

# जैव उर्वरक सूचना पत्र

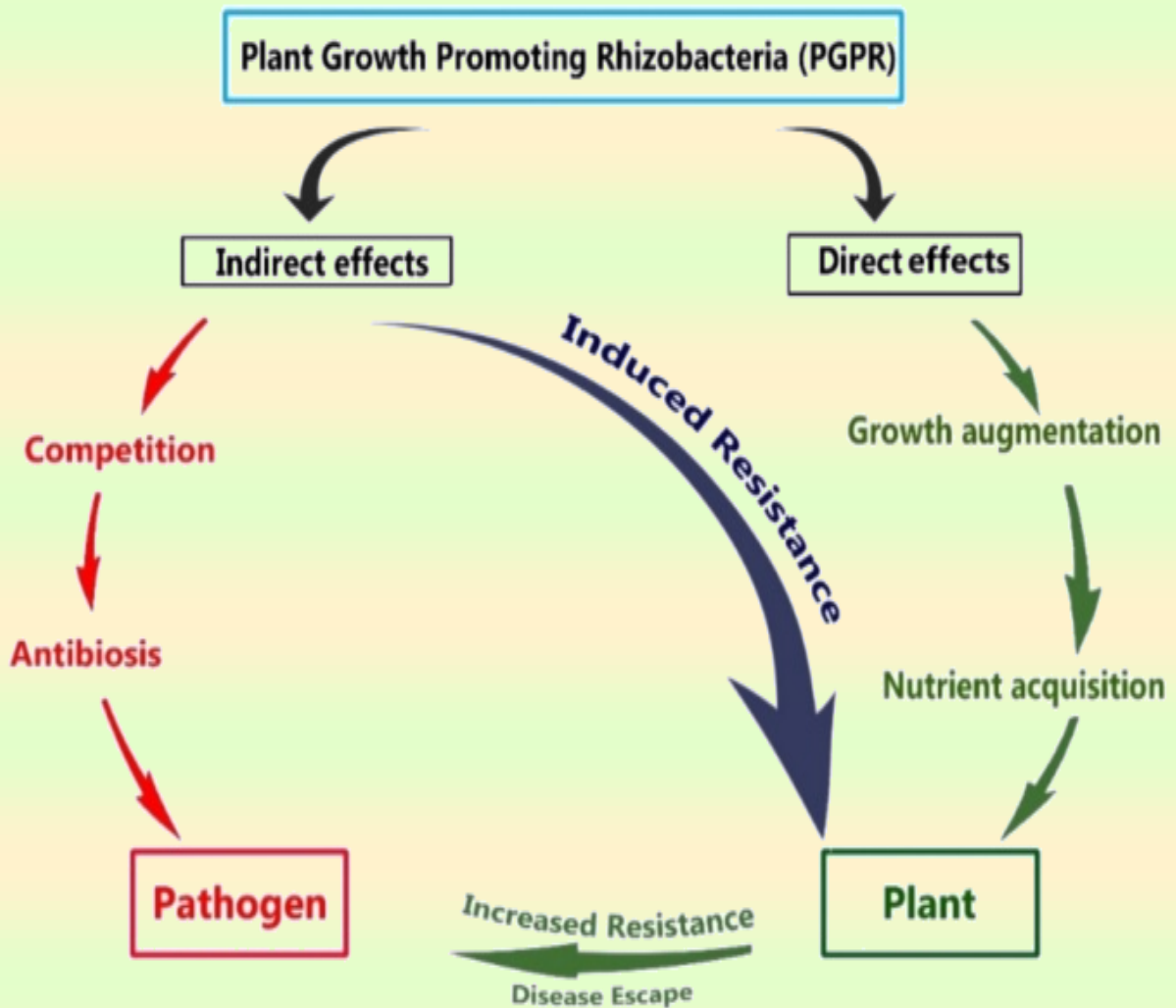
## BIOFERTILISER NEWSLETTER

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**Hapur Road, Kamla Nehru Nagar, Ghaziabad-201002**

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## BIOFERTILISER NEWS LETTER

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<p><b>प्रमुख संपादक Chief Editor</b> डा. गगनेश शर्मा Dr. Gagnesh Sharma राष्ट्रीय जैविक खेती केन्द्र, गाजियाबाद National Centre of Organic Farming, Ghaziabad</p> <p><b>संपादक Editor</b> डॉ. वी. के. वर्मा Dr. V.K.Verma क्षेत्रीय जैविक खेती केन्द्र, भुवनेश्वर Regional Centre of Organic Farming, Bhubaneswar</p> <p><b>सह संपादक Co-Editor</b> डॉ. सचिन कुमार वैद Dr. Sachin Kumar Vaid राष्ट्रीय जैविक खेती केन्द्र, गाजियाबाद National Centre of Organic Farming, Ghaziabad</p> <p><b>प्रकाशन सहायक Publication Assistant</b> हरि भजन Hari Bhajan</p> <p><b>संपादकीय कार्यालय Editorial Office</b> राष्ट्रीय जैविक खेती केन्द्र, गाजियाबाद National Centre of Organic Farming, Ghaziabad हापुड़ रोड, कमला नेहरू नगर, गाजियाबाद Hapur Road, Kamla Nehru Nagar, Ghaziabad ☎ 0120-2764212; 2764906; Fax 0120-2764901 Email :<a href="mailto:nbdc@nic.in">nbdc@nic.in</a>; website :<a href="http://ncof.dacnet.nic.in">http://ncof.dacnet.nic.in</a></p>	From the desk of Chief Editor	4
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From the desk of Chief Editor.....

Sustainable agriculture is vitally important in today's world because it offers the potential to meet our future agricultural needs, something that conventional agriculture will not be able to do. Recently there has been a great interest in eco-friendly and sustainable agriculture. PGPR are known to improve plant growth in many ways when compared to synthetic fertilizers, insecticides and pesticides. They enhance crop growth and can help in sustainability of safe environment and crop productivity. The rhizospheric soil contains diverse types of PGPR communities, which exhibit beneficial effects on crop productivity. Several research investigations are conducted on the understanding of the diversity, dynamics and importance of soil PGPR communities and their beneficial and cooperative roles in agricultural productivity.



Biofertilizers make nutrients available that are naturally abundant in soil and atmosphere to plants. In a nutshell, it provides "ecofriendly" organic agro-input which has the ability to convert nutritionally important elements from unavailable to available form through biological processes. So, it can be expected to reduce the use of chemical fertilizers and pesticides by introducing biofertilizers in agriculture sector. The microorganisms in biofertilizers are atmospheric nitrogen fixers, P-solubilizers, P-mobilizers like mycorrhizae, potash solubilizers and also those exhibit micronutrient transformations in the soil. Therefore, they are extremely advantageous in enriching soil fertility and fulfilling plant nutrient requirements by supplying organic nutrients. However, there are many challenges in biofertilizer production technology resulting into less production of biofertilizer in India which can't be ignored. The challenges in biofertilizer production include technological, infrastructural, quality testing and marketing constraints.

I wish, this edition will be helpful to researchers, Scientists, administrators and farmers / industrialists and others to understand the importance of Plant Growth Promoting Rhizobacteria (PGPR) for sustainable agriculture.

*Gagnesh Sharma*  
*Chief Editor*

## Introduction to Various Biopesticides and Their Role in Organic Farming

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### Abstract

Biopesticides are biological agents in the form of natural enemies or naturally occurring substances for the controlling pests. Synthetic pesticides are known for their effectiveness in disease management because of broad host range usefulness and less expensive production technology. However, they have devastating negative impacts on the environment as well as on soil health. Due to lack of suitable alternative, the use of synthetic and harmful pesticides in the agriculture is increasing day by day. The uses of biopesticides are emphasizing the agriculturists to move towards the direction of organic farming and sustainable disease management. Major advantages are that these are usually inherently no or less toxic than conventional pesticides affecting only the target pest and closely related organisms. In organic farming, organic growers have available a large array of biopesticides that may be applied for the management of plant diseases.

