Journal of the Indian Institute of Science
A Multidisciplinary Reviews Journal
ISSN: 0970-4140 Coden-JIISAD

REVIEWS

Biofertilizers for Sustainability

G.P. Brahmaprakash and Pramod Kumar Sahu*

Abstract | Biofertilizers are gaining importance in sustainable agriculture. Various complementing combinations of microbial inoculants for management of major nutrients such as nitrogen and phosphorus are necessary for sustainability. A broad canvas of biofertilizers that enhance nitrogen and specific to legumes and non legumes along with inoculants that enhance phosphorus nutrition are discussed from several perspectives. The mode of action of these microorganisms within and the transformation of nutrients is elucidated. In the Indian scenario, use of biofertilizers faces various constraints, such as longevity, etc, need to be overcome to achieve substantial fertilizer savings. One of the key issues that still remains is the method of formulation of these biofertilizers. Some of the key difficulties associated are brought out in this review.

1 Introduction

Nutrients are required for the growth of all living beings, be it babies, animals, plants or microbes. The conventional knowledge indicates farms manured regularly yield better.[1] Our understanding of nutritional needs of crop plants begin from the famous five year willow tree experiment conducted by Johann Baptista van Helmont (1579–1644). He observed that willow tree had gained nearly 74.4 kg but the loss of soil was only 56 g, and concluded that the tree drew its nutrients from water not soil. Now we are aware that the soil is the repository for most of the plant nutrients, hence the concern for its continued health and sustainability.

Seventeen plant nutrients are essential for proper crop development. Each is equally important to the plant, yet each is required in vastly different amounts. Liebig's law of minimum explains effect of limiting factor on crop production. These 17 nutrients are divided into three groups such as major, minor and micro nutrients based on plant requirement. Major nutrients are nitrogen, phosphorus and potassium; minor nutrients are calcium, magnesium and sulphur, and the micro-nutrients are boron, chlorine, copper, iron, manganese, molybdenum, zinc and nickel. In addition to the 13 nutrients listed above, plants require carbon, hydrogen and oxygen, which are extracted from air and water. Most plants take up nutrients as inorganic ions irrespective of the form in which it is applied to soil.

1.1 *Nitrogen: Chemical v/s biological nitrogen fixation*

In most agricultural systems nitrogen is most often the limiting nutrient that dictates crop production. Despite its presence in large quantities in the atmosphere, plants cannot utilize nitrogen since it is inert. Nitrogen is made available in the form of fertilizers which is chemical fixation of atmospheric nitrogen through the Haber-Bosch process. This process requires high temperature (400–500°C) and high pressure (20 MPa), and corresponds to energy inputs of about 875 cubic meters of natural gas, 5.5 barrels of oil, or 2 metric tons of coal to fix 1 metric ton of ammonia.[2] Dinitrogen is described as the most stable diatomic molecule known and two atoms are joined by a very stable triple bond. A lot of energy (945 kJ) is required to break this triple bond and therein lies one of the major challenge of dinitrogen fixation.[2]

Atmospheric dinitrogen can also be fixed biologically (by diazotrophs = *prokaryotes* that fix dinitrogen) to ammonia. This ammonia is available to crop plants. The ammonia is converted to nitrate by few microorganisms in soil which is then available to plants. The nitrate thus formed is amenable for denitrification reactions in deeper horizons of soil leading to formation of nitrogen gas which will escape to the atmosphere. This is the typical path of nitrogen cycle.

Prokaryotes: Unicellular organisms lacking a well defined nucleus.

Department of Agricultural Microbiology, UAS: GKVK: Bangalore, India.
**gpbrahmaprakash@yahoo.co.in*

Biofertilizer: Preparations containing beneficial micro-organisms which enhance plant growth.

Bacteria mediate fixation of nitrogen at ambient temperature and pressure enzymatically, by a process known as biological nitrogen fixation (BNF). Magnitude of naturally occurring nitrogen fixation in the biosphere is not easy to determine, but approximately it amounts to ~107 million metric ton/year compared to ~160 million metric ton/year of man made nitrogen fixation which is 1.5 times higher then the natural fixation.[3] In the global nitrogen cycle every nitrogen atom in the atmosphere cycles once in a million year.[4] BNF contributes 65% of nitrogen consumption in agriculture.[5] All the bacteria fixing atmospheric nitrogen catalyze the reaction through nitrogenase enzyme. This enzyme has two components—(1) Mo-Fe protein, called dinitrogenase and (2) Fe protein, called dinitrogenase reductase. First protein actually takes part in reducing dinitrogen to ammonia and second protein assists Mo-Fe protein by providing electrons for reduction of dinitrogen. The mechanism of nitrogen fixation is the same in all nitrogen fixing bacteria; the reduction of one molecule of dinitrogen requires 16 ATP *in vitro* and 20–30 ATP under field conditions, as it is less efficient (Figure 1).

1.2 *Biofertilizers*

The term '*Biofertilizer*' in India specifies fertilizers to meet the nutritional requirements of a crop through microbiological means, other countries use the term microbial inoculants. These biofertilizers are usually carrier based microbial preparations containing beneficial microorganisms in a viable state intended for seed or soil application, which enhance plant growth through nutrient uptake and/or growth hormone production. Important and popular microbial inoculants in our country are those that supplement nitrogen, phosphorus and plant growth promoting rhizobacteria (PGPR).

In India biofertilizers were introduced along with soybean since Indian soils was devoid of rhizobia nodulating the soybean crop. The crop response to rhizobial inoculation was fantastic, able to meet a significant portion of nitrogen requirement. Encouraged by the inoculation response in soybean, this technology was extended to other legumes, and later to cereals. The photograph in Plate 1 was taken in early seventies depicting *Rhizobium japonicum* inoculation response in soybean at University of Agricultural Science experimental farm at GKVK, Bangalore. Note the

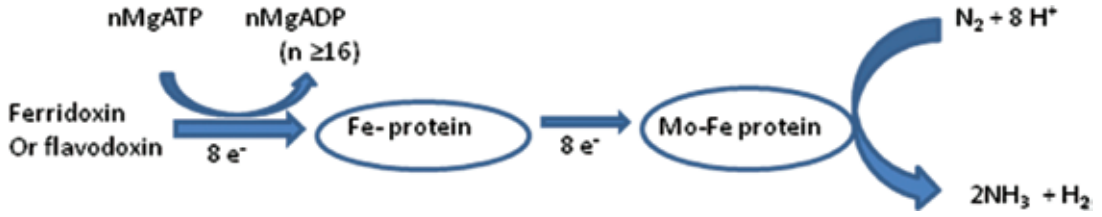

Figure 1: Reduction of dinitrogen to ammonia by nitrogenase enzyme complex.

Plate 1: *Rhizobium japonicum* inoculation response of soybean at UAS, GKVK campus Bangalore in early seventies. Note the yellowing (nitrogen deficiency symptom in plant) of border rows which were un-inoculated and rest of the crop which was inoculated appear lush green (nitrogen sufficiency symptom in plant).

yellowing (nitrogen deficiency symptom in plant) of border rows which were un-inoculated and rest of the crop which was inoculated appear lush green (nitrogen sufficiency symptom in plant). This kind of response was not being obtained for soybean alone, let alone other legume crops.

Microbial inoculants have been in vogue in the traditional farming in India without realizing their role as such. The importance of crop rotation in enhancing soil fertility could be directly attributed to the proliferation of these beneficial bacteria and their concomitant nitrogen fixation in soil. In Rajasthan, guar or cluster bean (*Cyamopsis tetragonoloba*) is a major legume cultivated. A pooja (ritualistic worship) is performed whenever new farms are brought under the cultivation of guar and the soil from the previously guar cultivated farm is ceremoniously sprinkled on to the new field. This ensures the nodulation of guar to be sown in the new soil. Those kind of rituals have been a part of Indian agriculture without often understanding the importance of the action.

Use of biofertilizers is gaining momentum especially with emphasis on organic farming and sustainable agriculture. They are an integral input of organic farming and have the following benefits:

- Low cost technology with a high cost-benefit ratio
- Improves soil fertility through their sustained activities in soil
- Increases plant growth and crop yield through increased nutrients availability and soil fertility
- Reduces the environmental pollution caused from the manufacturing of the fertilizers and chemicals used
- Improves soil health and conditioning
- Protects plants against many soil borne pathogens
- Helps plant to grow under stress conditions

1.2.1 Symbiotic nitrogen fixation: Legume—*Rhizobium* symbiosis: Legume—*Rhizobium* symbiosis is an important facet of symbiotic nitrogen fixation which is exploited to benefit agriculture and its sustainability. Over a century ago German scientists Hellriegel and Wilfarth experimentally demonstrated the nitrogen fixation in legume nodule by nodule inducing ferment (*Rhizobium*): the stage was set for the popularity of the *Rhizobium* inoculation technology world over.

In this symbiosis macro-symbiont is the legume plant and micro-symbiont is the prokaryotic bacteria *(Rhizobium)*. The Macro-symbiont legume belongs to Leguminaceae, divided into three subfamilies comprising of 700 genera and 14,000 species.[6] Only about 200 of these are cultivated by man. Important legumes cultivated in India are pigeonpea, chickpea, soybean, lentil, lathyrus, rajma, alfalfa, clover, beans and peas. India accounts for 25 per cent of world pulse production.

Micro-symbiont *Rhizobium* is a nitrogen fixing motile prokaryote defined solely by their ability to nodulate legumes. The taxonomy of *Rhizobium* is frequently changing. *Rhizobium* was initially classified into cross inoculation groups based on the ability of one rhizobia nodulating different legumes;[7] then on growth rate into fast and slow growing rhizobia[8] and now on 16s rRNA sequencing into ten genera, some are phylogentically outside traditional rhizobia but do carry *nod* genes encoding for Nod factors.[9]

The success of this *symbiosis* is a new tissue (nodule) in the plant which is the culmination of molecular dialogues between the legume plant and *Rhizobium*. Atmospheric nitrogen fixation is carried out by the enzyme nitrogenase of the bacterium with the assistance of nodulins (legume plant proteins) and transferred to plant in a true spirit of symbiosis. Estimates of nitrogen fixation in different Indian pulse crops are presented in Table 1.

Symbiosis: An interaction between two organisms wherein both are benefited.

Inoculation response of pulses is far from desirable, at best inconsistent and dependent on many variables. Most cultivated soils to legumes are known to harbor cowpea group of rhizobia and nodulation surveys indicate a need for inoculation every season for majority of the pulses cultivated in India. The competition of (inefficient) native strains to (efficient) inoculant strains appears to be a bottleneck in realizing

Table 1: Estimates of nitrogen fixation in different crops.[95]

Crop	N_2 fixed (kg/ha/yr)
Chickpea	26–63
Cluster bean	37–196
Cowpea	53–85
Groundnut	112–152
Lentil	35–100
Mungbean	50–55
Pigeonpea	68–200
Soybean	49–130
Peas	46
Alfalfa	100–300
Clover	100–150
Fenugreek	44

higher yields from *Rhizobium* inoculation. The yield increase due to inoculation in pigeonpea varied from 1.2–20.3%; 8–47.8% and 1.8–26.4% in 1992, 1993 and 1994 respectively, in different locations in India. The grain yield increase may also appear to be an interaction of varieties and strains of *Rhizobium*.[10] A complementary coordinated effort on the part of plant breeders and microbiologists is now necessary to successfully select a high yielding variety with elevated nitrogen fixing abilities for sustainable agriculture.

