack of gibberellins in plants, leads to occurrence of dwarfism in plant (Gopalakrishnan *et al*., 2014).

Cytokinins stimulate cellular division in some plants and in some cases the development of the root and absorbent hairs on the root (Gopalakrishnan *et al.*, 2014). Cytokinins also takes part in the development of plant callous and helps in differentiating shoots (Vijayabharathi *et al*., 2016).

Abscisic acid controls the physiological processes in plant (It is synthesized in the chloroplasts, while its entire biosynthesis primarily takes place in the leaves, initiated by the stressful environmental conditions such as absence of water and low temperatures (Gopalakrishnan *et al.*, 2014). It helps aggravate the germination of the seed, the closing of stomata and ability to withstand environmental stress (Vijayabharathi *et al*., 2016).

3.2.2 INDIRECT MECHANISM

PGPMs influences the induction of plant in-order to be resistant to pathogens by synthesizing various antibiotics, siderophores, cyanides or lytic enzymes (Bjelić, 2014). One of its major mechanism, is the ability to synthesize one or more antibiotics (Glick, 2005). Siderophores are low molecular mass organic compounds with strong chelating affinity towards ions of iron (Fe+3). In presence of oxygen, most of the iron particles are only partly soluble and thus are not completely available to the living organisms (Gopalakrishnan *et al.,* 2014). Bacteria siderophores have a positive effect on the growth of plants, and functions as a source of iron that is readily absorbed by the plant (Hassen *et al*., 2016).

In addition, PGPMs have an effective outcome on the soil characteristics itself and their consortiums are successfully used in the processes of bio-remediation. This is how adversely affected soil becomes fertile and available for agricultural production, since a transformation occurs in the hydrocarbons and other pollutants into less detrimental forms (Beškoski *et al.,* 2011). Microorganisms that can break down hydrocarbons and oil-based pollutants effectively includes:

Microorganisms that effectively break *Nocardia sp., Pseudomonas sp., Acinetobacter sp., Flavobacterium sp., Micrococcus sp., Arthrobacter sp., Corynebacterium sp., Mycobacterium sp., Bacillus sp., Nocardia sp., Pseudomonas sp., Acinetobacter sp., Flavobacterium sp., Bacillus sp., Micrococcus sp.,* etc. (Milic *et al*., 2009; Beškoski *et al*., 2011) *Nocardia sp., Pseudomonas sp., Acinetobacter sp., Flavobacterium sp., Micrococcus sp., Arthrobacter sp., Corynebacterium sp., Mycobacterium sp., Bacillus sp*., IAA induces the occurrence of lateral Gibberellin takes part *Azospirlillum sp., Azotobacter sp.*

TABLE 3.2: Effect of inoculation microbial fertilizer on plants (Adapted from Stamenkovic et al., 2018)

S/N	MICROORGANISM	PLANT	RESULT	REFERENCE
1	*Pseudomonas fluorescens* and *Azo-spirillum brasilense*	Paddy rice	Increased biomass production, harvest index, and grain yield. Reduced number of chaffy grains	García de Salamone et al., (2012).
2	*Pseudomonas fluorescens*	Peanuts	Reduced incidence of leaves dots Reduced incidence of leaves dot caused by pathogens	Meena *et al.* (2002).
3	*Pseudomonas putida*	Melon	Prevents melon wither	Bora *et al.* (2004

4	*Pseudomonas chlororaphis* TSAU13	Cucumber and Tomato	Protection of plants from pathogens and control of the production of phytohormone	Egamberdieva & Ade-semoye (2016)
5	*Bacillus sp.*	Maize	Increasing leaf area and dry biomass Increment in leaf area and dry biomass weight	Kavamura *et al.* (2013
6	*Bacillus aryabhattai*	Grape vine	Increase in the growth of *vitis vinifera*	Liu *et al.* (2016)
7	*Bacillus amyloliquefaciens*	Potato	Reduction of diseases caused by *Ralstonia solanacearum* stonia	Wei et al., (2011)

			solanacear	
8	Azospirullum spp. enriched with Azospirullum spp. enriched with *Azospiillum sp.* Enriched with metabolites of *Rhizobium tropici*	Maize	Increase in grain yield in relation to non-inoculated control in 5 out of 6 experiments	Marks et al., (2015)
9	*Azospirillum brasilense*	Cucumber	Increasing resistance to Fe-limiting Increase in Fe con-tent of leaves and biomass	Pii et al., (2015)
10	Azotobacter chroococcum	Maize	Production of auxin, phosphate	Rojas-Tapias *et al.*

			solubilization	(2012)
11	Rhizobium leguminosarium by phaseoli	Green beans	Increase in no of pod per plant gain weight	Mishra *et al.* (2011)
12	Bacillus cereus, Staphylococcus Bacillus cereus, staphylococcus sp., Pseudomonas fluorescensce	Cerasus sachalinensis	Increment in root viability and seedling growth	Qin *et al.*, (2016)

CHAPTER FOUR

4.1 FUTURE: BIO-PROSPECTIVE OF MICROBIAL FERTILIZERS (Adapted from SAJP, 2013)

Misuse of chemical fertilizers by farmers during rigorous agricultural practices, has led to the accumulation of excess nutrients especially phosphorus (P), causing the soil to become dead. Therefore, the major research interest is the development of effective and sustainable bio fertilizers for crop plants, where the use of in-organic fertilizers can be drastically reduced to prevent further problems with contamination.