**Keywords:** Biopesticides; disease management; agriculture; sustainable disease management; organic farming.

### 1.0 Introduction

With extensive use of synthetic chemicals inputs in the past decades has led to a number of long-term environmental problems and health hazard effects. In this situation, the conventional chemical pesticides have although enhanced the crop production, but also adversely affected the environmental and soil health. These synthetic pesticides are continuously accumulating in the soil and environment, harming and causing pollution in the ecosystem. Therefore, it is demand of time to identify alternatives to chemical pesticides for crop protection without effecting the productivity and prosperity of agriculture. Due to these side effects, sustainable crop production through eco-friendly management is basically required in the present scenario. Biopesticides present powerful eco-friendly approaches to generate a new generation of sustainable

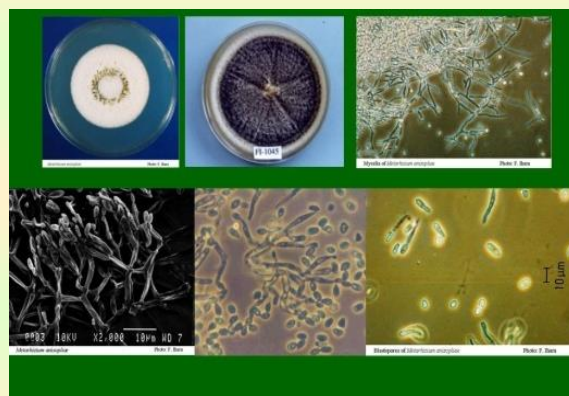
and chemical-free agriculture products. The biopesticides are pesticides derived from natural materials including animals, bacteria, plants, and minerals. Biopesticides separate into three major groups based on the type of active ingredient used including biochemical, plant-incorporated protectants, and microbial pesticides (**USEPA 2011**). Moreover, traditionally farmers use various plant-based products for controlling pests or insects like strong smell of garlic or chilly against any insects, sweet basil as repellent against mosquito and so on. Many such indigenous techniques using neem leaves, marigold cultivation, and ash application have gained popularity among the local farmers in different parts of India. Further such important practices can be improved or used more efficiently with the use of technologies.

So, keeping in view the above factors, the article is based on introduction to biopesticides which offer alternatives to concerns such as resistance towards pests, traditional and sustainable chemical pesticides, and public awareness about ill effects of conventional pesticides on the surrounding environment and ultimately on human health.

## 2.0 Microbial biopesticides

### 2.1 Bacterial Biopesticides

Bacterial biopesticides are the most common and broad form of microbial pesticides that function in multiple efficient ways. Generally, they are used as insecticides, along with to be used as biocontrol agent to control the growth of plant pathogenic bacteria and fungi. As an insecticide, they specifically attack on individual species of moths, butterflies, beetles, flies, and mosquitoes. Their effectiveness is based on the factor that they must come into contact with the target pest or insect and further required to be ingested. The most widely used bacterial pesticides are species and subspecies of *Bacillus thuringiensis* (Bt), accomplishing approximately 90% of the biopesticide market in the world. Since its discovery in 1901, Bt has been widely used world-wide to control insect-pests important in agriculture, forestry, and medicine (**Mazid and Kalita 2011**). Till date, around one hundred *B. thuringiensis* based bioinsecticides, biopesticides, and bio-fungicides have been reported and show insecticidal properties with the synthesis of endotoxins or Cry proteins (**Fig 1**). Biopesticides containing *B. thuringiensis* var. kurstaki kill the caterpillar stage of a wide range of butterflies and moths. In India, *Pseudomonas fluorescens* biopesticide is effectively used as biopesticide formulation and being used against phytopathogen *Ralstonia solanacearum* causing late blight of potato (**Chakravarty and Kalita 2011**).

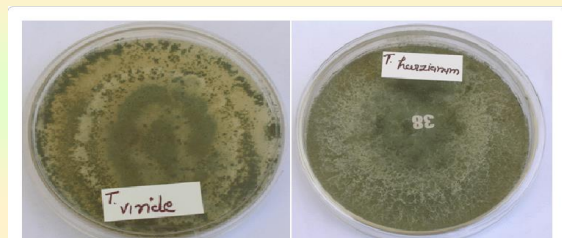


**Figure1.** Mode of action of bacterial pesticide *Bacillus thuringiensis* (Bt)

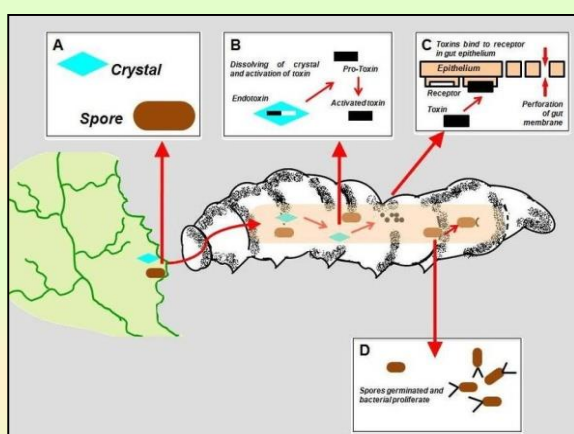
### 2.2 Fungal Biopesticides

The fungal phytopathogens play a significant role in the diseases occurrence in many agriculture and horticulture crops, resulting in severe crop yield losses. Excessive use of fungicides has resulted in accretion of toxic compounds and also responsible in the enhancing resistance in the pathogens. One advantage of fungal biopesticides is that they do not need to be ingested to be effective. However, these microbial pesticides often require a narrow range of conditions including low pH and moist soil and temperature to grow. Biocontrol agents like *Trichoderma* are reported as most effective, safe, and cheap, nullifying the side effects of chemicals pesticides. *Trichoderma* is an antagonist that proliferates into the mycelium of a disease-causing fungus and secretes enzymes that lead to degrade the cell walls of the other fungus and then consumes the degraded contents of the target fungus and reproduce its own spores. It is used worldwide for suitable to control various foliar and soil-borne phytopathogens like *Ceratobasidium*, *Fusarium*, *Macrophomina*, *Pythium*, *Phytophthora* spp., *Rhizoctonia*, and *Sclerotium*. Most commonly, *T. harzianum* fungal biopesticide is an antagonist of *Rhizoctonia*, *Pythium*, *Fusarium*, and other soil-borne pathogens. *Trichoderma viride* has reported to be very promising and potential candidate against soil-borne plant parasitic fungi (**Khandelwal et al. 2012**)(**Fig 2**). *Balsamo bassiana* and *Metarhizium anisopliae* are naturally

occurring entomo-pathogenic fungi that infect sucking pests including *Nezaraviridula* (L) (green vegetable bug) and *Creontiades sp.* (green and brown mirids) (Sosa-Gomez and Moscardi 1998) (Fig 3).



**Figure 2.** Fungal biopesticide *Trichoderma viridae*



**Figure 3:** Entomo-pathogenic fungi *Balsamobassiana*, *Metarhizium anisopliae* as fungal biopesticides

Viral biopesticides also play a promising role in antagonizing pathogens especially bacteria in the form of bacteriophages. Besides that, viruses are host specific and infect only one or a few closely related species, thus due to minimal off-target impacts getting less attention as biopesticides. If the virus possesses characteristic of attacking on bacteria that is phytopathogen, it can be used as a biopesticide. Patent protection and intellectual property are two critical factors in the production and commercialization of phage based biopesticides. The nuclear polyhedrosis virus (NPV), belong to the family of baculoviruses is a virus affecting insects, predominantly butterflies and moths. During ingestion by the host insect, infectious

virus particles are liberated in the insect's internal system and become active. The virus's protein capsule quickly disintegrates in the larval gut, and the viral DNA proceeds to infect digestive cells. After a few days, the host larvae become incapable to digest food and so weaken and depart their lives. NPV are particularly gaining attention for use as biopesticides due to their high host specificity. Another significant advantage is that in some cases, they can serve as alternative source to replace the antibiotics and chemical pesticides.

### 3.0 Plant product as biopesticides

Today there is a global search for alternatives to chemical pesticides and hence, there are various efforts to use natural products for pest control and crop protection. The traditional knowledge regarding the use of natural products for pest control and crop protection has been explored recently. A large number of plants and their extracts for different pests, crops and diseases have been used from past decades. Some of the renowned plants used for pest management in the sustainable agriculture are re neem, garlic, onion, persian lilac, turmeric, ginger, tobacco, papaya, leucas, pongam, tulsi, aloe, custard apple, vitex, sweet-flag, poison nut, calotropisetc (Balasubramanian et al., 2008). Farmers are used to pesticides which are packaged and available from the shelf, even though farmers realize the importance of using plant products as alternatives to chemical pesticides.

### 4.0 Limitation of application of biopesticides in agriculture

- **Lack of awareness among the farmers**  
Use of biopesticides in agriculture is neglected due to well- established chemical pesticide markets, lack of awareness about benefits, application and non-uniform efficiency of biopesticides.
- **Lack of trust of farmers and contradictory field response**  
Lack of trust of using biopesticides is one of the major factors responsible for their

declining popularity. Many farmers reported that reduced use of biopesticides was mainly because of the extremely unreliable supply and contradictory field performance.

- **Poor quality and low shelf life**

Poor quality and low shelf life of biopesticides ultimately lead to their weak performance in fields and are also one of the major problems that hindered their takeover on the market.

