EFFECTIVE PLANT GROWTH PROMOTING MICROORGANISMS (*Pseudomonas* spp and *Trichoderma* spp) IN LIQUID BIOFERTILIZER TO IMPROVE THE PLANT GROWTH: A REVIEW

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Abstract: Most of the fertilizers supply essential plant nutrients (N, P and K). These fertilizers increase the yield of the crop but they cause several health disorders. Due to this concern, consumer preferences look into the organic food production. In recent years, biofertilizers have emerged as an important tool for biological nitrogen fixation. They offer economically attractive and eco-friendly manner for providing nutrients to the plants. This is very low cost renewable source of nutrient that supplements the chemical fertilizers. Plant growth promoting microorganisms (PGPR) with carrier material (whey) is very effective for the cell maturation and long lasting of microbial cells.

Key words: Biofertilizer, PGPR, *Pseudomonas* spp. *Trichoderma* spp. Whey.

I. INTRODUCTION

The success of green revolution depends upon the availability of fertilizes, high yielding of seeds, improved agronomical practices and timely availability of water. The demand for nitrogenous fertilizers has been increasing but its production has always fallen short.In such a scenario, the use of microbes who do not need fossil energy is of immense value, low input sustain farming through biological nitrogen fixation or increased efficiency of fertilizers applied. The part of increasing deficit of nitrogenous fertilizers can be made up if part of the vast reservoir of atmospheric nitrogen in a simpler way-in the nodules of roots of legume plants e.g. soybean, chickpea etc (Borkar, 2015).

These are called natures mini fertilizer factories. Biofertilizers have an important role to play in improving nutrient supplies and their crop availability in the years to come. They are of environment friendly non-bulky and low cost agriculture inputs. Some specific bacteria or micro-organisms in the soil convert this nitrogen into ammonia and amino acids. These amino acids can be used by the plants to build up proteins. This process world wide is known as "biological nitrogen fixation" and the product is called biofertilizers. Biofertilizer is an organic product containing a specific micro-organisms in concentrated form which is derived either from the plant roots or from the soil root zone (Rhizosphere) (Arora *et al.*, 2010).

II. BIOFERTILIZER

A biofertilizer is a large population of a specific or a group of beneficial microorganisms, millions and billions of them incorporated aseptically into sterile carrier materials such as peat, lignite or charcoal. Such material is generally packed in plastic bags and sold to the farmers as biofertilizers for enhancing the productivity of soil either by fixing atmo*sp*heric nitrogen or by solubilising soil pho*sp*horus or by stimulating plant growth through synthesis of growth promoting substances. Biofertilizers are the most advanced biotechnology necessary to support developing organic agriculture, sustainable agriculture, green agriculture and non-pollution agriculture. This Bio-organic Fertilizer can increase the output, improve the quality and it is responsible for agriculture environment. Bio-fertilizers are an important option for agricultural sustainability, as they are conducive to long-term beneficial effects on the physical, chemical and biological aspects of soils (Mahdi *et al*., 2010).

2.1 Need of Bio-Fertilizers

Current soil and agriculture management strategies are mainly dependent on continuous use of inorganic chemical-based fertilizers which are industrially manipulated substances, largely water-soluble and contain high available nutrient concentrations. Sustainable agriculture offers the potential to meet our agricultural needs as it encompasses advances in agriculture by using special farming, management practices and technology at the same time ensuring that no harm done to the same. The levels of Inorganic fertilizer plays a significant role in environmental pollution. Among the inorganic fertilizers, nitrogen fertilizer increases denitrification, resulting in elevated emission of nitrous oxide (N2O) to the atmosphere which contributes to global warming. It has also been reported that application of nitrogen fertilizers may deplete soil organic carbon in the long run. Instead, biofertilizers, the products containing living cells of different microorganisms, can prevent the depletion of the soil organic matter. It has also been reported that application of biofertilizers increases yield and reduce environmental pollution (Mia and Shamsuddin, 2010).