The following points should highlight the most relevant and basic research needs;

i. **SELECTION OF EFFECTIVE AND MULTI FUCTIONAL MICROVIAL FERTILIZER (Adapted from SAJP, 2013)**

Special attention is currently given to microorganism(s) with multifunctional properties and bio fertilizers containing more than one microorganism. While most bio fertilizer products consist of a single microorganism function, such as Nitrogen fixing bacteria, emphasis are been placed on the development of bacterial isolates that could be produced as multifunctional biofertilizer microorganism. There is proof that a multifunctional consortium of various strains of Rhizobium, phosphate-solubilizing microbes and parasites, arbuscular mycorrhizal organisms, and free-living nitrogen-fixing Azotobacter strains improves the noduling capacity, nitrogen substance and herbage yield (up to two-overlay) of subabul seedlings (Leucaena leucocephala) in correlation with the utilization of every segment of the consortium alone. Another methodology for collecting multifunctional biofertilizer arrangements is to utilize indigenous microorganisms that have all the ideal qualities and are available in fertilizer. Among these significant qualities are plant-development advancing, phosphate-solubilizing and opposing activities towards microorganisms. Subsequently, multifunctional biofertilizer items dependent on fertilizing the soil are planned and created applying the accompanying methodological methodology:

a. Disconnection and screening for indigenous microorganisms at each phase of the fertilizing the soil cycle, that give in any event two significant qualities, for example capacity to solubilize phosphate and to create indole-3-acidic corrosive (IAA);

b. Improvement of these indigenous microorganisms into biofertilizer items;

c. Assessment of the impacts of the items on the development of a model plant and the commitment of N2 to the plants in a nursery preliminary. Choice of mixes of strains that essentially upgrade plant development through advancing nitrogen-fixing impacts or solubilizing insoluble inorganic phosphate mixes or hydrolyzing natural phosphate to inorganic P or incitement of plant development through hormonal activity, for example, creation of IAA.

ii. QUALITY CONTROL FRAMEWORKS FOR THE CREATION OF INOCULANTS AND THEIR FIELD APPLICATION (Adapted from SAJP, 2013)

The interest in biofertilizers is additionally expanding because of their potential for use in maintainable horticulture. Nonetheless, huge numbers of the items that are presently accessible overall are of low quality. The plan of an inoculant is a multistep cycle that outcomes in one/a few strains of microorganisms remembered for an appropriate transporter, giving a sheltered climate to shield them from the brutal conditions during capacity and guaranteeing endurance and foundation after presentation into soils. A central question in definition advancement and creation is the quality control of the items, at each phase of the creation cycle.

The successful application and use of biofertilizers for the agricultural system is restricted by several limitations:

a. Non-solid viability: the adequacy of most biofertilizers is farfetched, since their instrument of activity in advancing development isn't surely known, in spite of the broad exploration toward this path.

b. Impact of abiotic factors on biofertilizers viability: it is as yet not satisfactory how varieties in soil type, the board practices and climate influence the biofertilizer adequacy.

c. Field preliminaries execution: It is as yet hard to test inoculants in the field as standard investigations.

The best possible quality control component of biofertilizer creation and application covers the entire test measure: from microorganism segregation, through research center screening of the detached strains for plant development; nursery screening for plant development advancement; field screening of the best organisms in edited soil; correction and refining of inoculants; natural effect test and, at long last, creation.

Since quality is the boundary on which the acknowledgment or dismissal by the end-clients, the ranchers, depends, it is one of the main components impacting the advancement of the biofertilizer business.

iii. STUDY OF MICROBIAL PERSISTENCE OF BIOFERTILIZERS IN SOIL ENVIRONMENTS UNDER STRESSFUL CONDITIONS (Adapted from SAJP, 2013).

The evaluation of the tirelessness and recognizability in soil of the strains applied with biofertilizers can be a major test. There are a few significant explanations behind this.

a. The enormous and complex populace of microorganisms present in the dirt and the rhizosphere.

b. The high inconstancy of the microbial networks which reflects natural, ecological and auxiliary soil attributes.

c. The enormous assortment of horticultural administration frameworks.