- **High production cost and lesser Agribusiness and profit to the entrepreneurs**

Hi-tech and expensive instrumentation required for biopesticides production under completely sterile and aseptic conditions is not getting acceptance by the entrepreneurs. Screening of suitable strains exhibiting characteristics of efficient biopesticide, their identification and other research and development aspects leads to high production cost.

- **Regulatory framework**

Biopesticides registration is the main hurdle in the production. It is not only more expensive than production but also very time-consuming process.

- **Competition with chemical and synthetic pesticides**

Chemical pesticides are used in very high amounts all over the world and one-third of the global agricultural production is dependent on these synthetic pesticides. They show quick response in the fields and hence provide a tough competition to the biopesticides.

- **Health issues to living beings and ecological risks**

There may be some chances of adverse health effects have been noticed during the use of biopesticides when these are not used according to the instructions mentioned on the product, but in comparison to chemical pesticides, risks are far lower. Some studies suggest that spore of entomopathogenic fungi such as *Trichoderma*, *M. anisopliae*, and *B. bassiana* may cause allergy to

farmers. *M. anisopliae* is also reported to affect survival of non-target pests hence affecting the ecology of the ecosystem.

## Conclusions

Rich bio-diversity is a major factor, always providing a wide source of biopesticides which can be effectively used in agriculture at a large scale. The rich traditional knowledge through the highly diverse indigenous communities in world in combination with scientific approach may provide valuable indications for developing effective biopesticides. The traditional inputs using plant extracts can prove as effective biopesticides for broad range of insect-pests and may play important role in pest management in organic farming. The stress on organic farming-based agriculture and chemical free commodities would certainly necessitate increased adoption and application of biopesticides by the agriculturists.

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## Role of Plant Growth Promoting Rhizobacteria (PGPR)—in Sustainable Crop Production

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Plant rhizosphere, the narrow zone of soil surrounding the root system of growing plants, represents a hotspot for microbial activity in the soil (De la Fuente Cantó et al 2020). The rhizosphere is colonized by a wide range of microbial taxa, including both prokaryotes (archaea, bacteria, and viruses) and eukaryotes (fungi, oomycetes, nematodes, protozoa, algae, and arthropods), out of which bacteria and fungi comprise the most abundant groups (Kalam, et al 2017, Buée et al 2009) exhibiting fundamental ecological functions. Free-living soil bacteria that thrive in the rhizosphere, aggressively colonize plant roots, and facilitate plant growth are designated as plant growth promoting rhizobacteria (PGPR), a term introduced by Kloepper and Schroth in 1978 (Dutta et al 2010). This heterogeneous group of bacteria, representing a vital component of the soil microbiome, is known to produce and secrete various regulatory chemicals in the plant roots' vicinity that aid in plant growth promotion (Khoshru et al 2020, Ahemad and Kibret 2014). PGPRs influence plants' overall health by contributing to enhanced nutrient acquisition by host plants, protecting against phytopathogenic microbes, and promoting resistance to various abiotic stresses (Backer et al 2018, Parray et al 2016). Different PGPR strains are capable of increasing crop yields, exhibit biocontrol, enhance resistance to foliar pathogens, promote nodulation in legumes, and enhance the emergence of seedlings (Vejan et al 2016, Kalam, et al 2020, Swarnalakshmi et al 2020]. Reported PGPRs include members of the genera *Acinetobacter*, *Aeromonas*, *Agrobacterium*,

*Allorhizobium*, *Arthrobacter*, *Azoarcus*, *Azorhizobium*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Bradyrhizobium*, *Burkholderia*, *Caulobacter*, *Chromobacterium*, *Delftia*, *Enterobacter*, *Flavobacterium*, *Frankia*, *Gluconacetobacter*, *Klebsiella*, *Mesorhizobium*, *Micrococcus*, *Paenibacillus*, *Pantoea*, *Pseudomonas*, *Rhizobium*, *Serratia*, *Streptomyces*, *Thiobacillus*, and others (Parray et al 2016, Kalam, et al 2020).

### Characteristics of an Ideal PGPR

A rhizobacterial strain is considered to be a putative PGPR if it possesses specific plant growth promoting traits and can enhance plant growth upon inoculation. An ideal PGPR strain should fulfill the following criteria (Vejan et al 2016):

- i) It should be highly rhizosphere-competent and eco-friendly.
- ii) It should colonize the plant roots in significant numbers upon inoculation.
- iii) It should be able to promote plant growth.
- iv) It should exhibit a broad spectrum of action.
- v) It should be compatible with other bacteria in the rhizosphere.
- vi) It should be tolerant of physicochemical factors like heat, desiccation, radiations and oxidants.
- vii) It should demonstrate better competitive skills over the existing rhizobacterial communities.

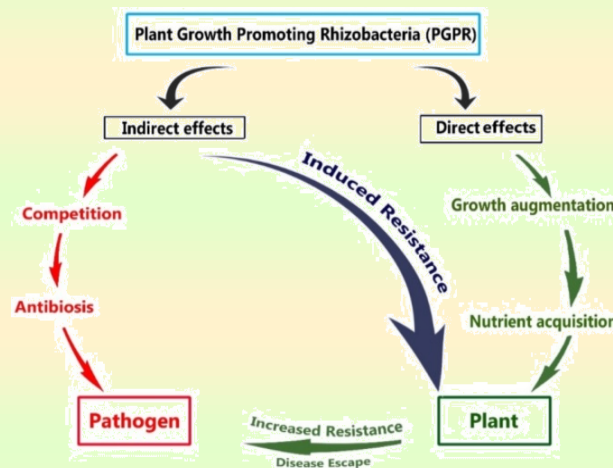
## Plant Growth Promoting Rhizobacteria in Agriculture

Agriculture is an age long practice, it involve the tilling of land and rearing of livestock for food and economic growth. This practice is considered to be the most important human occupation within the tropics, with over 70% of land in use for this purpose (Khan *et al.*, 2014). Rhizobacteria improves plant growth through synthesis of some secondary metabolites such as phytohormone, enzymes, siderophores, and antibiotics (Noordman *et al.* 2006; Ahmad *et al.* 2008), which are needed for the formation of specific enzymes required for plant growth and biochemical change. They help in fixing atmospheric nitrogen, provide nutritional uptake by solubilizing phosphate and producing biologically active molecules which enhances plant growth (Arshad and Frankenberger, 1992). Studies have shown that for PGPR to be utilized in crop production, it must be able to exert it effects in either one of these three ways; first by providing the plant with growth-promoting compounds (Glick 1995), secondly by uptake of certain essential nutrients such as phosphorus, nitrogen, sulfur, calcium and magnesium, (Bashan and Levanony 1990; Belimov and Dietz 2000; Cakmakci *et al.* 2006) and thirdly by averting plants diseases (Khan *et al.* 2002)

### Mechanisms of PGPR Action

Being the dominant rhizosphere microbial community, PGPRs are actively or passively involved in plant growth promotion. They can act as biofertilizers that promote plants' growth and development by facilitating biotic and abiotic stress tolerance and supporting host plants' nutrition [64,86,91,92]. These beneficial groups of bacteria, through their multi-faceted modes of action, including root colonization, positive effects on plant physiology and growth, biofertilization, induced systemic resistance, biocontrol of phytopathogens, etc., offer protection to plants and facilitate plant growth promotion. The detailed mechanisms of PGPR action and their specific contribution to plant

growth promotion have been reviewed comprehensively (Backer *et al* 2018, Dutta *et al* 2010, Khoshru *et al* 2020, Ahemad and Kibret 2014, Parray *et al* 2016]. The modes of action by which PGPRs promote plant growth have been traditionally classified into direct and indirect mechanisms occurring inside and outside the plant, respectively [Goswami *et al* 2016, Glick B R 2012] (Figure 1).



**Figure 1.** Overview of interactions between plant growth promoting rhizobacteria (PGPR), plants and pathogens. PGPRs directly promote plant growth by improving nutrient acquisition by the plant and growth augmentation via regulating phytohormone levels. The indirect effects of PGPRs include suppression of phytopathogens and inducing systemic resistance in plants against a wide range of pathogenic microbes.