A multidisciplinary coordinated research project on pulse crops such as pigeonpea, chickpea and MuLLaRP (mung, urd, lentil, lathyrus, rajma and pea) is in operation in India funded by Indian Council of Agricultural Research (ICAR), in many different centers for research on improvement of several aspects of these pulses. The emphasis in this project is on crop improvement, crop production and plant protection of these legumes.

1.2.2 Frankia—Casuarina symbiosis: Angiosperms belonging to several genera such as *Casuarina, Alnus, Myrica, Coriaria, Discaria, Hippophae* etc., form symbiotic association with actinomycetes *Frankia*. Nodules are formed by filamentous, spore forming actinomycetes on hundreds of plant species found in 25 genera in 8 families of dicotyledons. All are woody shrubs or trees and this kind of symbiosis has promising role not only in improving nitrogen economy by N_2 fixation but is also very important in agro forestry system and in stabilizing eroding land surfaces.

Actinorhizal symbiosis: Symbiosis between actinomycetes and plant roots. Actinomycetes are classified under bacteria.

The *actinorhizal symbiosis* are known to enhance fertility of temperate forests akin to what woody legumes do for tropics. Actinorhizal plants are capable of growing on nitrogen poor, eroded slopes and mining wastes. They provide shrubs and large trees of commercial importance. The present grasslands of North America do not have an extensive legume component and thus the actinorhizal symbiosis had played a major role in the past in improving nitrogen content in these sites. The actinorhizal nodules represent cluster of modified roots with the *Frankia* infected cells found in the cortex. Nodules first appear as swelling and later develop into lobes at their apices. It forms vesicles which are the site of nitrogen fixation. The magnitude of BNF in *Frankia* is about 90 kg N/ha/yr in *Coriaria arborea.*[11]

1.2.3 *Azolla—Anabaena* symbiosis: Azolla is used as a biofertilizer for rice production in several rice growing countries such as Philippines, China, Vietnam, Thailand and Sri Lanka. *Anabaena azollae* is a cyanobacteria, forms symbiotic association with a water fern *Azolla*. In India, for paddy fields *Azolla* is a promising biofertilizer and extensively used by farmers. It is grown in the slow flowing creeks or water beds and applied to crop between planting of rice. After a certain period of growth it is incorporated to soil before transplanting or left to be shaded out as rice canopy builds up. They are rapidly mineralized due to the narrow C:N ratio (as we use it in a succulent stage when there is not much lignification of cell walls and is easily degradable) and provide nitrogen to the plants. Apart from nitrogen fixation, azolla is also known to suppress weed population in wet land rice and hence provides an additional economic advantage to rice cultivation. Recently *Azolla* was also used as feed to enhance milk production in milching animals. *Azolla microphylla* (at 15 t/ha) increases grain yield by 29.2% (with neem cake).[12]

1.2.4 Asymbiotic nitrogen fixation: Most important bacteria in this group are *Azotobacter*, *Derxia* and *Beijerinkia* found in soil. Among these *Azotobacter* has a potential to be used as biofertilizers. Most common species is *A. chroococcum*, it is able to fix 10 mg N/g of carbon source supplied *in vitro*.[13] *Azotobacter* is also known to produce plant growth hormones like indole acetic acid (IAA), gibberellic acid and exhibit fungistatic activity. Increase in yield due to *Azotobacter* inoculation in many cereals such as maize, pearl millet, wheat and sorghum have been reported. India is one of the few countries that recommend the use of this microbial inoculant in agriculture. Yield increases due to inoculation of *Azotobacter* was 3000 kg in maize along with 66 kg of nitrogen/ha,[14] 570 kg in rice along with 60 kg N/ha[15], 600 kg in wheat along with 60 kg of N/ha, [14] 540 kg in sorghum along with 30 kg of N/ha.[16]

1.2.5 *Azospirillum* sp: *Azospirillum lipoferum* is a common soil inhabiting bacterium first described by Beijerinck in 1925. This is an associative type of symbiotic nitrogen fixing bacteria which produces growth-promoting substances such as IAA, and gibberellins, which promotes root proliferation. Plant growth promoting substances like pantothenic acid, thiamine and niacin are produced by *Azospirillum lipoferum* in large quantities that improve the plant growth and yield. *Azospirillum* is remarkably versatile; it fixes atmospheric N,[17] mineralizes nutrients from soil, sequesters Fe, survives in harsh environmental conditions, and favors beneficial mycorrhiza—plant associations.[18] Azospirillum in maize crop enhance the crop yield in the range similar to 60 kg urea N/ha.[19] Smith et al., have shown

a saving in 39–42 kg N in millets and guinea grass with *Azospirillum lipoferum*.[20]

Researchers have demonstrated the feasibility of *Azospirillum* inoculation to mitigate negative effects of NaCl on plant growth parameters. This beneficial effect of *Azospirillum* inoculation was previously observed in wheat (*Triticum aestivum* cv. 'Buck Ombú') seeds, where a mitigating effect of salt stress was also evident.[21] *Azospirillum*-inoculated wheat (*T. aestivum*) seedlings subjected to osmotic stress developed significant higher coleoptiles, with higher fresh weight and better water status than non-inoculated seedlings.[22,23] *Azospirillum* inoculation improve water status for plants.[24] In this sense inoculation technology with *Azospirillum* could be extended to arid soils in order to protect crops against drought. The main effect of *Azospirillum* is to promote a more developed radicle system; plant adaptation to water stress could be enhanced in inoculated crops.

Azospirillum spp. is not considered to be a classic biocontrol agent of soil-borne plant pathogens. However, there have been reports on moderate capabilities of *A. brasilense* in biocontrol of crown gall-producing *Agrobacterium*;[25] bacterial leaf blight of mulberry;[26] and bacterial leaf and/or vascular tomato diseases.[27,28] In addition, *A. brasilense* can restrict the proliferation of other nonpathogenic rhizosphere bacteria.[29] These antibacterial activities of *Azospirillum* could be related to its already known ability to produce bacteriocins[30] and siderophores.[31,32] It was recently reported that *A. brasilense* can synthesize phenylacetic acid (PAA), an auxin-like molecule with antimicrobial activity.[33] Biofertilizers made from *Azospirillum* is suitable for C_4 crops such as sugarcane, maize, bajra, sorghum; and other cereals like rice, wheat, barley, ragi and various horticulture crops. In this context, practices and potentialities still have a wider gap (it is not as popular as *Rhizobium)* and a lot can be done in sustaining cereal production.

1.2.6 *Gluconoacetobacter diazotrophicus*: This bacterium colonizes the internal root tissue of sugarcane and fix nitrogen. Since it occupies vascular tissue, it has the obvious advantage of being first in line and thus solves the problem of competition by non diazotrophs. *G diazotrophicus* is also found in *Pennisetum purpureum, Ipomoea batatas* and *Coffea arabica*—non legume plants. In the eighties Dobereiner and coworkers in Brazil discovered this association and named *Acetobacter diazotrophicus*. It is unique that it can grow at 30% sucrose concentration at 5.5 pH and is more tolerant to oxygen. These live in xylem vessels, intercellular space of root, shoot or leaf, ensuring proper supply of nutrients for nitrogen fixation. It was widely studied and used as a model system to assess the bacterial endophyte—plant interaction. After its discovery, it was reported from a variety of crops like coffee,[34] ragi[35] and pineapple[36] and a latest report throw light on *Gluconacetobacter* sp as a natural colonizer of the wild rice (*Porteresia cocarctata Tateoka, formerly Oryza coarctata Roxbi*) and a salt tolerant pokali rice variety.[37] In India its nitrogen fixing ability and plant growth promoting rhizobacteria (PGPR) production ability have been described, tested and found promising.[38]

Gluconacetobacter diazotrophicus is a nitrogen-fixing endosymbiont of sugarcane plants that antagonizes with *Xanthomonas albilineans* by impeding the production of the bacterial gum xanthum-like polysaccharide. Soybean and cereals could obtain up to 30% of their nitrogen from BNF when fertilized with ample supply of phosphorus, potassium and minor elements. The largest effect in this group was obtained with sugarcane, which can obtain up to 150 kg N/ha from BNF.[39] In Tamilnadu, 24 strains of *G.diazotrophicus* were isolated from sugarcane (root, stem and leaves) and screened for nitrogenase activity. The strain isolated from sugarcane stem (SoS2) showed maximum acetylene reduction assay, 410.92 nmoles of C_2H_4/hr/mg cell protein followed by SoL3 from sugarcane leaf and hence the strain from sugarcane stem was recommended as an effective biofertilizer.[40]

1.2.7 Free living nitrogen fixation—cyanobacteria: Several cyanobacteria (also known as blue green algae) such as *Anabaena, Nostoc, Cylindrospermum, Aulosira, Tolypothrix* are excellent nitrogen fixers. *Cyanobacteria* include unicellular and colonial species. Some filamentous colonies show the ability to differentiate into several different cell types: vegetative cells, normal photosynthetic cells that are formed under favorable growing conditions; akinetes, climate-resistant spores that may form when environmental conditions become harsh; and thick-walled heterocysts, which contain the enzyme nitrogenase, vital for nitrogen fixation. Heterocysts may also form under the appropriate environmental conditions (anoxic) when fixed nitrogen is scarce. Heterocyst-forming species are specialized for nitrogen fixation and able to fix nitrogen gas into ammonia (NH_3), nitrites (NO_2) or nitrates (NO_3), which can be absorbed by plants. The capacity of nitrogen fixation varies with agroclimatic conditions. Blue Green Algae (BGA) can provide 25 to 30% N/ha/season in Rice fields.[41,42] In addition to nitrogen, BGA enrich soil with extracellular carbohydrates,

Cyanobacteria: A prokaryotic microorganism capable of fixing nitrogen and carbon. These are usually considered as primary colonizers.

hormones, many secondary metabolites and improve soil health. It increases soil porosity, soil water holding capacity, ameliorates degraded soil due to excessive use of chemical fertilizers and also salt affected soils.[43] Based on their capacity to tolerate several stress factors like salinity, pH, pesticides, and desiccation.[44–46] Eight cyanobacterial species including *Anabaena, Nostoc, Calothrix,* and *Aulosira* were selected for field use in Orissa and coastal areas.

Nitrogen is one of the major nutrients required for crop growth and often limited. This is also highly mobile in the soil environment and amenable for losses. Biological nitrogen fixation by prokaryotes is a beneficial process in returning nitrogen to the soil towards crop production and leading to sustainable N management.

1.3 *Phosphorus nutrition—phosphate solubilizing microorganism*

Phosphorus is second only to nitrogen in mineral nutrients most commonly limiting growth of crops. Phosphorus is an essential element for plant development and growth making up about 0.2% of plant dry weight. Plants acquire P from soil solution as phosphate anions, however, these are extremely reactive and may be immobilized through precipitation with cations such as Ca^{2+}, Mg^{2+}, Fe^{3+} and Al^{3+} depending on the particular properties of a soil. In these forms, P is highly insoluble and a large portion of soluble inorganic phosphate applied to the soil as chemical fertilizer is immobilized rapidly and becomes unavailable to plants.[47] Hence, the amount available to plants is usually a small proportion of this total application.