That is the reason one can't pick a solitary subjective and quantitative way to deal with follow the tirelessness of bio-inoculants in the dirt on account of the assortment of living beings shaping the biofertilizers. This trouble, thus, brings up the issues about the techniques to be viewed as appropriate for observing the tirelessness of various immunized strains. The methodological methodology is of vital significance for assessment of the achievement of immunization, thus, the biofertilization.

iv. AGRONOMIC, SOIL AND FINANCIAL ASSESSMENT OF BIOFERTILIZERS FOR DIFFERENT AGRARIAN CREATION FRAMEWORKS (Adapted from SAJP, 2013).

The beneficial outcome of biofertilizer application relies upon numerous variables. Additionally, the assessment of the biofertilizer application is likewise mind boggling. The components engaged with plant advancement might be both host-plant-explicit and strain-explicit. Plant-development advancing microorganisms, when delivered into the dirt, are exposed to serious conditions that may seriously decrease their useful impacts. That is, the valuable impacts because of the utilization of a particular biofertilizer may contrast altogether under various agro-ecological conditions, scrutinizing the viability of microbial-based items.

v. TRANSFERRING TECHNOLOGICAL KNOW-HOW ON BIOFERTILIZER PRODUCTION TO THE INDUSTRIAL LEVEL (Adapted from SAJP, 2013).

Improvement in crop production due to application of biofertilizers has been reported extensively. At present various biofertilizers are produced in large scale industrially and are available for field application. For instance, inoculants using Rhizobium and Azotobacter are produced industrially following a production technology comprising three important steps:

a. Development of strains;

b. Upscale of biomass;

c. Preparation of inoculants.

vi. **FOUNDATION OF BIOFERTILIZER ACT AND SEVERE GUIDELINE FOR QUALITY CONTROL IN BUSINESS SECTORS AND APPLICATION (Adapted from SAJP, 2013).**

The Common Agricultural Policy (CAP) and its system of European Union agricultural subsidies and programs require farmland to be maintained in 'Good Agricultural Condition' and encourage application of particular land management activities to benefit the environment. Furthermore, some countries have included the principles of "humus/organic matter management" in these requirements and check it in the frame of the cross-compliance obligations.

CAP in EU is built on the following pillars:

a. Subsidizing production of basic foodstuffs in the interests of self-sufficiency.

b. Emphasis on direct payments to farmers as the best way of guaranteeing farms' income, food safety and quality, and environmentally sustainable production.

4.2 Benefit and limitation of biological fertilizer (Adapted from caevajal *et al.*, 2012)

Biological practices can provide a wide scope of chances for the advancement of better agrarian practices because of the benefits and advantages provided for the soil. However, restrictions of these practices are additionally all around considered and perceived, which recommends that achievability studies ought to be done to discover better answers for every specific case in rural exercises. Next in this segment, a few advantages and limits are referenced to feature the need of future exploration on certain issues.

4.2.1 Benefits of bio fertilizer (Adapted from Chen, 2006).

i. Biological fertilizers helps to mobilize nutrients which favors the expansion of biological activities in soils.

ii. Plant health is maintained and enhanced by the addition of balanced nutrients.

iii. Provision of food and adequate growth of beneficial microorganisms is impelled.

iv. Good soil root is promoted.

v. Promotes the development of mycorrhizal associations, which increases the availability of phosphorus (P) on the soil.

4.2.2 Limitations of bio fertilizer (Adapted from Chen 2006)

i. Extensive and long-term application of bio-fertilizer may result in salts accumulation, nutrients, and heavy metals that could lead to deleterious effects on plant growth, expansion of organisms of the soil, water quality, and human health.

ii. Implementation costs are higher than those of certain chemical fertilizers, i.e. it is expensive

iii. Biofertilizers are required in large quantity for land application due to low contents of nutrients, in comparison with chemical fertilizers.

iv. There could be deficiency in Nutrient caused by low transfer of micro- and macro-nutrients.

v. Unavailability of macro nutrient in sufficient quantities for growth and development of plants.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

Microbial fertilizer has been in a focal point of investigates for a serious long time. They are viewed as naturally adequate option in contrast to synthetic composts and agrochemicals, which are abused and destructive to the climate. Bio fertilizer, is seen to be eco-friendly and late progressions in the field of biofertilizers offer an occasion to natural cordial supportable farming

practices to diminish reliance on synthetic manures and subsequently decline unfriendly natural impacts. Consequently, utilization of PGPB as bio-fertilizer and biocontrol specialists is the practical technique for farming because of their less result and beneficial horticultural profitability. Other than bio-fertilizer and biocontrol, microbial inoculants or PGPB right now likewise utilized in the saltiness, draft, furthermore, other biotic and abiotic stress the executives. It opens another entryway for the ranchers, ventures, and analysts to utilize these PGPB strains in the resilience of saltiness, hefty metal and furthermore in xenobiotic debasement. These PGPB strains right now likewise utilized in bioactive mixes creation, which intervene in the medication disclosure and aides in infection the board.