Direct modes of PGPR action include improving plant nutrition by providing phyto-nutrients like fixed nitrogen or solubilized minerals from the soil (like P, K, Zn, Fe, and other essential mineral nutrients) and/or stimulating plant growth and development by regulating phytohormone levels (like auxins, cytokinins, gibberellins, abscisic acid, and ethylene) [Parray *et al* 2016, Kalam *et al* 2020, Gouda *et al* 2018]. The indirect effects of PGPRs include influencing the plant health by suppressing phyto pathogens and other deleterious microorganisms through parasitism,

competing for nutrients and niche within the rhizosphere, producing antagonistic substances (like hydrogen cyanid, siderophores, antibiotics, and antimicrobial metabolites) and lytic enzymes (like chitinases, glucanases, and proteases), and inducing systemic resistance in plants against a broad spectrum of root and foliar pathogens [Meena et al 2020, Islam et al 2016, Berg et al 2017, Sayyed et al 2019]. Due to these direct and indirect effects elicited by PGPRs on host plants, they prove to be ideal candidates to be formulated and commercialized as bio-inoculants and phytoprotective microbial products. However, the mode and mechanism of PGPR action vary with the host plant type [Garcia-Fraile et al 2015]. In addition to this, certain other factors also influence PGPR action, viz. biotic factors like plant genotype, developmental stages, plant defense mechanisms, and presence of other members of the microbial community and abiotic factors like soil type, composition, soil management history, and prevalent environmental conditions [Gouda et al 2018, Vacheron et al 2013].

#### **A. Direct mechanism of PGPR**

##### **a) Nitrogen fixation**

Nitrogen (N) is a vital element for all forms of life, it is the most important nutrient for plant growth. Nitrogen is an essential constituent of nucleotides, membrane lipids and amino acids (Marschner, 1995). It constitutes the fourth most important plants dry mass. The biological fixation of atmospheric nitrogen is an important microbial activity for the maintenance of life on earth through photosynthesis performed by photosynthetic organisms. This process occurs when atmospheric nitrogen is converted to ammonia by an enzyme called nitrogenase; a highly complex oxygen labile enzyme conserved in free-living symbiotic diazotrophs (Franche *et al.*, 2009). The process is coupled with the hydrolysis of 16 equivalents of ATP and is accompanied by the co-formation of one molecule of H<sub>2</sub>. Considering the two types of nitrogen fixation (symbiotic and non-symbiotic

process) base on the plant involve and the associated group of organisms, it is agreed generally that non-symbiotic bacteria fix lesser amount of nitrogen than the root nodule bacteria (rhizobia) (James and Olivares, 1997). In spite of their low fixing capacity, some PGPR have shown to be very effective in augmenting this process by making the scarce essential nutrient (nitrogen) available to plants.

In non-symbiotic nitrogen fixation process, free living diazotrophs perform their role by stimulating the growth of non-leguminous plants. The genera identified in this group include *Azoarcus*, *Azotobacter*, *Acetobacter*, *Azospirillum*, *Burkholderia*, *Diazotrophicus*, *Enterobacter*, *Cyanobacteria*, *Pseudomonas* and *Gluconacetobacter* (*Anabaena*, *Nostoc*) (Bhattacharyya and Jha, 2012; Vessey, 2003). While in symbiotic association, bacteria such as *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, and *Mesorhizobium* interact with leguminous plants, while *Frankia* (a nitrogen fixing Actinomycete) makes association with non leguminous trees and shrubs (Zahran, 2001). The inoculation of several cultures with diazotroph PGPR (non-symbiotic nitrogen fixing organisms) especially *Azotobacter* and *Azospirillum* have improved the yield of annual and perennial grasses (Tilak et al, 2005). Additionally, Cyanobacterial nitrogen fixation has been essential in the cultivation of rice by increasing the rice-field fertility. *Azotobacter* inoculant on its own also encourage high yield of wheat by over 30% (Gholami et al., 2009).

##### **b) Phosphate solubilization**

Phosphate is next to nitrogen in the list of essential minerals mostly required by plants. However, there deficiency in soil limits plant growth (Nisha et al., 2014) in a number of ways. It is an insoluble inorganic element which increases the economic viability of any agricultural product when solubilized. The organic forms are found mostly in humus and decayed organic materials. Phosphate represent about

0.2% of plant dry weight as it is essential constituent of nucleic acids, phytin and phospholipids. Additionally, it plays a key role in photosynthesis, respiration, storage and transfer of energy cell division and elongation (Sagervanshi et al., 2012). A large portion of soluble inorganic P is applied to the soil as fertilizer. Due to its rapid rate of fixation and complex formation with other soil elements, it is speedily immobilized and become unavailable to plants (Iordanis et al., 2013; Vikram and Hamzehzarghani, 2008). Organic materials constitute an important reservoir of immobilized phosphate, accounting for about 20-80% of total soil phosphorus. The greater proportion of insoluble inorganic phosphate (apatite) or insoluble organic phosphates (inositol phosphate, phosphomonoesters and phosphotriesters) are inaccessible by plant (Pérez-Montano et al., 2014; Iordanis et al., 2013; Khan et al., 2007).

Microorganisms auspiciously have been identified to play an important role in mediating phosphorus available to plants through their participation in the soil phosphorus cycle. These organisms (PGPRs) either directly solubilize and mineralize inorganic phosphorus or facilitate the mobility of the organic form through biogeochemical cycle for more efficient root uptake (Richardson and Simpson, 2011). Specifically, Phosphate Solubilizing Bacteria (PSB) are *Arthrobacter*, *Pseudomonas*, *Alcaligenes*, *Bacillus*, *Burkholderia*, *Serratia*, *Enterobacter*, *Acinetobacter*, *Azospirillum*, *Azotobacter*, *Flavobacterium*, *Rhizobium*, and *Erwinia* (Zaidi et al., 2009). The PSBs secrete different types of organic acids e.g., carboxylic acid, formic acid, propionic acid, lactic acid, glycolytic acid, fumaric and succinic acid (Vazquez et al., 2000). Kaur et al., (2016) in their discovery established that these organic acids lowers the pH in the rhizosphere, thus causing release of the bound forms of phosphate like  $\text{Ca}_3(\text{PO}_4)_2$  in the calcareous soils. Apart from creating the availability of accumulated phosphate, phosphorus biofertilization also help in increasing the efficiency of biological

nitrogen fixation and render availability of Fe, Zn, etc., through production of plant growth promoting substances. PSB are also able to mineralize the insoluble organic phosphate through the excretion of extracellular enzymes such as phytases and C-P lyases phosphatases (Weyens et al., 2010).

### c) Siderophore production

Iron is a vital element needed by all forms of life. It is one of the most abundant mineral deposits on earth. The unavailability of this element in its biological form for plant utilization creates perplexing circumstances for its growth. Siderophore which literally means iron carrier or iron chelating is an important strategy developed to increase iron ( $\text{Fe}^{3+}$ ) bioavailability as a unique constituent of cytochrome, enzymes co-factor and heme or non-heme proteins. Siderophores are low molecular weight biomolecules produced by microorganisms, and has strong affinity with  $\text{Fe}^{3+}$  ions while moving into the cell (Sureshbabu et al., 2016; Neilands, 1989). When Fe is limited, microbial siderophores scavenge and provide plants with Fe from the mineral phase through the formation of soluble  $\text{Fe}^{3+}$  complexes. Suppression of soil borne plant pathogens by siderophore producing *Pseudomonas* has been reported (Buysens et al., 1996; Loper, 1988). Related study has shown that siderophore production occurs in both gram positive and gram negative organisms with specific example of *Bacillus*, *Rhodococcus*, *Pseudomonas* and *Enterobacter* genera (Tian et al., 2009). Consequently, this property is also exhibited by some plant especially grasses (phyto-siderophores) (Van der Helm and Winkelmann, 1994), as they form constituent in fertilizer formulation, regulate iron intake capacity in plants and facilitate growth (Miller and Malouin, 1994).

One of the major challenges limiting efficient production of siderophore is environmental factor. These include pH, soil level of iron and their forms, presence of other trace elements, inadequate supply of carbon, nitrogen and phosphorus (Duffy and

Défago, 1999). However, siderophore mediated growth promoting activity of PGPR is associated with the suppression of root pathogens by competitive exclusion, hindering deleterious microorganisms access to environmental iron by extracellular siderophores complex formation (Podile and Kishore, 2006; Ahemad and Khan, 2011; Saharan and Nehra, 2011). Elsewhere, works have shown that PGPR synthesis of siderophore improve not only the growth performance of plants and their adaptation in stress environment, but also enhance their ability to absorb both radioactive iron and rhizospheric metals iron even at low concentration (Dimkpa et al., 2009; Robin et al., 2008).

Apart from creating favourable competitive room for bacteria against some pathogenic microorganisms by removing iron from the environment (O'Sullivan and O'Gara 1992; Persello-Cartieaux *et al.* 2003), chelated iron have also proven to poses one of the weakest affinity with fungi (Loper, and Henkels. 1999). This condition seems possible considering the fact that many bacterial siderophores differ in their strength or abilities to sequester iron leading to it biological and/or adaptive deprivation of the scares commodity (iron) to the pathogenic organisms. Generally, production of siderophore by PGPR is most efficient in controlling the plant root pathogens (Diaz *et al.*, 2002; and Dey *et al.*, 2004). Siderophores has also been linked with potential of promoting bacterial auxin synthesis by reducing the detrimental effects of heavy metals through chelation mechanism (Dimkpa *et al.*, 2008).