Indiscriminate use of synthetic fertilizers and toxic pesticides has led to the pollution and contamination of the soil, has polluted water basins, destroyed micro-organisms, ground water pollution by eutrophication of water bodies and friendly insects, making the crop more prone to diseases.Conventional, chemically processed fertilizers also subvert the soil ecology, disrupt environment, degrade soil fertility and consequently shows harmful effects on human health. Demand is much higher than the availability. It is estimated that by 2020, to achieve the targeted production of 321 million tones of food grain, the requirement of nutrient will be 28.8 million tones, while their availability will be only 21.6 million tones being a deficit of about 7.2 million tones. Depleting feedstock/fossil fuels (energy crisis) and increasing cost of fertilizers. This is becoming unaffordable by small and marginal farmers, depleting soil fertility due to widening gap between nutrient removal and supplies, growing concern about environmental hazards, increasing threat to sustainable agriculture. Besides above facts, the long term use of bio-fertilizers is economical, eco-friendly, more efficient, productive and accessible to marginal and small farmers over chemical fertilizers (Rembiałkowska, 2007).

2.2 Biofertilizer making

Soil micro-organisms play a significant role in regulating the dynamics of organic matter decomposition and the availability of plant nutrients such as N, P and S. It is well-recognized that microbial inoculants constitute an important component of integrated nutrient management that leads to sustainable agriculture. In addition, microbial inoculants can be used as an economic input to increase crop productivity; fertilizer doses can be lowered and more nutrients can be harvested from the soil.Biofertilizer is defined as a substance which contains living micro-organisms and is known to help with expansion of the root system and better seed germination. A healthy plant usually has a healthy rhizosphere which should be dominated by beneficial microbes. Conversely, in unhealthy soil, dominated by pathogenic microbes, optimum plant growth would not be possible.Biofertilizers differ from chemical and organic fertilizers in the sense that they do not directly supply any nutrients to crops and are cultures of special bacteria and fungi. The production technology for biofertilizers is relatively simple and installation cost is very low compared to chemical fertilizer plants (Subba Rao, 1982).

There are several things need to be considered in biofertilizer making such as microbes' growth profile, types and optimum condition of organism, and formulation of inoculum. The formulation of inocula, method of application and storage of the product are all critical to the success of a biological product. In general, there are 6 major steps in making biofertilizer. These includes choosing active organisms, isolation and selection of target microbes, selection of method and carrier material, selection of propagation method, prototype testing and large scale testing. First of all, active organisms must be decided. For example, it is the decision to use whether organic acid bacteria or nitrogen fixer or the combination of some organisms. Then, isolation is made to separate target microbes from their habitation. Usually organism are isolate from plants root or by luring it using decoy such as putting cool rice underground of bamboo plants. Next, the isolated organisms will be grown on Petri plate, shake flask and then glasshouse to select the best candidates. It is also important to decide form of our biofertilizer product wisely so that the right carrier material can be determined. If it is desired to produce biofertilizer in powder form, then tapioca flour or peat are the right carrier materials (Gaur, 2010).

Selection of propagation method is mainly to find out the optimum condition of organism. This can be achieved by obtaining growth profile at different parameter and conditions. After that, prototype (usually in different forms) is made and tested. Lastly, biofertilizer is testing on large scale at different environment to analyze its effectiveness and limitability at different surrounding. Biofertilizers are usually prepared as carrier based inoculants containing effective microorganisms. Incorporation of microorganisms in carrier material enables easy-handling, long-term storage and high effectiveness of biofertilizers. Sterilization of carrier material is essential to keep high number of inoculants bacteria on carrier for long storage period. Gamma irradiation or autoclaving can be used as method for sterilization. Various types of material can be used as carrier for seed or soil inoculation. The properties of a good carrier material for seed inoculation are inexpensive and available in adequate amounts. It must non-toxic to inoculants bacterial strain and non-toxic to plant itself. Because it acts as carrier for seed inoculation, it should have good moisture absorption capacity and good adhesion to seeds. Last but not the least; carrier should have good pH buffering capacity, easy to process and sterilized by either autoclaving or gamma radiation (Anonymous, 2010).