There is a significant correlation between the numbers of phosphate-solubilizing bacteria and fungi and the levels of total P in soil was observed.[48] The principal mechanism for mineral phosphate solubilization is the production of organic acids,[49,50] and acid phosphatases play a major role in mineralization of organic phosphorus in soil. It is generally accepted that the major mechanism of mineral phosphate solubilization is through the action of organic acids synthesized by soil microorganisms. Production of phosphatase enzyme is another mechanism for P mineralization and concomitant P—solublization. Strains from the genera *Pseudomonas, Bacillus* and *Rhizobium* are among the most powerful phosphate solubilizing bacteria,[51] and among fungi, *Penicillium* and *Aspergillus* are promising P—solublizers.[52,53] Mineral phosphate solubilizing activities of several isolates were tested on tricalcium phosphate medium by analyzing the soluble-P content after 72 h of incubation at 30°C. HPLC analysis detected eight different kinds of organic acids, namely, citric acid, gluconic acid, lactic acid, succinic acid, propionic acid and three unknown organic acids from the cultures of these isolates.[54] Phosphate solubilizing bacilli have received considerable attention as inoculants for crops. *B. circulans* and *B. megaterium* var. *phosphaticum* inoculants increased plant weight and P-uptake of pea, respectively in green house experiments.[55,56] Similarly, Gaind and Gaur[57] reported that a *B. subtilis* inoculant increased biomass, grain yield, P and N-uptake of mung bean grown in a P-deficient field soil amended with rock phosphate.

Phosphate rock minerals are often too insoluble to provide sufficient P for crop uptake. Use of phosphate solubilizing microorganisms increase crop yields up to 70 per cent.[58] Combined inoculation of arbuscular mycorrhiza and phosphate solubilizing bacteria enhanced uptake of both native P from soil and P coming from the phosphatic rock.[59,60] Microorganisms with phosphate solubilizing potential increase the availability of soluble phosphate and enhance the plant growth by improving biological nitrogen fixation.[61,62]

The distribution of depositions of rock phosphate (raw material for phosphate fertilizers) is patchy, in India it is found in Udaipur, Mussorie but not in large quantities. P—solubilizing microorganisms make available the insoluble phosphate into soluble forms and can reduce the demand of P-fertilizers to a greater extent and sustain crop production by increasing the inherent capacity of soil to supply soluble form of phosphate.

Bioactivation of rock phosphate and seed treatment with phosphorus solubilizing microbes (PSM) was found to be effective in enhancing P nutrition in cowpea and ragi.[63] Significant increases in total dry mater accumulation of chickpea and groundnut due to inoculation with PSMs have been reported by Gaur,[64] Hebbara and Suseeladevi.[65] Similar studies of yield increases due to PSM inoculation of pulses such as green gram, black gram and chick pea are available.[66,67]

1.3.1 P—mobilizers: P—mobilizers facilitate mobilization of soluble phosphorus from distant places in soil where plant roots cannot reach and thus increase availability of P to plants. Mycorrhiza are prominent P mobilizers. Mycorrhiza is a symbiotic association between plant roots and a few fungi. The fungal partner is benefited by obtaining its carbon requirements from host's photosynthates and the plant in turn gains the much needed nutrients especially phosphorus, calcium, copper and zinc which would otherwise be inaccessible to the host. This uptake of nutrients is facilitated

with the help of a fine absorbing hyphae of the fungus. These fungi are associated with majority of agricultural crops. There are seven genera of these fungi that produce Arbuscular mycorrhizal symbiosis with plants. They are *Glomus, Gigaspora, Scutellospora, Acaulospora Entrophospora, Archaeospora* and *Paraglomus.* Probably the most abundant fungi in agricultural soils are the arbuscular mycorrhizal (AM) fungi. They account for 5–50% of the biomass of soil microbes.[68] Hyphal biomass of AM fungi may amount to 54–900 kg/ha,[69] and some products formed by them may account for another 3000 kg.[70] Pools of organic carbon such as glomalin produced by AM fungi may even exceed soil microbial biomass by a factor of 10–20.[71] Approximately 10–100 m mycorrhizal mycelium can be found per cm root.[72] The mechanism that is generally accepted is a wider physical exploration of the soil by mycorrhizal fungi (hyphae) rather than by roots. A speculative mechanism to explain P uptake by mycorrhizal fungi involves the production of glomalin.[70]

The microorganisms always participate in cycling of phosphorus in the environment hence there is recycling and not exhaustion.

AM fungi play an important role in water economy of plants. Their association improves the hydraulic conductivity of the root at lower soil water potentials, and this improvement is one of the factors contributing towards better uptake of water by plants.[73] A few proposed mechanisms by which AM fungi also help in activation of plant defense systems include changes in exudation patterns and concomitant changes in mycorrhizosphere populations, increased lignification of cell walls, and competition for space for colonization and infection sites.[74]

1.4 *Plant Growth Promoting Rhizobacteria (PGPR)*

PGPR were first defined by Kloepper[75] to describe soil bacteria that colonize roots of plants following inoculation onto seed and that enhance plant growth.

Beneficial effects of PGPR are as follows:

- Production of plant hormones like IAA, GA_3, cytokinin and induce formation of ethylene
- Reduces deleterious effects of pathogens on crop growth by protection against pathogens by production of antibiotics
- Solubilization of mineral nutrients by inducing specific ion flux in plant cell
- Fixation of atmospheric nitrogen that is transferred to the plant
- Solubilization of phosphorus
- Production of siderophores that chelate iron and make it available to the plant root
- Induce systemic resistance in plant

In a study in Allahabad, efficiency of plant growth promoting rhizobacteria were found to enhance seed germination, plant growth and yield of *Cicer arietinum* L. by producing IAA and other growth promoters by 10 rhizosphere bacterial isolates.[76] The effect of PGPR on seed germination, seedling growth and yield of field grown maize were to be significantly enhanced.[77] Stimulation of different crops by PGPR has been demonstrated in both green house and field trials. Strains of *Pseudomonas putida* and *Pseudomonas fluorescens* have increased root and shoot elongation in canola, lettuce, and tomato; yields in potato, radishes, rice, sugar beet, tomato, lettuce, apple, citrus, beans, ornamental plants, and wheat.[78,79] Wheat yield increased by 30% with *Azotobacter* inoculation; 43% with *Bacillus* inoculants,[80] and a 10–20% in field trials using a combination of *Bacillus megaterium* and *Azotobacter chroococcum.*[81]

There is been a large body of literature describing potential uses of plants associated bacteria as agents stimulating plant growth and managing soil and plant health.[82] Plant growth-promoting bacteria (PGPB) are associated with many, if not all plant species, and are commonly present in many environments and protect plant against pathogens. Enhanced growth of cotyledons in the bacterial supernatants suggest that cytokinins are implicated in the mechanisms of plant growth promotion by bacteria *Bacillus benzoevorans* and *Ralstonia* sp. Under iron-limiting conditions PGPR produce low-molecular-weight compounds called siderophores to competitively acquire ferric ion. The basis of antibiosis as a biocontrol mechanism of PGPR has become increasingly better understood over the past two decades.[83] A variety of antibiotics have been identified, including compounds such as amphisin, 2,4-diacetylphloroglucinol (DAPG), hydrogen cyanide, oomycin A, phenazine, pyoluteorin, pyrrolnitrin, tensin, tropolone, and cyclic lipopeptides produced by pseudomonads.[84]

PGPR: Soil bacteria that colonize plant roots and enhance plant growth.

A variety of microorganisms also exhibit hyperparasitic activity, attacking pathogens by excreting cell wall hydrolases.[85] Chitinase produced by *Serratia. plymuthica* C48 inhibited spore germination and germ-tube elongation in *Botrytis cinerea*.[86] Induced systemic resistance (ISR) is one of the mechanisms of plant protection by PGPR.[87] PGPR-elicited ISR was first observed in carnation (*Dianthus caryophillus*) with reduced susceptibility to wilt caused by *Fusarium* sp.[88] and on cucumber

(*Cucumis sativus*) with reduced susceptibility to foliar disease caused by *Colletotrichum orbiculare*.[89] The ineffectiveness of PGPR in field appears to be because of their inability to colonize plant roots.[90] PGPR has dual role in enhancing crop production. The main role is production of growth harmones. However, many PGPR have a potential role as biocontrol agents. Further researches is required to examine the replacement of insecticides.

1.5 *Formulations of biofertilizers for sustainable agriculture*

The success of inoculation technology depends on two factors such as the microbial strain and inoculants formulation. In practical terms, formulation determines potential success of inoculants.[91] The technical optimization of an *inoculant* formulation is independent of strains used, since most of the strains of same bacterial species share many physiological properties, it may be assumed that a technological progress developed for a particular strain is readily adaptable to another strain of same species with only minor modifications.[92] In spite of a central role of formulation in successful commercialization of inoculant products, research in this area has been largely ignored. In addition to limited availability of published scientific information with regard to inoculant formulation, the information available is fragmented.[93] A survey of bibliographic database of scientific literature shows that major emphasis was given to the development of improved strains through different approaches. Indeed many such strains have been constructed and granted patent in many developed countries but failed to appear on the commercial market, perhaps because of inappropriate formulation.

Inoculant: (Synonym for biofertilizer) preparations containing beneficial microorganisms which enhances plant growth.

Development of improved formulations often rests with inoculant manufacturer's research and development facility which are primarily located in developed countries where target market exist, but they fail to consider the unique problems in applying these inoculants in developing countries. The most important characteristic common to most of biofertilizers is unpredictability of their performance. In order to harness the benefits of biofertilizers in agriculture, the consistency of their performance must be improved.[94]

Carriers: Inert materials, used for transporting mircobes from laboratory to land.

Bacteria introduced to soil may fail to establish in sufficient numbers in the *rhizosphere* because of competition from native numbers, and little is known about the factors controlling competitiveness of bacterial strains, more so under field conditions. Agricultural practices in developing countries and under semi arid conditions are two examples wherein biofertilizers may find their greatest challenges. Farmers in developing nations follow low input agriculture in which fertilizers, pesticides and agro technical machinery are scarce. Application or uses of biofertilizers in such systems requires additional infrastructure, cost, labour and technical knowledge. Semiarid conditions make survival difficult for introduced bacteria, harsh conditions including droughts, lack of sufficient irrigation, high salinity and soil erosion may quickly diminish the introduced bacteria; even in developed nations. However, bacterial inoculants may have greatest contribution if inexpensive and easy to use formulations can be developed.[92] Most important constraints for adoption of biofertilization in India have been attributed to poor quality of inoculants produced, lack of knowledge about inoculation technology for extension personnel and farmers; effective inoculants delivery/supply system and lack of committed policy to exploit biofertilizers successfully.[95]

Rhizosphere: Region of the soil under the influence of plant roots.