#### **d. Plant Growth Regulator**

These are organic chemical compounds that influence plant growth. They are in other words called plant growth regulator or phytostimulant e.g.; Auxin (indole- 3-acetic acid (IAA), Gibberellic acid (GA), cytokinins, and ethylene. These chemical molecules are recognized over the years as four major plant hormones needed for biochemical,

physiological and morphological development. PGPR species belonging to the genera *Azospirillum*, *Pseudomonas*, *Xanthomonas*, *Rhizobium*, *Bradyrhizobium*, *Alcaligenes*, *Enterobacter*, *Acetobacter* and *Klebsiella*, and also the species of *Bacillus pumilus*, *B. licheniformis*, *Paenibacillus polymyxa*, *Phosphobacteria* sp, *Glucanacetobacter* sp, *Aspergillus* sp and *Penicillium niger* possess the ability to produce phytohormones (Lordanis et al., 2013; Shobha and Kumudini, 2012).

#### **i) Auxin**

Auxin is an essential molecule that regulates directly or indirectly most plant processes. Being the first phytohormone discovered by Darwin (1880) using *Phalaris canariensis* seeds, it has since paved way for more discovery leading to identification of indole-3- acetic acid (IAA) as the most active and famous plant hormones of auxin group. Irrespective of plant being able to synthesis this chemical molecule (endogenous supply), they still depend largely on external supply (exogenous) for their optimum performance. This exterior meet up is predominantly oversee by PGPR and it associate soil bacterial (Khalid et al., 2006; Patten and Glick, 2002). Auxin triggers a number of cellular function ranging from differentiation of vascular tissues, initiation of lateral and adventitious roots, stimulation of cell division, elongation of stems and roots, and orientation of root and shoot growth in response to light and gravity (Glick, 1995). For PGPR to produce IAA more efficiently, consideration of the type of specie and strain, its culture condition, developmental stage cum availability of nutrient in the rhizosphere are of important (Ashrafuzzaman et al., 2009).

Although other auxins, such as indole 3 butyric acid (IBA) and phenyl acetic acid (PAA) have also been identified in plants (Normanly, 1997), scientist are yet to understand their complexity and most importantly their mode of action. Contrary, bacteria IAA producers (BIPs) are found to

be most abundant in the soil/plant auxin pool and L-Tryptophan (L-TRP) as a precursor that aid in the increase and production of auxin. This was demonstrated in *Bacillus amyloliquefaciens* FZB42 (Idris *et al.*, 2007), Fluorescent *Pseudomonas* (Karnwal, 2009) and *Azotobacter* and *Azospirillum* strains in canola plant (Yasari and Patwardhan, 2007). Findings suggest that rising level of L-Tryptophan increases the biochemical and metabolic activities of BIPs or auxin producing bacterial (APBs), with a corresponding response in root length and modifications of root architecture. The four main metabolic pathways dependent of tryptophan are; tryptophol, tryptamine, indole-3-pyruvic acid and indole-3-acetamide pathway (Bartel, 1997). Emerging evidence illustrate that organisms which produces low quantity of auxins as a result of absence of L- Tryptophan have the propensity of turning up high amount of auxins when augmented with L-tryptophan, especially in the presence of viable strain of *Rhizobium* (Zahir *et al.* 2004; Zahir *et al.*, 2010). Importantly, it interesting to note that the indigenous auxin (IAA) produced by plant though will contribute to plant growth, it might still not be necessarily enough for the optimal performance of the plant (Pilet and Saugy, 1987).

## ii) Gibberellic Acid (GA)

The exact mechanisms by which PGPR promote plant growth via the synthesis of gibberellic acid are still not yet fully understood. In GA promote the development of stem tissue, root elongation and lateral root extension (Yaxley *et al.*, 2001). GA constitute a group of tetracyclic diterpenes that greatly influence the processes of seed germination, leaf expansion, stem elongation, fruit development, flower and trichome initiation (Yamaguchi 2008). Because of their vital role in improving efficient photosynthetic processes in plants, gibberellins remain the primary target during environmental stress

condition, making it an important plant growth bioregulator that can increase the stress tolerance of many crop plants. The improvement of plant growth by some rhizobacteria (PGPR) producing gibberellins was reported by Kang *et al.*, 2009. The exogenous application of this growth hormone may be useful in amendment of polluted soil and improvement of crop performance (Iqbal *et al.* 2011). Application of GA has shown to increase considerably the grain yield in wheat (Iqbal *et al.* 2011; Radi *et al.* (2006), barley (Vettakkorumakankav 1999) and tomato by decreasing stomatal resistance and improved water use efficiency (Maggio *et al.*, 2010). Conclusively, gibberellin is involved in plant morphology modification by stimulating the development of aerial part (Van Loon, 2007) as they remain an excellent alternative for inducing stress tolerance.

## iii) Cytokinins

Cytokinin play a significant role during cell division, vascular differentiation nutrient mobilization, chloroplast biogenesis, shoot differentiation, leaf senescence, apical dominance, anthocyanin production, and photomorphogenic development (Davies, 2004). It participates in vascular cambium sensitivity, proliferation of root hairs and contrarily in inhibition of lateral root formation and primary root elongation (Aloni *et al.*, 2006). This molecule can be acquired endogenously and exogenously by either plant or PGPR respectively. Plant increases uptake of endogenous cytokinin via the promotion of biosynthesis (Pospíšilová 2003b). Studies have shown that during plant growth, cytokinin perfectly regulates plant adaptation especially in salt polluted site (Hadiarto and Tran 2011). Biochemical processes revealed that cytokinin serve as a major antagonist to abscisic acid (ABA), thus resulting in metabolic alteration of other phytohormones (Pospíšilová 2003a). During water scarcity, the plant cytokinin content reduces drastically with a resultant positive increase in ABA concentration. Assessing the production of plant hormones by different

*Streptomyces* strains in broth medium, shows that all strains of *Streptomyces* synthesizes cytokinins and gibberellins (Mansour et al., 1994). Though this is vital for phyto development, its mechanism of action is still not well elucidated. Cytokinin receptor gene of most plants and organisms are regulated by changes in osmotic conditions and as well demonstrate a complex osmotic stress response (Merchan et al., 2007). Research has shown that inoculating seedling with cytokinin producing strains of *Bacillus subtilis* confer the plants resistance against environmental stress.

#### iv) Ethylene

This is a unique phytohormone with wide range of biological activities. The beneficial role of this biomolecule is best recorded at low concentration. It hinders some key developmental properties e.g., root elongation, induce defoliation and other cellular processes at high concentration resulting to reduced crop performance (Bhattacharyya and Jha, 2012). Pierik, *et al.* (2006) was of the opinion that its classification as a senescence hormone was due to its inhibitory role to plant growth. To overcome this alarming consequence, an enzyme 1- aminocyclopropane-1 carboxylic acid (ACC) deaminase is needed. The role of this biocatalyst is to degrade the plant ACC which is the direct precursor of ethylene synthesis in plant to  $\alpha$ -ketobutyrate and ammonium (Glick et al., 2007). The result of the degradation is the reduction of plant ethylene production through a range of mechanisms, while the PGPR producing ACC-deaminase regulates the ethylene level in plant and prevents the growth inhibition caused by high levels of ethylene (Noumavo et al., 2016). PGPR capable of inducing exogenous production of ethylene via degradation of the endogenous product using enzyme include *Acinetobacter*, *Achromobacter*, *Agrobacterium*, *Alcaligenes*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Pseudomonas*, *Ralstonia*, *Serratia* and *Rhizobium*. Works have shown that PGPR ACC deaminase activities was vital for

*Brassica napus* growth (Dell'Amico et al., 2008). Pierik et al., (2006) suggested that at low concentration of ethylene mediated by PGPR, the plant yield, growth performance and germination properties of *Arabidopsis thaliana* get accelerated. However, this vaporous hormone regulate also root initiation, fruit ripening, seed germination, leaf abscission and wilting (Kaur et al., 2016).

### B. Indirect Mechanism of PGPR

#### a) BIOCONTROL

PGPR has been identified as biocontrol agent with capacity to suppress a wide range of organisms possible of presenting disease condition to plant. For PGPR to be an efficient biocontrol agent against pathogenic bacteria, fungi and viruses, it must utilize one of the following mechanisms; production of antibiotics, competition for nutrients and niche, signal interference, induced systemic resistance, hydrogen cyanide and lytic enzymes production. (Podile and Kishore, 2006; Lugtenberg and Kamilova, 2009). Generally, these modes of actions are classified as either direct or indirect form of antagonism, with fungi, bacteria and nematode being the most pathogenic organisms of interest in their order of severity. Consequently, this means of plant disease control involves application of beneficial rhizobacteria or their metabolites in minimizing/neutralizing the negative impact of pathogens while promoting healthy living in plants (Junaid et al. 2013).