2.3 Most important microorganisms used in biofertilizer

Organisms that are commonly used as biofertilizers component are nitrogen fixers (N-fixer), potassium solubilizer (K-solubilizer) and pho*sp*horus solubilizer (P- solubilizer), or with the combination of molds or fungi. Most of the bacteria included in biofertilizer have close relationship with plant roots. *Rhizobium* has symbiotic interaction with legume roots, and Rhizobacteria inhabit on root surface or in rhizo*sp*here soil. The pho*sp*ho-microorganism mainly bacteria and fungi make insoluble pho*sp*horus available to the plants. Several soil bacteria and a few *sp*ecies of fungi possess the ability to bring insoluble pho*sp*hate in soil into soluble forms by secreting organic acids. These acids lower the soil pH and bring about the dissolution of bound forms of pho*sp*hate. While Rhizobium, Blue Green Algae (BGA) and Azollaare crop *sp*ecific, bio-inoculants like *Azotobacter*, *Azospirillum*, Pho*sp*horus Solubilizing Bacteria (PSB), and Vesicular Arbuscular Mycorrhiza (VAM) could be regarded as broad *sp*ectrum biofertilizers. VAM is fungi that are found associated with majority of agriculture crops and enhanced accumulation of plant nutrients. It has also been suggested that VAM stimulate plant growth by physiological effects or by reducing the severity of diseases caused by the soil pathogens. Examples of free living nitrogen fixing bacteria are obligate anaerobes (*Clostridium pasteurianum*), obligate aerobes (*Azotobacter*), facultative anaerobes, photosynthetic bacteria (*Rhodobacter*), cyanobacteria and some methanogens. The example of K solubilizer is Bacillus mucilaginous while for P-solubilizer are *Bacillus megaterium*, *Bacillus circulans*, *Bacillus subtilis* and *Pseudomonas straita* (Gupta, 2002).

Nitrogen is one of the major important nutrients which is very essential for crop growth. Atmo*sp*here contains about 80 percent of nitrogen volume in Free State. The major part of the elemental nitrogen that finds its way into the soil is entirely due to its fixation by certain *sp*ecialized group of microorganisms. Biological Nitrogen Fixation (BNF) is considered to be an important process which determines nitrogen balance in soil ecosystem. Nitrogen inputs through BNF support sustainable environmentally sound agricultural production. The value of nitrogen fixing legumes in improving and higher yield of legumes and other crops can be achieved by the application of biofertilizers. Biological nitrogen fixation is one way of converting elemental nitrogen into plant usable form. Nitrogen-fixing bacteria (NFB) that function transform inert atmo*sp*heric N_2 to organic compounds. Nitrogen fixer or Nfixers organism are used in biofertilizer as a living fertilizer composed of microbial inoculants or groups of microorganisms which are able to fix atmo*sp*heric nitrogen. They are grouped into free-living bacteria (Azotobacter and Azo*sp*irillium) and the blue green algae and symbionts such as Rhizobium, Frankia and Azolla. The list (Table 1) of nitrogen fixing bacteria associated with nonlegumes includes *sp*ecies of Achromobacter, Alcaligenes, Arthrobacter, Acetobacter, Azomonas, Beijerinckia, Bacillus, Clostridium, Enterobacter, Erwinia, Derxia, Desulfovibrio, Corynebacterium, campylobacter, Herba*sp*irillum, Klebsiella, Lignobacter, Mycobacterium, Rhodo*sp*irillum, Rhodo-pseudomonas, Xanthobacter, Mycobacterium and Methylosinus (Gahukar, 2005-06)).

Table 1: Biofertilizers can be grouped in different ways based on their nature and function.