1.6 *Inoculant formulations*

Formulation step is a crucial aspect for producing microbial inoculants and determines the success of a biological agent. Formulation typically consists of establishing viable bacteria in a suitable carrier together with additives that aid in stabilization and protection of microbial cell during storage, transport and at the target. The formulation should also be easy to handle and apply so that it is delivered to target in most appropriate manner and form, one that protects bacteria from harmful environmental factors and maintain or enhance the activity of the organisms in the field. Therefore, several critical factors including user preference have to be considered before delivery of a final product.[93]

1.6.1 *Carriers* for inoculant formulations: A suitable carrier plays a major role in formulating microbial inoculants. Carrier is a delivery vehicle which is used to transfer live microorganism from an agar slant of laboratory to a rhizosphere. A good quality inoculant should be made of a superior carrier material. Hence Smith[96] has listed the characters of a superior quality carrier material for microbial inoculants, which includes:

- High water holding and water retention capacity
- No heat of wetting
- Nearly sterile, chemically, physically and uniform
- Non toxic in nature, easily biodegradable and non polluting
- Nearly neutral pH or easily adjustable
- Supports growth and survival of bacteria

- Amenable to nutrient supplement
- Rapid release of bacteria in soil
- Manageable in mixing, curing and packaging operations
- Available in powder or granular form in adequate quantities and at reasonable cost

Considering the above characteristics it is clear that not a single universal carrier is available which fulfill all the desirable features, but good ones should have as many as possible. Peat was the carrier of choice and most commonly used for *Rhizobium* inoculants worldwide for decades. Though its popularity is primarily due to successful field results obtained under commercial cultivation, it has many drawbacks like, variability in quality which is dependent on source, heat sterilization of some peat may release components toxic to bacteria,[97] and its availability is restricted to a very few countries. All these factors have forced researchers to look for alterative carrier materials. Some of the alternative carriers evaluated for bacterial inoculants include lignite and coal[98,99] clays and inorganic soils,[97] compost, farm yard manure, soybean meal,[100] wheat bran,[101] pressmud,[102] spent agricultural waste material,[103] spent mushroom compost.[104] Apart from these many other synthetic and inert material like vermiculite,[105,106] perlite, ground rock phosphate, calcium sulphate, polyacrylamide gels[107] and alginate[108,109] have also been evaluated. Most of the evaluated carriers are either naturally abundant resources or available waste materials. Little research has been conducted with objectives of synthesizing a carrier with superior characteristics.[92]

Target microorganism can be introduced into a sterile or non sterile carrier to produce inoculants. A sterile carrier has distinct advantages from a purely microbiological point of view. Disadvantages with sterilized carriers include a higher cost of production, increased labor, necessity for a sterilizing unit, and the necessity for aseptic procedures during packaging. The type of carrier used in inoculants production usually depends on the mode of application. There are two types of inoculants commonly produced. Those for seed treatment and for direct application to the soil. Owing to differing methods of delivery, these formulations can either be powder for seed treatment or granulated for soil application.[110]

Principal drawbacks of solid carrier based inoculants originate from a great variability in quality of carriers which is source dependent, and composition of carriers are undefined and complex. This greatly affects the final product and cause difficulties in inoculant dosage, storage conditions[111], and variation of inoculant effectiveness between different manufacturers and between different batches from the same manufacturer.[92] In carrier based inoculants, bacteria have a lower tolerance for physical stress during storage, particularly for temperature variations. Some types of peat can even reduce plant growth.[112] They are often prone to contamination that can reduce the shelf life of the inoculant.[91,111,113,114] Addition of adhesives to inoculant during its application to seeds, or slurry application will improve its adhesion, but that requires additional time and labor for a process that is already labor-intensive.[115] Solid carriers based inoculant production involves a significant amount of cost, labor, energy intensive processing such as mining, drying, milling and neutralization before its use in a commercial production.

The carrier based inoculants produced in India generally have a short shelf life, poor quality, high contamination and unpredictable field performance. High quality biofertilizers would be expected to have higher population of desired microorganisms, sufficient viability, and remain uncontaminated for longer period of storage. The carriers used in India are nearly inert material and forms clumps upon drying, which leads to significant loss of viability. Seed is not a favorable environment for most of the plant growth promoting bacteria, as they are soil bacteria, yet seed inoculation is a common practice for microbial inoculation. Hence it is important for a high quality biofertilizer to maintain viability of bacteria on seed upon inoculation. The usual carrier based biofertilizers do have some drawbacks for seed inoculation like seed coat damage, seed coat toxicity, death of cells due to desiccation and possible contact of microorganisms with agricultural chemicals. Today, advances in inoculant technology are concerned with improving quality, extending useful shelf life and developing new formulations for use under less favorable conditions. Liquid inoculants and alginate based granular formulations are two important new inoculant formulations which are an alternative to peat/lignite based ones.

1.6.2 Liquid inoculants: Liquid inoculants are not the usual broth culture from a fermenter or water suspension of the carrier based biofertilizers, as often made out to be. It is a special liquid formulation containing not only the desired microorganisms and their nutrients but also contains special cell protectant and amendments that promote cell survival in a package and after application to seed or soil. Various liquid media are being used to culture bacteria. These media normally consist of carbon, nitrogen and vitamin sources, which promote growth of bacteria.

However, additives used in liquid inoculants improve quality of inoculants by increasing the population density and enhanced shelf life.[116]

Additives used in the preparation of liquid inoculants have been selected based on their ability to protect bacterial cells in package and on seeds at extreme conditions such as high temperature, desiccation and toxic condition of seeds and seed chemicals. Most of the additives are high molecular weight polymers with good water solubility, non-toxicity and complex chemical nature[117] and are able to limit heat transfer, posses good rheological properties and high water activities.[118] Some of the polymers which are presently used in preparation of liquid inoculants includes polyvinyl pyrrolidone (PVP), methyl cellulose, polyvinyl alcohol, polyethylene glycol, gum Arabica, trehalose, glycerol, Fe-EDTA, sodium alginate, tapioca flour etc.[116,119] Polyvinylpyrrolidone was known to bind toxic compounds present in seed exudates that are mobilized during inoculation and seed germination. PVP has a high water-binding capacity and appears to cause slow drying of an inoculant after application. PVP solution tends to coalesce into ridges on their seed coat as it dries, perhaps providing a thicker layer of protection than some other compounds. Its sticky consistency may also enhance cell and inoculants adherence to seeds.[119] Some time seed-released compounds may bind iron in yeast extract, making it unavailable to cells. Supplementary iron may, therefore, replace Fe bound by seed exudates.[120] Glycerol has a high water-binding capacity and may protect cells from effects of desiccation by slowing the drying rate. Its flow characteristics appear to promote rapid and even coating on seeds.[121,122] Trehalose is widely reported to enhance cell tolerance to desiccation, osmotic and temperature stress. It acts by stabilizing both enzymes and cell membranes, is a compatible osmoticum as well, and readily manufactured by *Bradyrhizobium* given ideal conditions.[123,124] Addition of PVP in a medium was known to protect both fast and slow growing *Rhizobium.*[125] Bushby and Marshall and Vincent et al. have showed that addition of maltose (9%) and montmorillonite clay could protect *Rhizobium* against high temperature and desiccation.[125,126] Polymers that are soluble in liquid inoculant formulations are convenient for batch processing of inoculants and make seed application a simpler process for farmers. Liquid *Rhizobium* inoculants prepared with PVP as an osmo-protectant had improved shelf life, nodulation and nitrogen fixation on par with lignite-based inoculants in cowpea.[127]

Bacteria respond to not only the type of polymer in liquid inoculants and its concentration in a medium. *Pseudomonas* maintained highest population density in the presence of PVP formulations, but population density of *Acinetobacter* was highest in the presence of PEG. Marked response was noticed to the addition of glycerol in case of *Azotobacter* and to PVP K-15 with *Bacillus.* In general, the addition of various osmolytes at the concentration of 1% or higher results in maintaining a population higher than 0.5% level of amendment.[128]

Liquid inoculants can be produced by a simple fermentation process, packed directly from the fermentor aseptically, and stored. It minimizes the production cost by avoiding processing and sterilization of solid carrier material. The complete sterilization could be achieved with liquid formulations and any contamination during the storage can be easily detected. Liquid inoculants could be produced with minimum labour, space and energy and also the quantity of inoculum required is less compared to carrier based formulations, hence easier for farmers to handle. The first yard stick to measure the quality of biofertiliser is the viable cell density of desired microorganisms which essentially provides adequate number of microorganisms on each seed. The liquid inoculants developed were known to have population of *Rhizobium* sp., *Azotobacter* sp., *Azospirillum* sp. and PSB up to the level of 10^8 cells per ml.[128–131] A strong correlation existed between the number of surviving cells on seeds and nodulation in legumes, hence it is important to have more number of cells per seed, which are sufficient to compete with native *Rhizobium* and to offset death of cells due to biotic and abiotic stresses. Since the liquid biofertiliser have high cell count, each seed receives more than thousands of cells. Additives in liquid biofertiliser protect the cells on the inoculated seeds against toxicity, desiccation and osmotic shock.[130] Studies at University of Agricultural Sciences, Bangalore showed that the liquid inoculants of *Rhizobium*, *Azotobacter, Azospirillum* and PSB may be stored at ambient temperature without significant loss in viability for more than one year (Figure 2).[128,132] The storage and transportation conditions are not congenial many a times for the bio-inoculants as temperature in many parts of the country may reach up to 45°C, in such condition the quality of biofertilizer will decline drastically. Liquid biofertilizers were known to have more than one year shelf life compared to carriers. Studies have revealed that these liquid inoculants can be stored without losing viability in high temperature (45°C) conditions also.[130]

Imposition of stress to bacteria results in an adaptive response. This necessitates changes in

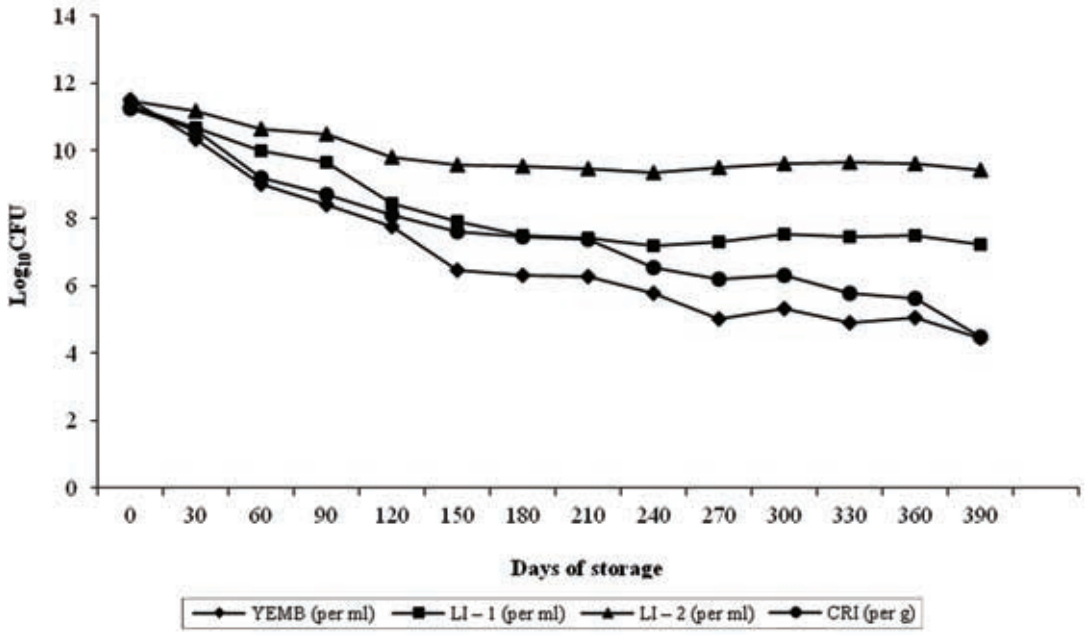

Figure 2: Survival of *Bradyrhizobium* sp. stored at room temperature in different inoculant formulations.
Note: YEMB: Yeast Extract Mannitol Broth; LI: Liquid inoculant; CRI: Carrier based inoculant.