#### b) Antibiotics Production

Antibiotics production is one of the most studied biocontrol strategies display by PGPR. A good example include amphisin, 2,4-diacetylphloroglucinol (DAPG) oomycin-A, phenazine, pyoluteorin, pyrrolnitrin, tensin, tropolone, and the cyclic lipopeptides (Loper and Gross, 2007) synthesis. Others include oligomycin A, kanosamine, zwittermicin A, and xanthobaccin (Compant, *et al.*, 2005). Basically, these biochemical are produced by *Pseudomonas* strains. *Bacillus*, *Streptomyces*, and



*Stenotrophomonas* sp. As an active chemical agent, they are influenced by biotic and abiotic factor and environmental stress. Antibiotics are low weight molecular compound that suppress the development of plants pathogenic microorganisms. Phloroglucinols (Phl), 2-hexyl-5-propyl resorcinol (HPR), D-gluconic acid, hydrogen cyanide (HCN) and 2-hydroxymethyl-chroman-4-one have successfully been utilized as biocontrol agent (Perneel et al. 2008; Kang et al. 2004; Kaur et al. 2006; Cazorla et al. 2006). Elsewhere, increase productivity as a result of biocontrol inoculant was reported in *S. rochei* inhibition of pepper root rot caused by phytophthora (Ezziyyani et al., 2007); *S. platensis* against *R. solani* leaf blight/seedling blight of rice (Wan et al., 2008);

Fusarium root rot and tomato wilt caused by *S. griseoviridis* (Minuto et al., 2006); *S. hygroscopicus* infection of *Colletotrichum gloeosporioides* anthracnose and in wide range of crops (Prapagdee et al., 2008) Lugtenberg and Kamilova 2009). The demonstration of increased growth and productivity of many commercial crops including maize (Sandhya et al., 2010), rice (Ashrafuzzaman et al., 2009), black pepper (Dastager et al., 2010), wheat (Cakmakci et al., 2007), sugarcane (Sundara et al., 2002), cotton (Anjum et al., 2007), Banana (Mia et al., 2010), and cucumber (Maleki et al., 2010).

### c) Nutrients and niche competition

For rhizospheric bacteria to claim dormant over the rest of soil microorganisms, it must be able to compete favourably for the available nutrient and space. This is a vital strategy needed to limit the incidence and severity of plant disease (Kamilova et al. 2005). Consequently, this adaptation makes the root unfit to host phytopathogens as a result of PGPR rapid and abundant colonization. As a negative form of association, the most competent group of microorganisms takes charge and controls the whole metabolic activities. Aside the

inherent growth which PGPR acquires via competition as a result of sufficient nutrient availability, other properties such as presence of flagellium, lipopolysaccharide, chemotaxis and the usage of secreted root exudate enhanced their survival (Lugtenberg and Kamilova, 2009). A good illustration can be seen in unavailability of iron to phytopathogenic fungi when chelated by siderophores synthesized by PGPR. Conversely, iron is one of the essential nutrients required by all microorganisms for synthesis of ATP, formation of heme, reduction of ribotide precursors of DNA, and a number of functions (Saraf et al. 2011). In niche competition, a physical occupation of site by PGPR is enhanced through delay tactics, by preventing the colonization of pathogens until the available substrate is exhausted (Heydari and Pessarakli 2010). This feature has been an age long adaptive property exerted by beneficial soil microorganisms to occupy the root rhizosphere and make available scarce nutrient for their upkeep (Lugtenberg et al. 2001).

### d) Induced Systemic Resistance (ISR)

PGPR trigger inducement of some kind of defense system that is capable of fighting some pathogenic bacteria, fungi and viruses. This potentially positions the plant as much stronger and highly adapted specie (Van Loon, 2007). The gene and gene product involve in this form of biological control phenomenon has not been well documented. Unlike the systemic acquired resistance (SAR) (Handelsman and Stabb 1996), which is a state of defense that is activated all through the plant following the primary infection by pathogens (Ryals et al. 1996), Induce systemic resistance (ISR) utilize organic acid and plant hormones (salicylic acid, jasmonic acid, and ethylene) in plants signaling and stimulation of the host plant defense response against variety of plant pathogens (Niranjan et al. 2005; Beneduzi et al. 2012; Pieterse et al. 2014). PGPR response to ISR is usually felt by increased physical and mechanical strength of the cell wall as well as adjusting their

physical and biochemical reaction to environmental stress (Labuschagne et al. 2010). ISR in PGPR can be in the form of salicylic acid, siderophores production, lipopolysaccharide, flagella, N-acyl homoserine lactone (AHL) molecules (Van Loon 2007; Shuhegger et al. 2006) and antibiotics. The participating organisms in this form of biocontrol include *Bacillus pumilus*, *Pseudomonas* sp and enterobacteria (Jourdan et al., 2009). In a wider scale, application of PGPR strain as seed coat have improved tremendously the ISR against *Colletotrichum lagenarium* which causes anthracnose in cucumber, *Pseudomonas syringae* causing angular leaf spot and bacterial wilt by *Erwinia tracheiphila* (Zehnder et al. 2001).

#### e) Signal Interference

For an organism (beneficial or pathogenic) to exert its function, a particular number or quorum is required. This requirement especially in gram negative organisms is communicated via a small diffusible signaling molecule called N-acyl homoserine lactone (AHL). This regulatory agent allows the cells to sense the population of their kind and to express certain character. The development of essential physiological characters such as production of pathogenicity/virulence factors, swarming, swimming and twitching motilities, rhizosphere colonization can also be credited to cell signaling (Gray and Garey 2001; Miller and Bassler 2001). The discovery of enzyme capable of degrading AHL is considered to be a fight in the right direction against phytopathogens quorum-sensing system, as *Bacillus thuringiensis* has shown to efficiently decrease the incidence and development of potato soft rot caused by *E. carotovora* using signal interference strategy (Dong et al. 2004).

#### f) Lytic enzymes production

The production of extracellular enzymes such as chitinases,  $\beta$ -1-3 glucanases, lipases, cellulases, and proteases by rhizobacteria has been suggested to be a vital form of biocontrol (Markowich and Kononova 2003). They are hydrolytic

enzymes that are capable of degrading wide range of compound usually of plant origin. For plants to be hydrolyzed, chitinases, glucanases, cellulases, proteases, dehydrogenases, lipases, phosphatases, exo and endo- poly galacturonases, pectinolyases must be secreted (Joshi et al., 2012; Whipps, 2001), more so for the lysing of fungal cell wall (Mabood et al. 2014). Palumbo et al. (2005) has suggested the significance of beta-1, 3-glucanase on the biocontrol activities of *Lysobacter enzymogenes* strain C3 against Bipolaris leaf spot caused by *Phytophthora* sp. This innate properties shield the plants from the attack of foreign pathogens. As multifunctional organic protein, these enzymes form protection from desiccation and against abiotic and environmental stress (Qurashi and Sabri, 2012). Lytic enzyme can be used in the control of blight in pepper by *Phytophthora capsici* (Jung et al. 2005), Fusarium infection (Hariprasad et al. 2011) and sugar beet by *Pythium ultimum* (Dunne et al., 1997). Chaiarn et al., (2008) illustrate the antagonistic potential of PGPR by production of chitinase,  $\beta$  1, 3 glucanase, proteolytic enzymes and cellulase at low concentration, even as *Pseudomonas* sp has proven to be a good candidate in the synthesis of lytic enzymes (Cattelan et al., 1999). Mycoparasitic and Trichoderma species have also been implicated in its antagonistic biocontrol activities against R. necatrix and other plant pathogens using chitinases (Hoopen and Krauss 2006; Harman et al. 2004).