S. No.	Groups	Examples
Nitrogen (N2) fixing Biofertilizers		
1	Free-living	Azotobacter, Clostridium, Anabaena, Nostoc,
2	Symbiotic	Rhizobium, Frankia, Anabaena azollae
3	Associative Symbiotic	Azospirillum
P Solubilizing Biofertilizers		
1	Bacteria	Bacillus megaterium var. phosphaticum
		Bacillus circulans, Pseudomonas striata
2	Fungi	Penicillium sp, Aspergillus awamori
P Mobilizing Biofertilizers		
1	Arbuscular mycorrhiza	Glomus sp.,Gigaspora sp.,Acaulospora sp.,
		Scutellospora sp. & Sclerocystis sp.
2	Ectomycorrhiza	Laccaria sp., Pisolithus sp., Boletus sp., Amanita sp.
3	Orchid mycorrhiza	Rhizoctonia solani
Biofertilizers for Micro nutrients		
1	Silicate and Zinc solubilizers	Bacillus sp.
Plant Growth Promoting Rhizobacteria		
1	Pseudomonas	Pseudomonas fluorescens

III. PHO*SP*HATE SOLUBILIZER

Several reports have examined the ability of different bacterial *sp*ecies to solubilize insoluble inorganic pho*sp*hate compounds, such as tricalcium pho*sp*hate, dicalcium pho*sp*hate, hydroxyapatite, and rock pho*sp*hate. Among the bacterial genera phos*p*hate solubilizer are *Pseudomonas*, *Bacillus*, *Rhizobium*, *Burkholderia*, *Achromobacter*, *Agrobacterium*, *Microccocus*, *Aereobacter*, *Flavobacterium* and *Erwinia*. There are considerable populations of pho*sp*hate-solubilizing bacteria in soil and in plant rhizo*sp*heres. These include both aerobic and anaerobic strains, with 11 a prevalence of aerobic strains in submerged soils. A considerably higher concentration of pho*sp*hate solubilizing bacteria is commonly found in the rhizo*sp*here in comparison with nonrhizo*sp*here soil. Visual detection and even semiquantitative estimation of the pho*sp*hate solubilization ability of microorganisms have been possible using plate screening methods, which show clearing zones around the microbial colonies in media containing insoluble mineral pho*sp*hates (mostly tricalcium pho*sp*hate or hydroxyapatite) as the single pho*sp*hate source(Gaur,1990).

Akhtar and Siddiqui (2009) have isolated and grouped pho*sp*hate solubilizing bacteria into four different types, according to kinetics and rate of pho*sp*hate accumulation. These groups range from a linear increase of pho*sp*hate concentration along with the growth of the culture, to oscillating behavior with variations in the soluble pho*sp*hate levels giving rise to several peaks and troughs of pho*sp*hate concentration. This last type of kinetic behavior has also been observed. These changes in pho*sp*hate concentration could be a consequence of pho*sp*hate precipitation of organic metabolites and the formation of organo-pho*sp*hate compounds with secreted organic acids, which are subsequently used as an energy or nutrient source, this event being repeated several times in the culture.

3.1 Plant growth promotion

For many years, the ability of these fungi to increase the rate of plant growth and development, including, especially, their ability to cause the production of more robust roots has been known. The mechanisms for these abilities are only just now becoming known. Some of these abilities are likely to be quite profound. Recently, we have found that one strain increases the numbers of even deep roots (at as much as a meter below the soil surface). These deep roots cause crops, such as corn, and ornamental plants, such as turfgrass, to become more resistant to drought. Perhaps even more importantly, our recent research indicates that corn whose roots are colonized by *Trichoderma* strain T-22 require about 40% less nitrogen fertilizer than corn whose roots lack the fungus. Since nitrogen fertilizer use is likely to be curtailed by federal mandate to minimize damage to estuaries and other oceanic environment, the use of this organism may provide a method for farmers to retain high agricultural productivity while still meeting new regulations likely to be imposed.

3.2 *Pseudomonas fluorescens*

Pseudomonas fluorescens has multiple flagella. It has an extremely versatile metabolism, and can be found in the soil and in water. It is an obligate aerobe but certain strains are capable of using nitrate instead of oxygen as a final electron acceptor during cellular respiration. Certain *Pseudomonas fluorescens* isolates produce of the secondary metabolite 2, 4-diacetylphloroglucinol (2,4-DAPG), the compound found responsible for antiphytopathegenic and biocontrol properties in these strains. The phlgene cluster encodes factors for 2, 4-DAPG biosynthesis, regulation, export, and degradation.