regular metabolic processes in cells, which are then reflected in an alteration of protein profiles.[133] Synthesis of additional 19 salt stress proteins (SSPs) in *Rhizobium* (40–52 kDa), 10 SSPs (ranging from 19 to 82 kDa) in *Anabaena* sp. L-31 under salt stress[134] and synthesis of 19 heat shock proteins (ranging from 8–60 kDa) in *Bradyrhizobium japonicum* at 43°C have been reported.[135] *Bradyrhizobium* sp. (*Arachis*) grown at room temperature in liquid inoculant synthesized 60 and 47 kDa proteins of higher intensity but the same proteins of lower intensity in *YEMB*. *Bradyrhizobium* sp. (*Arachis*) on exposure to heat stress showed the presence of bands of same proteins (60 and 47 kDa) in liquid inoculant. Similarly, under salt stress (0.05 M NaCl), *Bradyrhizobium* sp. (*Arachis*) grown in liquid inoculant synthesized the extra proteins of 66 kDa but not in YEMB.[132] This kind of mechanisms provides potential to grow at different types of soil as we know that performance of inoculants depends largely on soil conditions.

The amount of inoculant needed for seed inoculation is less and there is no need of any sticker material unlike carrier-based inoculants. Liquid inoculants can easily be adopted to advanced seeding equipments, since it can be sprayed on to seeds as it passes through seed drill and dries before it travels in to the seed bin on the planter.

1.6.3 Field response of liquid *Rhizobium* inoculant formulation: Researchers have shown that the performance of liquid rhizobial formulations is comparable to that of peat-based products under field conditions.[136] The field efficiency of the liquid inoculant was tested by on farm trails under different agroclimatic conditions of India for two successive years in four different legumes like groundnut, pigeonpea, chickpea and soybean. Results of trials showed that liquid *Rhizobium* inoculants performed better than the carrier based *Rhizobium* inoculants (Table 2: Figure 3 and Plate 2 and 3).[132] There is a need to improve formulation for better field performance. In this direction the research is in progress indicates that solid carrier materials have been replaced with microbe friendly liquid formulations. Further a formulation containing a consortium is to be developed for field application.

YEMB: Yeast Extract Mannitol Broth.

1.6.4 Polymer entrapped inoculants formulation: During the last decade, several experimental formulations based on polymers have been evaluated. These polymers have demonstrated potential as bacterial carriers for microbial inoculants[137] that offered substantial advantages over peat. These formulations encapsulate living cells, protect microorganisms against many environmental stresses and release them to soil, gradually but in large quantities, where the polymers are degraded by soil microorganisms. They can be dried stored at ambient temperatures for prolonged periods, offer a consistent batch quality and a better defined environment for the bacteria and can be manipulated easily according to the needs of specific

Table 2: Yield of pulses in response to inoculation with liquid *Rhizobium* inoculant.

Treatments	Yield kg/ha			
	Soybean	Chickpea	Pigeonpea	Groundnut
Uninoculated	1803.00[c]	1024.00[b]	702.40[c]	982.21[c]
LRI	2074.00[a]	1121.00[a]	854.90[a]	1131.21[a]
CRI	1959.00[b]	1124.00[a]	802.71[b]	1048.74[b]
CD at p = 0.05	47.28	33.63	37.76	30.65

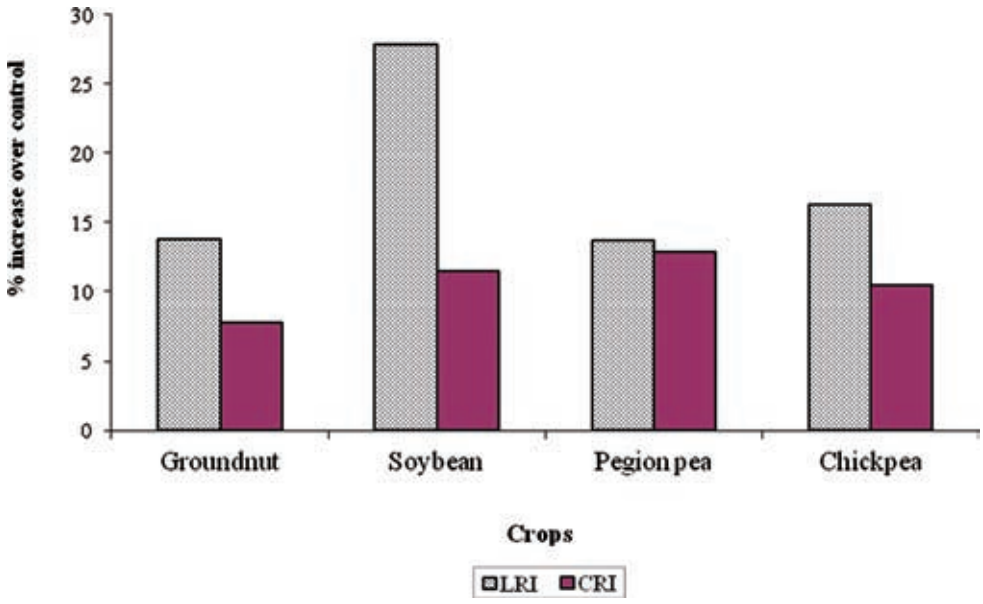

Figure 3: Percent increase in the yield of pulses over uninoculated control due to inoculation with liquid *Rhizobium* inoculant.
Note: LRI—Liquid *Rhizobium* Inoculant; CRI—Carrier based *Rhizobium* Inoculant.

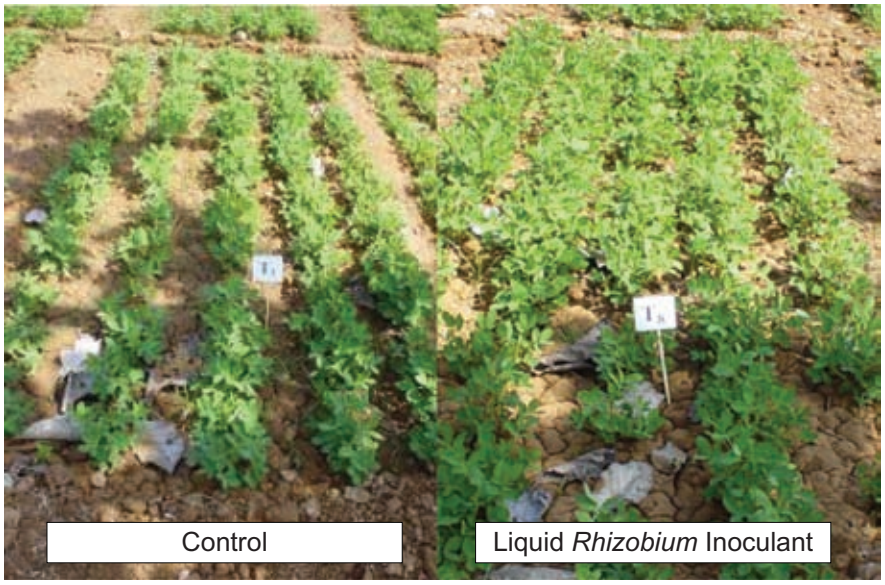

Plate 2: Field experiment to study the effect of liquid *Rhizobium* inoculant on Groundnut.

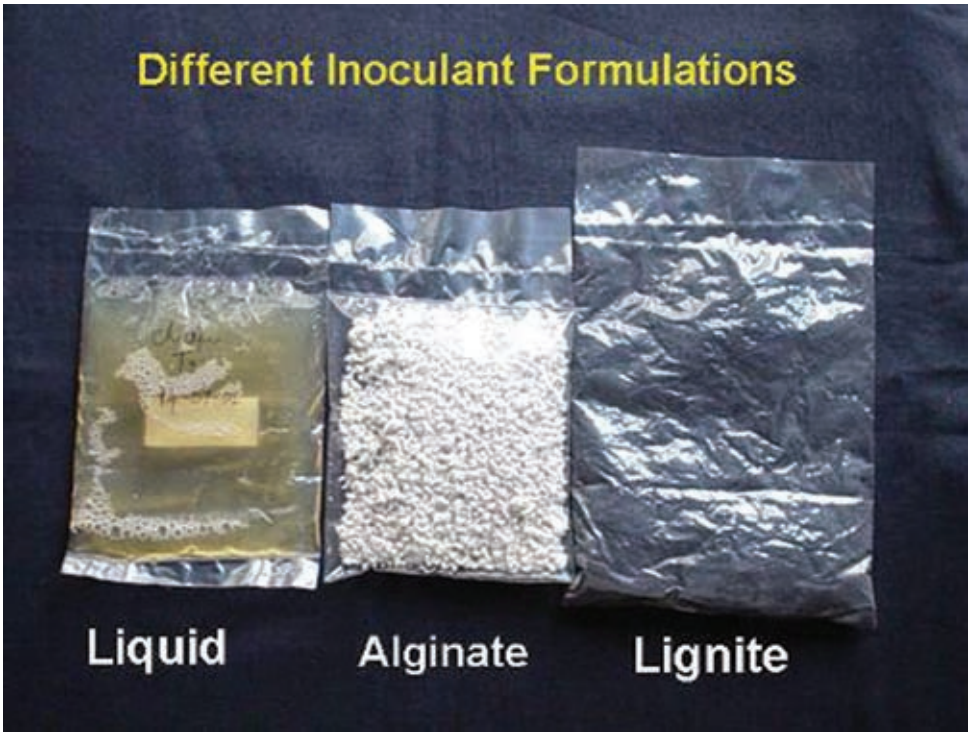

Plate 3: *Rhizobium* biofertilizer in different inoculant formulations.

bacteria. These inoculants can be amended with nutrients to improve short-term survival of bacteria upon inoculation, which is essential to the success of an inoculation process, especially with associative PGPB.[92] However, major constraints for the inoculation industry is that polymers are expensive compared to peat-based inoculants and require more handling by the industry.[91]

The encapsulation of microorganisms into a polymer matrix is still experimental in the field of bacterial-inoculation technology. At present there is no commercial bacterial product using this technology. The concept underlying immobilized microbial cells is to entrap beneficial microorganisms into a matrix. The formulation (bacteria-matrix) is then fermented in a bacterial growth medium. Immobilized microbial cells are easy to produce, store, and handle during industrial operations. Encapsulated bacterial formulations in agriculture have two advantages (i) to temporarily protect the encapsulated microorganisms from the soil environment and microbial competition, and (ii) to release them gradually for the colonization of plant roots.[108,138,139]