#### g) Hydrogen cyanide (HCN)

Production of hydrogen cyanide (cyanogenesis) is predominantly associated to *Pseudomonas* sp quantitatively; this can be detected according to the techniques described by Lorck (1948). HCN a well studied biocontrol agent is known to be a volatile compound. Its cyanide ion inhibits most metalloenzymes, especially copper containing cytochrome c oxidases (Blumer and Haas 2000). Cyanide produced by *Pseudomonas* strains has successfully

been used to curb canker of tomato (Lanteigne et al., 2012). As a secondary metabolite produced by gram negative bacteria, it is formed from glycine and catalyzed by HCN synthase (Castric, 1994). *P. fluorescens* strain CHA0 (Voisard et al., 1989) was used to control tobacco black root rot caused by *Thielaviopsis basicola* (Laville et al., 1998). However, because of the aggressive colonizing strength of *Fluorescent pseudomonas*, it has effectively been used in the control of soil-born plant pathogens (Lugtenberg et al. 2001). There are still indications that a good number of rhizobacteria are cyanogenic when provided with glycine in their culture medium

### Conclusion:

In the past century, for an agricultural practice to the successful one must not neglect the use of chemical fertilizer, herbicides and pesticides. Initially, they aid in plant growth while at a long run exert their negative effect. This norm has not only affected the soil and its inhabitant but also renders threat to human life through the food chain. With rise in soil pollution, climatic condition, soil-born pathogen and extensive land overuse, the soil has become grossly infertile and unproductive. As evident in the low agro output, food insecurity couple with rising human population. To achieve self-sufficiency, effort must be made especially in the tropics to key into scientific knowledge through broad understanding of soil-plant-microbial interaction and their mechanism of action. This will not only lead to bumper harvest but keep the soil safe and healthy. However, actions should focus on substituting agrochemicals with bioproduct such as biofertilizer, bioinsecticide and bioherbicide with consortium of beneficial PGPR. Highlighted advantages of these bioinoculant in terms of increased plant nutrient, and biocontrol through, induction of systemic resistance and nutrients or space competition must be carefully stated and comprehended by farmers to enhance crop yield while retaining soil quality.

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## Specification of Biofertilisers in schedule –III (Part A ) under FCO-1985

- as amended vide Gazette Notification, dated 02.07.2021

### 1. Rhizobium

Total Viable count	CFU minimum $5 \times 10^7$ cell/g of powder, granules or carrier material/ or per gram capsule content in gelatin base or $1 \times 10^8$ cell/ml of liquid
Contamination level	No contamination at $10^5$ dilution
pH	6.5-7.5
Efficiency character	Should show effective nodulation on all the species listed on the packet and there should be minimum of 25% increase in dry matter yield in test plant, after 25 Days after sowing (DAS) when tested as per the method given under controlled conditions

### 2. Azotobacter

Total Viable count	CFU minimum $5 \times 10^7$ cell/g of powder, granules or carrier material/ or per gram capsule content in gelatin base or $1 \times 10^8$ cell/ml of liquid
Contamination level	No contamination at $10^5$ dilution
pH	6.5-7.5
Efficiency character	The strain should be capable of fixing at least 10 mg of nitrogen per g of sucrose consumed.

### 3. Azospirillum

Total Viable count	CFU minimum $5 \times 10^7$ cell/g of powder, granules or carrier material/ or per gram
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	capsule content in gelatin base or $1 \times 10^8$ cell/ml of liquid
Contamination level	No contamination at $10^5$ dilution
pH	6.5-7.5
Efficiency character	Formation of white pellicle in semisolid N-free bromothymol blue media.

### 4. Phosphate solubilising Bacteria

Total Viable count	CFU minimum $5 \times 10^7$ cell/g of powder, granules or carrier material/ or per gram capsule content in gelatin base or $1 \times 10^8$ cell/ml of liquid
Contamination level	No contamination at $10^5$ dilution
pH	6.5-7.5 for moist/dry powder, granulated carrier based and 5.0 – 7.5 for liquid based
Efficiency character	The strain should be capable of solubilizing at least 30 mg/litre of Phosphorus in liquid broth when tested as per the method given using Tricalcium Phosphate or Aluminium Phosphate or Iron Phosphate as Phosphate source.

### 5. Mycorrhizal Biofertilizers

Total viable spores/gm of product, minimum	10 viable spore per gram of finished product
pH	6.0-7.5
Inoculum potential	1200 IP per gram of finished product by MPN method with 10 fold dilution

### 6. Potassium Mobilizing Biofertilisers (KMB)

Total Viable count	CFU minimum $5 \times 10^7$ cell/g of powder, granules or carrier material/ or per gram capsule content in gelatin base or $1 \times 10^8$ cell/ml of liquid
Contamination	No contamination at $10^5$ dilution
pH	6.5-7.5 for moist & dry powder granulated carrier based and 5.0-7.5 for liquid based.
Efficiency character	The strain should be capable of solubilizing at least 20 mg/litre of Potash in liquid broth when tested as per the method given using Aluminium Potassium Silicate as K source.

### 7. Zinc Solubilizing Bacteria (ZSB)

Total Viable count	CFU minimum $5 \times 10^7$ cell/g of powder, granules or carrier material/ or per gram capsule content in gelatin base or $1 \times 10^8$ cell/ml of liquid
Contamination	No contamination at $10^5$ dilution
pH	6.5-7.5 for moist & dry powder granulated carrier based and 5.0-7.5 for liquid based.
Efficiency character	The strain should be capable of solubilizing at least 20 mg/litre of Zinc in liquid broth when tested as per the method given using Zinc Oxide/ Zinc Carbonate/ Zinc Phosphate as Zinc source.

### 8. Acetobacter

Total Viable count	CFU minimum $5 \times 10^7$ cell/g of powder, granules or carrier material/ or per gram capsule content in gelatin
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	base or $1 \times 10^8$ cell/ml of liquid
Contamination	No contamination at $10^5$ dilution
pH	5.5-6.0 for moist/ dry powder, granulated or carrier and 3.0-6.0 for liquid
Efficiency character	Formulation of yellowish pellicle in semisolid medium N free medium

### 9. Carrier Based Consortia

Individual organism Viable cell count	CFU minimum in a mixture of any 2 or maximum three of following microorganisms: CFU minimum: Rhizobium or Azotobacter or Azospirillum: $1 \times 10^7$ /g PSB: $1 \times 10^7$ /g KSB: $1 \times 10^7$ /g
Total viable count of all the biofertilizer organisms in the product	CFU minimum $3 \times 10^7$ /g of carrier/powder
Efficiency character :	The efficiency character of individual microorganism to be determined as mentioned in case of individual biofertilizers through quantitative estimation methods.

### 10. Liquid Consortia

Individual organism Viable cell count	CFU minimum in a mixture of any 2 or maximum three of following microorganisms: CFU minimum: Rhizobium or Azotobacter or Azospirillum: $5 \times 10^7$ /mL PSB: $5 \times 10^7$ /mL KSB: $5 \times 10^7$ /mL
Total viable count of all the biofertiliser organisms in the product	CFU minimum $1.5 \times 10^8$ /mL of liquid based

Contamination	No contamination at any dilution
pH	5.0-7.0
Efficiency character :	The efficiency character of individual microorganism to be determined as mentioned in case of individual biofertilizers through quantitative estimation methods.

Efficiency character	The strain should be capable of solubilizing at least 30 mg/litre of Phosphorus in liquid broth when tested as per the method given using Tricalcium Phosphate or Aluminium Phosphate or Iron Phosphate as Phosphate source.
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### 11. Phosphate Solubilizing Fungal Biofertilizer

Spore count	Minimum $1 \times 10^6$ / spores/gram Minimum $1 \times 10^7$ viable fungal spores/ ml of liquid
Contamination level	Nil for liquid inoculum $1 \times 10^3$ cells/g for carrier based preparation
pH	Liquid: 3.5-5.5 Carrier: 6.0-7.7

### Part-B

#### Tolerance limit of Biofertilizers

1. In case of Rhizobium, Azotobacter, Azospirillum, PSB, KMB, ZSB, the total viable count shall not be less than  $1 \times 10^7$  CFU/g of carrier or  $5 \times 10^7$  per ml of liquid material.
2. In case of consortia, the total viable count shall not be less than  $1 \times 10^7$  in case of carrier based and  $1 \times 10^8$  in case of liquid formulations.
3. In case of mycorrhizal biofertilisers, total viable spores shall not be less than 8/ gm of finished product.

## **Task Force on "Production and Promotion of Bio fertilizers with special Focus on Improving Economic Viability of *Gaushalas*"**

In pursuance of the decision taken in the meeting held under the Chairmanship of Hon'ble Vice Chairman, NITI Aayog on "Organic Fertilizers and Marketing Constraints", on July 9, 2021 with representatives of Gaushalas, a Task Force on "Production and Promotion of Bio fertilizers with special Focus on Improving Economic Viability of *Goshalas*" was constituted under the Chairmanship of the Member (Agriculture), NITI Aayog. Director, NCOF Dr. Gagnesh Sharma has been nominated as one of member of this task force.

Terms of Reference of the Task Force will be as follows :

- (i) To assess the status of bio fertilizer production and consumption in the country and briefly present its implications.
- (ii) Assessment of the existing standards of bio fertilizers through the Organic Fertiliser Control order of the Government of India for organic fertiliser and suggest changes to expand its scope and the existing testing facilities including recommendations thereof.
- (iii) To devise innovative ways and policy to promote cow economy and use of cow manure as bio-fertilizer

and bio-energy especially for gaushalas to convert cattle into economic assets.