3.3 Nitrogen fixation by *Pseudomonas* sp

Nitrogen is one of the most common nutrients required for plant growth and productivity, as it forms an integral part of proteins, nucleic acids and other essential biomolecules. About 80 per cent of nitrogen is present in the atmosphere but is unavailable to plants. It needs to be converted into ammonia, a form available to plants and other eukaryotes.Fixation of nitrogen in non-leguminous plants by rhizobacterial strains. This phenomenon by PGPR is correlated with the activity of ACCD. Similarly, PGPR strains utilizing ACC as sole nitrogen source increased the fixation of ebiological nitrogen, nodulation and growth of Lupinus albus cv. Mutolupa. Application of PGPR in apricot increased the yield, growth and nutrient element composition of leaves viz., N, P, K, Ca and Mg (Esitken *et al*., 2003).

3.4 Mineral phosphate solubilization (MPS) by Pseudomonads

Involvement of microorganisms in the solubilization of insoluble phosphate was first shown by Stalstorm (1903). Since then, lot of work has been done on the isolation, enumeration, efficiency screening, mechanisms of solubilization and crop response to their inoculation. The biological process of conversion of unavailable/fixed form of inorganic phosphorus into primary orthophosphate and secondary orthophosphate has been termed as mineral phosphate solubilization (Goldstein, 1986).

The solubilization of the precipitated calcium phosphate on agar medium has been used as the criterian for isolation and enumeration of MPS microorganisms. Among the groups of mineral phosphate solubilizing bacteria (MPSB), Pseudomonas assume importance since they are the most common and frequency occurring group in the rhizosphere and are capable of utilizing a wide array of compounds as carbon and energy sources. They are also known to have wide range of plant growth promotional activity by virtue of nutrient mobilization, P-solubilization, production of plant hormones and biocontrol potential (Sperber, 1957).

Neelam and Meenu (2003) reported high tricalcium phosphate solubilizing ability of *Pseudomonas* sp isolated from rhizosphere of Trigonella. The tricalcium phosphate solubilizing activity of *Pseudomonas fluorescens* and their cold-tolerant mutants and reported that the cold-tolerant mutants were more efficient than their respective wild type counterparts for P-solubilization at low temperatures.

Gupta *et al*. (2002) described *Pseudomonas* species as a potent phosphate solubilizer while they were developing heavy metal resistant mutants of phosphate solubilizing Pseudomonas NBRI 4014.

Disimine *et al*. (1998) recorded an interesting observation of solubilization of ZnPO4 by a phosphate solubilizing *Pseudomonas fluorescens* only in the presence of glucose as the carbon source. *Pseudomonas* sp. isolated from Maharashtra soils could solubilize 13-58 per cent of tricalcium phosphate added to liquid medium.

Sardina *et al*. (1986) reported 50 per cent solubilization of phosphorus from low grade mineral residues by *Pseudomonas* sp. when they were added at the rate of 1 g per l medium. Different mechanisms of mineral phosphate solubilization included synthesis of organic acids by the phosphate solubilizing bacteria, CO_2 and H_2S production and chelation of other acids.

The amount of organic acid liberated by these microorganisms is said to be roughly about five per cent of the carbohydrate consumed. It is also shown that solubilization of mineral phosphate by bacteria is the result of acidification of the periplasmic space by the direct oxidation of glucose or other aldose sugars (Goldstein, 1995).

Among the different organic acids, gluconic acid seems to be most commonly produced acid by phosphate solubilizing *Pseudomonas aeruginosa*, *Pseudomonas cepacia* and *Pseudomonas fluorescens* (Disimine *et al*., 1998).