1.6.5 Alginate based formulations: *Alginate* is a commonly used polymer for encapsulation of microorganisms and is naturally occurring, composed of β-1,4-linked D-mannuronic acid and L-glucuronic acid. It is available from different macroalgae[140] as well as several bacteria.[141] Alginate cost has recently dropped because of its massive production in the Far East, making it potentially more attractive to the inoculant industry. The preparation of beads containing bacteria is fairly easy and involves a multistep procedure.[91,139]

The main advantages of alginate preparations are their nontoxic nature, biodegradability, and their slow release of microorganisms into a soil.[91,142] This technology was used to encapsulate the plant-beneficial bacteria *Azospirillum brasilense* and *Pseudomonas fluorescens*,[91] which were later successfully used to inoculate wheat plants under field conditions. The bacteria survived in the field long enough and their populations were comparable to the survival of bacteria originating from other carrier-based inoculants.[143] Furthermore, the addition of clay and skim milk to the beads significantly increased bacterial survival over alginate beads alone. Alginate mixed with perlite was used to entrap *Rhizobium*.[144] Colonization of wheat roots by beneficial cells released from the beads was superior to that achieved by direct soil inoculation. These studies provide clear evidence that alginate beads are efficient slow-release carriers for plant inoculants, providing a protective environment in the soil. Several other alginate-based preparations have been tried for the encapsulation of VAM fungi,[145] ectomycorrhizal fungi,[146,147] *Frankia* inoculation,[148] and

Alginate: Biopolymer extracted from microalgae.

fungi used as biocontrol agents against soil-borne pathogens.[149,150]

Alginate preparations may have solved many of the problems associated with traditional peat inoculants. These inoculant formulations may solve the problems associated with tropical, low input agriculture. In many parts of tropical region there is always a chance of prolonged dryness after sowing and microbial inoculation. Alginate encapsulated formulations are already desiccated due to lower water activity, microorganisms will be at a low metabolic activities, and are released into soil only after sufficient moisture is available, which always coincide with the germination of seeds. Considering the cost involved in production of alginate formulations, attempts have been made to amend these formulations with material like rock phosphate, cement, bentonite clays, granite powder, gypsum, lignite, talc by which cost of production can be minimized besides adding bulkiness to formulation.[150]

1.7 *VAM inoculants*

VAM: (Vesicular-Arbuscular Mycorrihizae) Fungus capable of symbiosis with plant roots.

The *VAM* being an obligate symbiont there are many constraints in its large scale commercial production and application. The only method of production is in association with host plant by pot culture, as production of VAM in the artificial media have met with little or no success. There are different types of VAM inoculum required for different purposes. The spores of VAM fungi are used as inocula generally for experiments *in vitro* conditions. Large scale production of spores is difficult.[152]

1.7.1 Infected root inoculum: Large scale production of infected root is possible in aeroponic cultures. Infected roots contain internal mycelium and external mycelia (may have spores). Infected roots colonize a host after one or two days of inoculation. Root inocula without spores should be used within a week. *In vitro* reproduction of some VAM fungi on tissue cultured roots has been demonstrated.[153] The production process is difficult and expensive. Other problems are a) infected root introduced as inocula acts as an attractive nutrient source for several saprophytic and parasitic microorganisms, b) short survival time and c) large quantities of inocula required.

1.7.2 Soil based inoculums: Soil inoculum is produced using a traditional pot-culture techniques containing all VAM fungal structures and is highly infective. The success of good soil inoculum production depends on the selection of host-plant and the ambient conditions under which a defined VAM fungus can be mass multiplied.[152]

1.7.3 Peat based inoculants (Nutrient film technique): VAM inocula obtained from pot cultures were incorporated into peat and compressed into blocks. Lettuce plants are allowed to grow in the peat block for 2–5 weeks then the blocks are transferred to nutrient film technique (NFT) channels.[154] The NFT channels slope and nutrient solution flows at 200 ml per minute. Plants are allowed grow in NFT system for 8–10 weeks. During this time, mass reproduction of the fungus takes place. The peat blocks are allowed to dry, chopped and used as VAM inoculums. The shelf life of such peat based inoculants are around six months.[152] Recently Government of India vide Gazette notification dated 8 November 2010 has included mycorrhizal biofertilizer under the ambit of Fertilizer control order 1985 and set the standards for this (Table 3).[155]

1.8 *Mixed bacterial inoculants*

Numerous recent studies show a promising trend in the field of inoculation technology. Mixed inoculants (combinations of microorganisms) that interact synergistically are currently being devised. Plant studies have shown that the beneficial effects of *Azospirillum* on plants can be enhanced by co-inoculation with other microorganisms. Co-inoculation, frequently, increased growth and yield, compared to single inoculation, provided the plants with more balanced nutrition, and improved absorption of nitrogen, phosphorus, and mineral nutrients.[156] Thus, plant growth can be increased

Table 3: Specification of Mycorrhizal biofertilizers.[155]

(i)	Form/base	Fine power/tablets/granules/root biomass mixed with growing substrates
(ii)	Particle size in case of carrier based materials	90% should pass through 250 miccron IS sieves (60 BSS)
(iii)	Moisture content percent maximum	8–12
(iv)	pH	6.0–7.5
(v)	Total viable propagules/gm of product, minimum	100/gm of finished product
(vi)	Infectivity potential	80 infection points in test roots/gm of mycorrhizal inoculums used

by dual inoculation with *Azospirillum* and phosphate-solubilizing bacteria.[157,158] *Azospirillum* is also considered to be a *Rhizobium*-"helper" stimulating nodulation, nodule activity, and plant metabolism, all of which stimulate many plant growth variables and plant resistance to unfavorable conditions.[159,160] Synergestic interaction between *Rhizobium* and VAM fungi in legume plants is well established.[161] Mixed inoculation with diazotrophic bacteria and arbuscular-mycorrhizal fungi creates synergistic interactions that may result in a significant increase in growth, phosphorus content, enhanced mycorrhizal infection, and an enhancement in the uptake of mineral nutrients such as phosphorus, nitrogen, zinc, copper, and iron.[161–165]

1.9 *Regulation and quality control of biofertilizers*

Naturally, an inoculant should contain a level of bacteria sufficient enough to inoculate plants and produce an economic gain. The required level of bacteria cannot be established as a general standard because it varies from one bacterial species to another and under different conditions. Hence different artificial standards for level of viable cells in inoculants have been established in different countries.[96] In majority of nations only rhizobial inoculants have legally established standards. Since this is a new research field, standards for PGPB numbers in inoculants do not yet exist, and every manufacturer can claim whatever he deems appropriate for his product.[91] Many developed countries have regulations for inoculant quality, but in most of the developing countries, inoculant quality is not regulated, nor are the existing regulations well enforced. The level of rhizobia required in the inoculant varies worldwide (between 10^7 and 4×10^9 cfu/g inoculant) and no set of common international standards exist.[113] To enumerate the bacterial number, commonly known methods in microbiology are used; the traditional Plate Count methods, Most Probable Number,[166] ELISA, and Immunoblot.[167–169]

One should consider two important aspects in establishing a quality standard for microbial inoculants, one is to maintain a minimum level of viable cells per unit and second is the level of contaminant. In most countries, there are no regulations of the level of *contaminants* in the most commonly used nonsterile peat preparations. Australia permits low levels of contaminants (0.1% of the total bacterial population), but at the same time requires high population levels of rhizobia.[170] Even some developing countries have very high standards for inoculants. In Rwanda, high rhizobia counts and no more than 0.001% contaminants are allowed.[171] Since the introduction of governmental regulations, there has been an improvement in the quality of commercial inoculants in several countries, including Australia, Canada, and the UK.[172] Surprisingly, the USA and UK have no regulations, perhaps because there have been no reported adverse effects where, quality control is left to market forces and the manufacturers' discretion.[96]

Contaminants: Growth of non-target microorganisms in growth medium.

In India Ministry of Agriculture and Cooperation has devised the specification on registration, standards, procedures and testing protocol for *Rhizobium, Azotobacter, Azospirillum* and Phosphate solubilizing bacteria (Table 4–7).[155] Bureau of Indian standard is the nodal agency for formulating the standard for biofertilisers, they have specified that all the bacterial inoculants should have minimum CFU of 5×10^7 per g of carrier and 10^8 CFU per ml of liquid inoculants, and should not have contamination at 10^{-5} dilution. Olsen et al.,[113] noted that Canadian regulations sometimes allow even low levels of rhizobia to be legally acceptable, perhaps because the cost of regulation is too high, compared to the risk of misuse.

Table 4: Specification of biofertilizers—*Rhizobium.*[155]

(i)	Base	Carrier based* in form of moist/dry powder or granules, or liquid based
(ii)	Viable cell count	CFU minimum 5×10^7 cell/g of carrier material or 1×10^8 cell/ml of liquid.
(iii)	Contamination level	No contamination at 10^{-5} *dilution*
(iv)	pH	6.5–7.5
(v)	Particle size in case of carrier based materials	All materials should pass through 0.15–0.212 mm IS sieve
(vi)	Moisture percent by weight maximum in case of carrier based	30–40%
(vii)	Efficiency character	Should show efficient nodulation on all the species listed on the packet

Dilution: Reducing the density of microorganisms serially to a manageable number so as to enable plating it on an agar medium and count the number of colonies that grow from it.

**Type of carrier:* The carrier materials such as peat, lignite, peat soil and humus, wood charcoal or similar material favoring growth of organism.

Table 5: Specification of biofertilizers—*Azotobacter*.[155]

(i)	Base	Carrier based* in form of moist/dry powder or granules, or liquid based
(ii)	Viable cell count	CFU minimum 5×10^7 cell/g of carrier material or 1×10^8 cell/ml of liquid.
(iii)	Contamination level	No contamination at 10^{-5} dilution
(iv)	pH	6.5–7.5
(v)	Particle size in case of carrier based materials	All materials should pass through 0.15–0.212 mm IS sieve
(vi)	Moisture percent by weight maximum in case of carrier based	30–40%
(vii)	Efficiency character	The strain should be capable of fixing at least 10 mg of nitrogen per g of sucrose consumed

**Type of carrier:* The carrier materials such as peat, lignite, peat soil, humus, wood charcoal or similar material favoring growth of organism.

Table 6: Specification of biofertilizers—*Azospirillum*.[155]

(i)	Base	Carrier based* in form of moist/dry powder or granules, or liquid based
(ii)	Viable cell count	CFU minimum 5×10^7 cell/g of carrier material or 1×10^8 cell/ml of liquid.
(iii)	Contamination level	No contamination at 10^{-5} dilution
(iv)	pH	6.5–7.5
(v)	Particle size in case of carrier based materials	All materials should pass through 0.15–0.212 mm IS sieve
(vi)	Moisture percent by weight maximum in case of carrier based	30–40%
(vii)	Efficiency character	Formation of white pellicle in semisolid nitrogen free bromothimol blue media.

**Type of carrier:* The carrier materials such as peat, lignite, peat soil, humus, wood charcoal or similar material favoring growth of organism.