- (iv) To devise the measures/policy initiatives for encouraging the commercial production, packaging, marketing & distribution of bio fertilizers including development of Brandis and address difficulties in marketing & certification of bio fertilisers.
- (v) To explore Public Private Partnership model with various stakeholders including goshalas, Dairy cooperatives and Farmer Producer Organisations for production of enriched solid and liquid bio fertilizers.
- (vi) Suggest mechanism and policy support for encouraging the commercial production, packaging, marketing, & distribution and use of bio fertilizers to create level playing field with inorganic fertilisers.

The Task Force may submit its report within three months.

(Source; Web portal of Niti Aayog vide F.No. Q-11/2/2021-Agri. Comp.# 36181 Government of India, National Institute for Transforming India (NITI) Aayog Knowledge & Innovation Hub (KI), Dated: September 2021.

## Research Abstract

Drought is one of the major abiotic stresses that affects crop yield worldwide. An eco-friendly tool that can broadly improve plants' tolerance to water stress is bioinoculation comprising plant growth-promoting rhizobacteria (PGPR). In this study, the effect of two PGPR *Cupriavidus necator* 1C2 (B1) and *Pseudomonas fluorescens* S3X (B2), singly and/or co-inoculated at two inocula sizes (S1 -  $3 \times 10^3$  cells  $g^{-1}$  dry weight (dw) soil and S2 -  $3 \times 10^6$  cells  $g^{-1}$  dw soil), on growth, nutrient uptake, and use efficiency was assessed in maize (*Zea mays* L.) plants grown at three levels of irrigation (80% of water holding capacity (WHC) – well-watered, 60% of WHC - moderate water deficit stress, and 40% of WHC - severe water deficit stress) in a greenhouse experiment. The impact of water deficit and bioinoculants on soil microbial activity (fluorescein diacetate hydrolysis) was also evaluated. Moderate and severe water deficit negatively affected soil microbial activity, as well as, maize growth, by reducing plants' shoot biomass and increasing root/shoot ratio at 60 and 40% of WHC. Bioinoculants mitigated the negative effects on shoot biomass, especially when PGPR were co-inoculated, increasing up to 89% the aerial biomass of plants exposed to moderate water deficit. Bioinoculation also increased nitrogen (N) and phosphorous (P) use efficiency, which may have led to higher maize growth under water deficit conditions. The size of the inocula applied had marginal influence on biometric and nutrient parameters, although the higher concentration of the mixture of PGPR was the most effective in improving shoot biomass under moderate water deficit.

This study shows that rhizobacterial strains are able to increase nutrient use efficiency and to alleviate water stress effects in crops with high water demands and have potential applications to keep up with productivity in water stress scenarios. **(Plant growth-promoting rhizobacteria (PGPR) improve**

**the growth and nutrient use efficiency in maize (*Zea mays* L.) under water deficit conditions** S.I.A. Pereira, D. Abreu, H. Moreira, A. Vega, P.M.L. Castro Heliyn6 (2020) e05106

The study aimed to investigate an effect of organic fertilizer applied to rice in the first planting season for unfertilized soybean as second crop followed by inorganic and biofertilizer applied in the third season on soybean growth and yield under the rice-soybean-soybean cropping pattern in 2016/2017. The main plot was organic: (1) without organic amendment, (2) 10 t  $ha^{-1}$  of chicken manure, and (3) 10 t  $ha^{-1}$  precomposted rice straw. The subplot was inorganic N and P fertilizers and commercial biofertilizer (consisting of *Rhizobium*, nitrogen-fixing bacteria, and P-solubilizing bacteria) applied at planting of soybeans in the second dry season: (1) control (no inorganic and biofertilizer), (2) 50 kg urea  $ha^{-1}$ , (3) 100 kg SP36  $ha^{-1}$ , (4) 50 kg urea + 100 kg SP36  $ha^{-1}$ , (5) biofertilizer, (6) biofertilizer + 25 kg urea  $ha^{-1}$ , (7) biofertilizer + 75 kg SP36  $ha^{-1}$ , and (8) biofertilizer + 25 kg urea + 75 kg SP36  $ha^{-1}$ . Soybean planted on the first dry season after rice harvested was not fertilized (untreated). The results showed that the chicken manure amendment increased grain yield of soybean in the second season, i.e., from 1.03 t  $ha^{-1}$  (without organic amendment) to 1.27 t  $ha^{-1}$ , an increase of 23%. There was no effect of rice straw on soybean grain yield. In the third season, however, the residual effect of straw compost or chicken manure increased soybean grain yield by 8% and 20%, respectively. Both straw compost and chicken manure also showed a positive effect on the use of inorganic and biofertilizers in increasing soybean productivity.

**Impact of organic matter, inorganic and biofertilizers combination on Soybean yield in Entosol soil of Indonesia (Didik Sucahyono, Yudi Widodo, Runik D.**

**Purwaningrahayu, Henry Kuntastuti, Herdina Pratiwi, Sri Wahyuningsih, Titik Sundari, Rudy Soehendi, I. Gusti K., D. Arsana and Made J. Mejaya: International Journal of Agronomy, Vol. 2021, Article ID 7222217, 7 Pages**

In order to reduce chemical fertilization and improve the sustainability of common wheat (*Triticum aestivum* L.) cultivation, maintaining at the same time high production and quality standards, this study investigated the effects of three commercial biofertilizers on rhizosphere bacterial biomass, biodiversity and enzymatic activity, and on plant growth and grain yield in a field trial. The wheat seeds were inoculated with the following aiding microorganisms: (i) a bacterial consortium (*Azospirillum* spp. + *Azoarcus* spp. *Azorhizobium* spp.); and two mycorrhizal fungal-bacterial consortia, viz. (ii) *Rhizophagus irregularis* + *Azotobacter vinelandii*, and (iii) *R. irregularis* + *Bacillus megaterium* + *Frateuria aurantia*, and comparisons were made with noninoculated controls. We demonstrate that all the biofertilizers significantly enhanced plant growth and nitrogen accumulation during stem elongation and heading, but this was translated into only small grain yield gains (+1%–4% vs controls). The total gluten content of the flour was not affected, but in general biofertilization significantly upregulated two high-quality protein subunits, i.e., the 81 kDa high-molecular-weight glutenin subunit and the 43.6 kDa low-molecular-weight glutenin subunit. These effects were associated with increases in the rhizosphere microbial biomass and the activity of enzymes such as  $\beta$ -glucosidase,  $\alpha$ -mannosidase,  $\beta$ -mannosidase, and xylosidase, which are involved in organic matter decomposition, particularly when *Rhizophagus irregularis* was included as inoculant. No changes in microbial biodiversity were observed. Our results suggest that seed-applied biofertilizers may be effectively exploited in sustainable wheat cultivation without altering the biodiversity of the resident microbiome, but attention should be paid to the

composition of the microbial consortia in order to maximize their benefits in crop cultivation.

Effects of Seed-Applied Biofertilizers on Rhizosphere Biodiversity and Growth of common Wheat (*Triticum aestivum* L.) in the field by Cristian Dal Cortivo, Manuel Ferrari, Giovanna Visioli, Marta Lauro, Flavio Fornasier, Giuseppe Barion, Anna Panozzo and Teofilo Vamerali) published:26 February 2020 doi: 10.3389/fpls.2020.00072

Rice production in Ghana has become unsustainable due to the extremely nutrient-poor soils. It is caused by inadequate soil fertility management, including the inefficient application of fertilizers. A practical solution could be the biofertilizers, *Azospirillum* sp. B510. We performed field trials in Ghana and Japan to compare the effects of B510 colonization on selected Ghanaian rice varieties grown. The B510 inoculation significantly enhanced the rice cultivars' growth and yield. The phenotypic characteristics observed in rice varieties Exbaika, Ex-Boako, Agra Rice, and Amankwatia were mainly short length and high tillering capacity. These features are attributed to the host plant (cv. Nipponbare), from which the strain B510 was isolated. Furthermore, *Azospirillum* species has been identified as the dominant colonizing bacterium of rice rhizosphere across a diverse range of agro ecologies in all major rice-growing regions in Ghana. Our results suggest that the utilization of B510 as a biofertilizer presents a promising way to improve rice growth, enhance soil fertility, and sustain rice productivity in Ghana Biofertilizer Activity of *Azospirillum* sp. B510 on the Rice Productivity in Ghana

Elsie Sarkodee-Addo,, Chihiro Tokiwa, Patrick Bonney, Daniel Asiamah Aboagye, Alex Yeboah , Samuel Oppong Abebrese , Ralph Bam , Eric Kwesi Nartey , Shin Okazaki and Michiko Yasuda: Microorganisms 2021, 9, 2000.