IV. What is *Trichoderma*?

Trichoderma is a genus of asexually reproducing fungi that are often the most frequently isolated soil fungi; nearly all temperate and tropical soils contain 101-103 culturable propagules per gram. These fungi also colonize woody and herbaceous plant materials, in which the sexual Teleomorph (genus Hypocrea) has most often been found. However, many strains, including most biocontrol strains, have no known sexual stage. In nature, the asexual forms of the fungi persist as clonal, often Heterokaryotic, individuals and populations that probably evolve independently in the asexual stage .They show a high level of genetic diversity, and can be used to produce a wide range of products of commercial and ecological interest. They are prolific producers of extracellular proteins, and are best known for their ability to produce enzymes that degrade cellulose and chitin although they also produce other useful enzymes. For instance, different strains produce more than 100 different metabolites that have known antibiotic activities (Sivasithamparam and Ghisalberti, 1998). *Trichoderma* species have long been recognized as agents for the control of plant disease and for their ability to increase plant growth and development, high reproductive capacity, ability to survive under very unfavorable conditions, efficiency in the utilization of nutrients, capacity to modify the rhizosphere, strong aggressiveness against phytopathogenic fungi and efficacy in promoting plant growth and defense mechanisms. They are becoming widely used in agriculture, and the most useful strains show a property that is known as ‘rhizosphere competence’ that is, the ability to colonize and grow in association with plant roots. These properties have made *Trichoderma* a ubiquitous genus present in any habitat and at high population densities. *Trichoderma* is more efficient in acidic than alkaline soils. *Trichoderma* BCAs control *Ascomycetous*, *Deuteromycetous* and *Basidiomycetous* fungi, which are mainly soil borne but also air borne pathogens (Jayaraj and Ramabadran, 1996).

4.1 Benefits of *Trichoderma* (Hjeljord and Tronsmo, 1998)

4.1.1 Disease Control

Trichoderma is a potent biocontrol agent and used extensively for soil born diseases. It has been used successfully against pathogenic fungi belonging to various genera, viz., *Fusarium*, *Phytopthara*, *Scelerotia*, etc.

4.1.2 Plant Growth Promoter

Trichoderma strains solubilize phosphates and micronutrients. The application of Trichoderma strains with plants increases the number of deep roots, thereby increasing the plant's ability to resist drought.

4.1.3 Biochemical Elicitors of Disease

Trichoderma strains are known to induce resistance in plants. Three classes of compounds that are produced by *Trichoderma* and induce resistance in plants are now known. These compounds induce ethylene production, hypersensitive responses and other defense related reactions in plant cultivars.

4.1.4 Transgenic Plants

Introduction of endochitinase gene from *Trichoderma* into plants such as tobacco and potato plants has increased their resistance to fungal growth. Selected transgenic lines are highly tolerant to foliar pathogens such as Alternaria alternata, *A. solani*, and *Botrytis cirerea* as well as to the soil-borne pathogen, *Rhizectonia* spp.

4.1.5 Bioremediation

Trichoderma strains play an important role in the bioremediation of soil that are contaminated with pesticides and herbicides. They have the ability to degrade a wide range of insecticides: organochlorines, organophosphates and carbonates.

4.1.6 Carrier-based biofertilizers

Carrier-based biofertilizers are prepared with the help of whey, which act as a carrier for microbial inoculants. Biofertilizer consumption is not very satisfactory due to certain disadvantages associated with carrier-based biofertilizers like low shelf life (3-4 months), storage conditions (stored in cool temperature) as it is temperature sensitive, bulky to transport, therefore, high transport cost, less scope for export, more chances of contamination, problem of proper packing, poor cell protection, poor moisture retention capacity and restriction on use of whey as a measure of conservation (Kharbanda and Stallworthy, 1990).

IV. CONCLUSION

So from the various above cited studies, it can be concluded that biofertilizers are eco-friendly, low cost agricultural inputs that have an important role to play in improving nutrient supply to crops. Plant Growth Promoting microorganisms have the potential to increase the availability of primary nutrients and other growth inducing factors to the plants. To achieve this, it is imperative that efficient organisms adapted to the local conditions are required to be isolated for making region specific preparations. Carrier is the most important component of biofertilizer technology, and the selection of an economically viable and easily available carrier, capable of maintaining high viable count is an important area of research.

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