Table 7: Specification of biofertilizers—Phosphate solubilizing bacteria.[155]

(i)	Base	Carrier based* in form of moist/dry powder or granules, or liquid based
(ii)	Viable cell count	CFU minimum 5×10^7 cell/g of carrier material or 1×10^8 cell/ml of liquid.
(iii)	Contamination level	No contamination at 10^{-5} dilution
(iv)	pH	6.5–7.5 for moist or dry power granulated carrier based and 5.0–7.5 for liquid based
(v)	Particle size in case of carrier based materials	All materials should pass through 0.15–0.212 mm IS sieve
(vi)	Moisture percent by weight maximum in case of carrier based	30–40%
(vii)	Efficiency character	The strain should have phosphate solubilizing capacity in the range of minimum 30%, when tested spectrophotometrically. In terms of zone formation, minimum 5 mm solubilization zone in prescribed media having at least 3 mm thickness.

**Type of carrier:* The carrier materials such as peat, lignite, peat soil, humus, wood charcoal or similar material favoring growth of organism.

Olsen et al.,[114] concluded that increased standards not only ensure that a farmer is provided with effective inoculants but are also in the best interest of the inoculation industry. Outlawing low quality inoculants from the market will help prevent a bad public image for the industry and will facilitate the introduction and acceptance of inoculants. It should be noted that the percentage of sub-standard inoculants in the market is not known, and perhaps the problem is just hypothetical.

2 Formulations for Microbial Consortia—Inoculants for Future

Despite progress in research on mixed inoculants, microbial inoculants with multiple organisms are not yet produced commercially. Until now, the research on mixed microbial inoculation was only confined to development and inoculation of each bacterium in separate formulation. But developments of new inoculant formulation like polymer entrapped desiccated inoculants have opened new vistas in mixed microbial inoculants. In this direction concept of "*microbial consortium*" assumes greater importance for sustainable agriculture.

Feasibility of production of microbial consortium using *Rhizobium* and PSB using lignite, liquid and alginate granules have been tested (Figure 4).[151,173] It was observed that microbial consortium developed using alginate encapsulation was able to conserve the viability of both the organisms used for more than 6 months. But in liquid formulations, fast growing *bacilli* had outnumbered the slow growing *rhizobia*. There is a need to exercise caution in selecting the bacterial strains and formulation in development of microbial consortium. Care should be taken to avoid bacterial strains which have antagonistic interactions among themselves. Alginate encapsulation is a promising inoculant formulation for microbial consortia as they are desiccated formulation, microorganisms will be in metabolically inactive state. The development of microbial consortium may minimize cost, labour and energy involved production of inoculants. But more and more single strains microbial inoculants must be registered, before inoculation industry can contemplate the development and commercialization of multi-bacterial inoculants.[174]

At the University of Agricultural Sciences Bangalore, consortium of agriculturally beneficial microorganisms (ABM) such as *A. chroococcum, Acinetobacter* sp. and *P. fluorescens* was constituted in alginate by Archana[175] and in soybean flour by Swapna.[176] Consortium containing AM fungi and Agriculturally beneficial microorganisms such as *Azotobacter*, *Acinetobacter* and *Pseudomonas* is constituted by Subramanyam (personal communication, Plate 4–6). Processing of material in Fluid bed dryer (FBD) involves forced air application at

Microbial consortium: A group of microbial species that work together to carry out an overall reaction or process, in our case beneficial organisms that together help promoting plant growth.

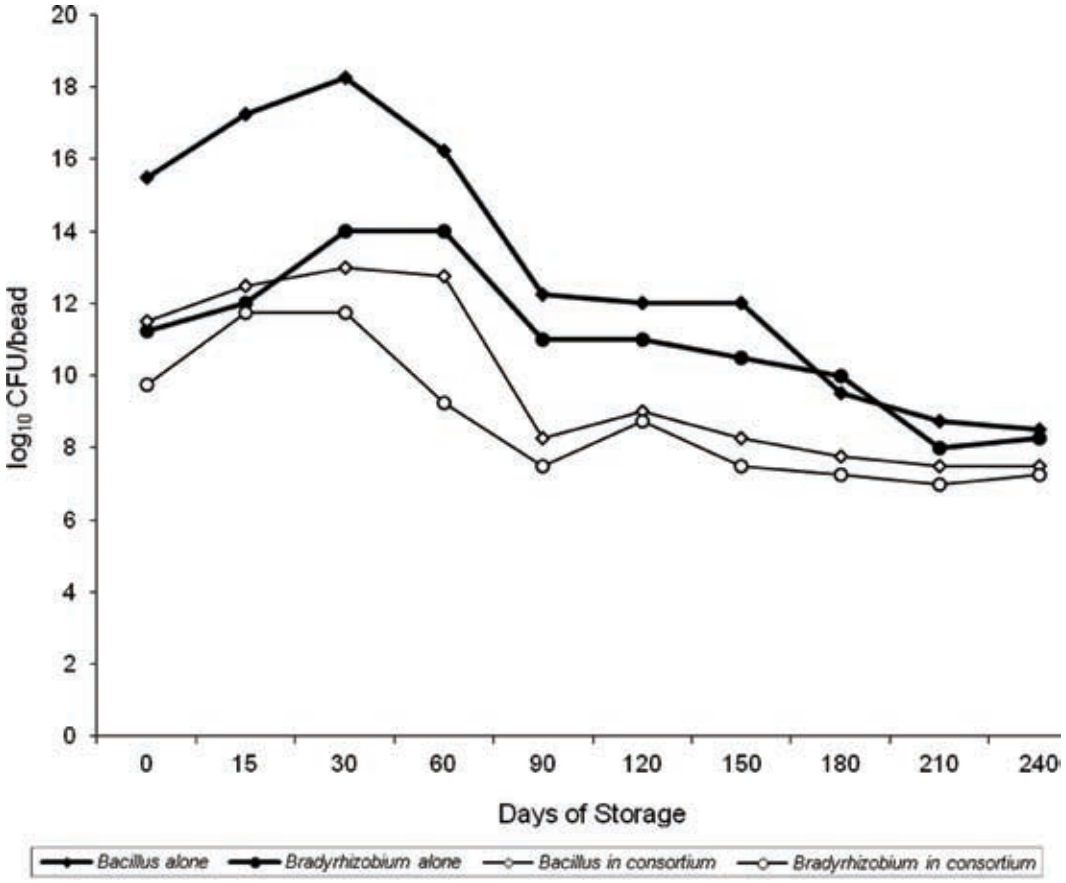

Figure 4: Survival of *Bacillus megaterium* and *Bradyrhizobium* in alginate based microbial consortium.

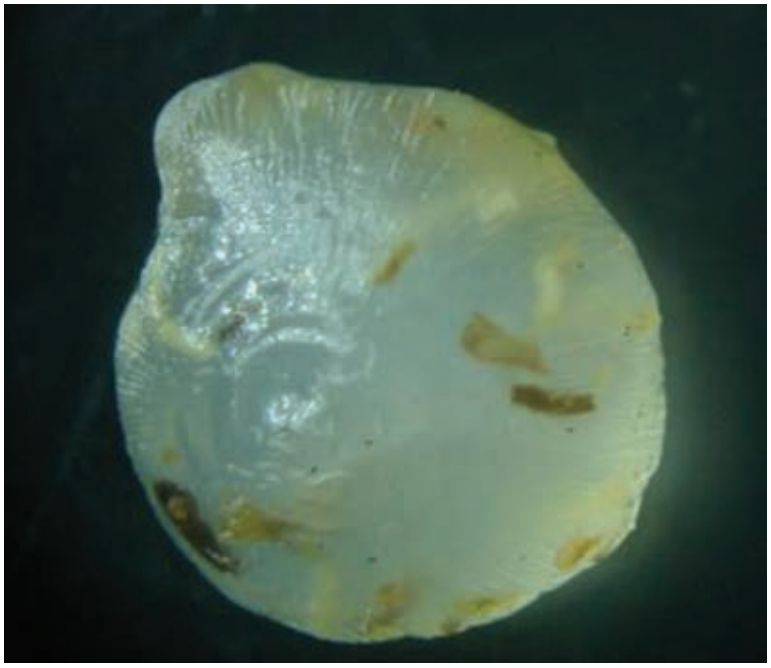

Plate 4: AM fungi colonized corn root bits entrapped in Ca-alginate bead.

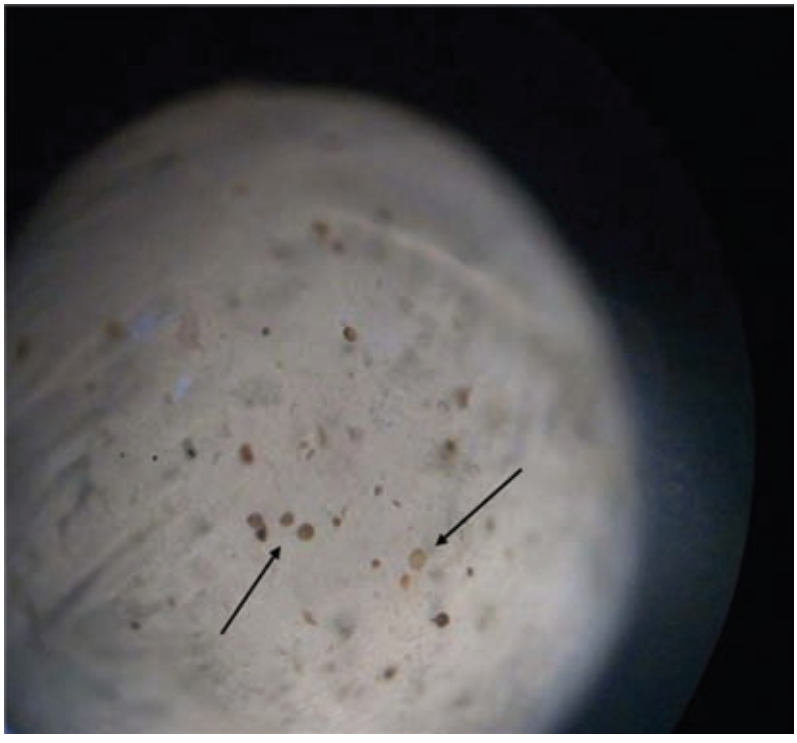

Plate 5: AM fungal spores entrapped in Ca-alginate bead.

a critical velocity so that fluidized state is attained, which facilitate higher contact area for drying in a natural state. A common example of a product from fluid bed dryer is instant coffee powder. FBD is being employed for the manufacture of consortium inoculants of ABMs. In this direction Sahu (personal communication) has constituted a consortium of *Pseudomonas*, *Azotobacter* and *Acinitobacter* in talc employing FBD, and the survival of individual microbes is above the BIS standards at 180 days of storage. Further the contamination is negligible. Lavanya (Personal communication)

Plate 6: Consortia of ABMs (*Azotobacter* sp., *Acinetobacter* sp., *Pseudomonas fluorescence* as well as AM spores and AM fungi infected corn root bits) entrapped in Ca-alginate bead.

has constituted a FBD inoculant from skim milk, gelatin and sugar containing two microbes (*Pseudomonas* and *Acinetobacter*).

Indian agriculture, since 1960s have progressed tremendously due to introduction of high yielding varieties responding to high fertilizer inputs leading to enhanced food grains production. This high input agriculture has also lead to undesirable effects on environment and overall sustainability of farming system such as adverse effects of agrochemicals. Fertilizer contamination of ground water has led to, over a period of time, eutrophication of lake and river water, caused decrease in oxygen content and death of aquatic life, nitrate pollution, increased emission of gaseous nitrogen and metal toxicity. The nitrate toxicity causes health hazards such as birth defects, impaired nervous system, cancer and methaemoglobenemia (blue baby syndrome).

In this context, every unit of chemical fertilizers getting substituted by biofertilizers adds to sustainability and in the long run reduces the hazardous load of chemicals in ecosystem. A rough estimate of the chemical fertilizers that may be substituted by biofertilizers is presented in Table 8.

Future of inoculant technology and its benefits for sustainable agriculture depends on improving inoculant quality and effectiveness. Hence, the challenge is to develop and popularize an inoculant formulation with long shelf life and effective in its response once inoculated, be it seed or soil. There is a need for extensive research to synthesize a new inoculant formulation like freeze dried and fluid bed dried inoculants. More recently mixed microbial inoculants have become popular, hence further research work is required in this area and also appropriate regulations and quality control guidelines are needed.

Table 8: Substitution of chemical fertilizers by biofertilizers.

Sl. no	Biofertilizers	Substitutes/ha/year
1	*Rhizobium*	108.6–217.3 kg of urea,[73]
2	*Azospirillum*	60 kg urea in maize,[19]
3	Azolla	20–40 kg urea/10 T,[73]
4	BGA	54–65 kg urea,[41,42]
5	*Frankia*	195 kg urea,[11]

Calculated based on Kg N fixed × 2.17/ha/year.

Biofertilizers are low cost inputs with high benefits in agriculture. There is a need to popularize this low cost technology with the farming community to reap higher dividends. Biofertilizers supplementing phosphorus nutrition in agriculture may be vital in saving the much needed foreign exchange if we succeed in making the 'fixed' phosphorus available to crops. However, achieving this would be difficult. A concerted effort between soil chemists, microbiologists and agronomists is needed to facilitate judicious use of inorganic and microbiological inputs to realize better yields while ensuring the agriculture remains sustainable.

Acknowledgements

Funding received from National Agricultural Technology Project (NATP) for development of liquid inoculant of *Rhizobium* and Department of Biotechnology (DBT) for development of liquid inoculants of *Azotobacter*, *Azosprillum* and Phosphobacteria (PSB) and consortium of Agriculturally beneficial microorganisms is gratefully acknowledged.

Received 22 February 2012.

References

1. Anonymous: *Handbook of Agriculture*. ICAR, New Delhi. pp. 203–247, (1987).
2. Dixon, R.O.D.; Wheeler, C.T.: *Nitrogen fixation in plants*. Chapman and Hall, New York, (1986).
3. Galloway, J; Raghuram, N; Abrol, Y.P.: 'A perspective on reactive nitrogen in a global, Asian and Indian context'. *Current Science.* 94(11), pp. 1375–1381, (2008).
4. Postgate, J.R.: 'Trends and perspectives in nitrogen fixation research'. *Advances in Microbial Physiology.* 30, pp. 1–22, (1989).
5. Burris, R.H.; Roberts. G.P.: 'Biological nitrogen fixation'. *Annual Review of Nutrition.* 13, pp. 317–335, (1993).
6. Beringer, J.E.; Berwin, A.V.B.; Johnston, Schulman, J.B.; Hopwood, D.A.: 'The *Rhizobium*-legume symbiosis, in the cell as a habitat'. University press, Cambridge. pp. 105–121, (1979).
7. Fred, E.B.; Baldwin, I.L.; McCoy, E.: *Root nodule bacteria and leguminous plants*. Univ. Wisconsin, Madison. U.S.A, (1932).
8. Quispel, A.: *Biology of nitrogen fixation*. North Holland Press, Amsterdum. pp. 748, (1974).
9. Raychaudhuri, S.; Yadav, A.K.; Mousami Raychaudhuri.: 'Changing face of Rhizobium Taxonomy'. *Biofertilizer News letter,* 15(1), pp. 3–10, (2007).
10. Brahmaprakash, G.P.; Hegde, S.V.: 'Nitrogen fixing in Pigeonpea. In: *Advances in Pigeonpea Research*' (eds. M. Ali and Shivkumar). Indian Institute of Pulses Research, Kanpur. pp. 214–228, (2005).
11. Silvester, W.B.: 'Ecological and economical significance of the non- legume symbioses'. In *1st Int. symposium on nitrogen fixation.* (eds. W.E. Newton and C.J. Nyman). Washington state univ. press, Washington, pp. 489–586, (1975).
12. Sundaravarathan, S.; Kannaiyan, S.: 'Influence of *Azolla* and *Sesbania rostrata* application on changes In microbial population and enzymes in rice soils'. *Biotechnology of Biofertilizers* (ed. S. Kannaiyan). pp. 251–225, (2002).
13. Thomas, G.V.: 'Biological nitrogen fixation by asymbiotic and non leguminous symbiotic systems'. In: *Organics in soil health and crop production*, (ed. P.K. Thampan). 'Peekey Tree Crops Development Foundation. pp. 105–124, (1993).
14. Konde, B.K.; Shinde, P.A.: 'Effects of *Azotobacter chroococcum* and *Azospirillum brasilense* inoculation and nitrogen on yield of sorghum, maize, pearl millet and wheat'. In *Proc. Working group meeting cereal nitrogen fixation* (ed. S.P. Wani). 'ICRISAT, Patancheru, India. pp. 85–92, (1986).
15. Shinde, S.T.: 'The role of Azotobacterin in increasing the yield of rice'. *Rice News Letter.* 13, pp. 92–95, (1965).
16. Shinde, S.T.; Rudraksha, G.B.; Rajani Apte and Raut, R.S.: '*Azotobacter* inoculation: nitrogen economy and response of sorghum CSHI. In *Proc. Working group meeting on cereal nitrogen fixation* (ed. S.P. Wani). ICRISAT, pp. 75–76, (1986).
17. Dobereiner, J.: 'Biological Nitrogen fixation in tropics: Social and economic contributions'. *Soil Biology and Biochemistry.* 29(5–6), pp. 771–774, (1997).
18. Bashan, Y.; Holguin, G.; de-Bashan, L.E.: '*Azospirillum*-plant relationships: physiological, molecular, agricultural, and environmental advances'. *Canadian Journal of Microbiology.* 50, pp. 521–577, (2004).
19. Fulcheri, E.; Frioni, D.: '*Azospirillum* inoculation of maize: effects on yield on a field experiment in Central Argentina'. *Biophysics.* 244, pp. 686–691, (1994).
20. Smith, R.L.; Bouton, J.H.; Schank, S.C.; Quesenberry, K.H.; Tyler, H.E.; Milam, J.R.; Gaskins, M.H.; Littell, R.C.: 'Nitrogen fixation in grasses inoculated with *Spirillum lipoferum*'. *Science.* 1093, pp. 1003–1005, (1976).
21. Creus, C.M.; Sueldo, R.J.; Barassi, C.A.: 'Shoot growth and water status in *Azospirillum*-inoculated wheat seedlings grown under osmotic and salt stresses'. *Plant Physiology and Biochemistry.* 35, pp. 939–944, (1997).
22. Alvarez, M.I.; Sueldo, R.J.; Barassi, C.A.: 'Effect of *Azospirillum* on coleoptiles growth in wheat seedlings under water stress'. *Cereal Research Communications.* 24, pp. 101–107, (1996).
23. Creus, C.M.; Sueldo, R.J.; Barassi, C.A.: 'Water relations in *Azospirillum* inoculated wheat seedlings under osmotic stress'. *Canadian Journal of Botany.* 76, pp. 238–244, (1998).
24. Okon, Y.: '*Azospirillum* as a potential inoculant for agriculture'. *Trends in Biotechnology.* 3, pp. 223–228, (1985).
25. Bakanchikova, T.I.; Lobanok, E.V.; Pavlova-Ivanova, L.K.; Redkina, T.V.; Nagapetyan, Z.A.; Majsuryan, A.N.: 'Inhibition of tumor formation process in dicotyledonous plants by *Azospirillum brasilense* strains'. *Mikrobiologiya.* 62, pp. 515–523, (1993).
26. Sudhakar, P.; Gangwar, S.K.; Satpathy, B.; Sahu, P.K.; Ghosh, J.K.; Saratchandra, B.: 'Evaluation of some nitrogen fixing bacteria for control of foliar diseases of mulberry' (*Morus alba*). *Indian Journal of Sericulture.* 39, pp. 9–11, (2000).
27. Bashan, Y.; de-Bashan, L.E.: 'Reduction of bacterial speck *(Pseudomonas syringae* pv. tomato) of tomato by combined treatments of plant growth-promoting bacterium, *Azospirillum brasilense*, streptomycin sulfate, and chemothermal seed treatment'. *European Journal of Plant Pathology.* 108, pp. 821–829, (2002).
28. Bashan, Y.; de-Bashan, L.E.: 'Protection of tomato seedlings against infection by *Pseudomonas syringae* pv.

tomato by using the plant growth-promoting bacterium *Azospirillum brasilense*'. *Applied and Environmental Microbiology.* 68, pp. 2637–2643, (2002).

29. Holguin, G.; Bashan, Y.: 'Nitrogen-fixation by *Azospirillum brasilense* Cd is promoted when co-cultured with a mangrove rhizosphere bacterium (*Staphylococcus* sp)'. *Soil Biology and Biochemistry.* 28, pp. 1651–1660, (1996).
30. Oliveira, R.G.B.; Drozdowicz, A.: 'Inhibition of bacteriocin producing strains of *Azospirillum lipoferum* by their own bacteriocin'. *Zentralblatt für Mikrobiologie.* 142, pp. 387–391, (1987).
31. Tapia-Hernández, A.; Mascarúa-Esparzá, M.A.; Caballero-Mellado, J.: 'Production of bacteriocins and siderophore-like activity in *Azospirillum brasilense*'. *Microbios.* 64, pp. 73–83, (1990).
32. Shah, S.; Karkhanis, V.; Desai, A.: 'Isolation and characterization of siderophore, with antimicrobial activity, from *Azospirillum lipoferum* M'. *Current Microbiology,* 25, pp. 34–35, (1992).
33. Somers, E.; Ptacek, D.; Gysegom, P.; Srinivasan, M.; Vanderleyden, J.: '*Azospirillum brasilense* produces the auxin-like phenylacetic acid by using the key enzyme for indole-3-acetic acid biosynthesis'. *Applied and Environmental Microbiology.* 71, pp. 1803–1810, (2005).
34. Jimnez-Salgado, T.; Fuentes-Ramirez, L.E.; Tapia-Herandez, A.; Mascarua, M.A.; Martinez-Romero, E.; Caballero-Mellado, J.: '*Coffea Arabica L.;* a new host plant for *Acetobacter diazotrophicus* and isolation of other nitrogen fixing cyanobacteria'. *Applied and Environmental Microbiology.* 63, pp. 3676–3683, (1997).
35. Loganathan. P.; Sunitha. R.; Parida. A.K.; Nair. S.: 'Isolation and characterization of two genetically distant groups of *Acetobacter diazotrophicus* from a new host plant *Eleusine coracana L.' Applied Microbiology.* 87, pp. 167–172, (1999).
36. Herandez, A.T.; Bustilos-cristales, M.R.; Jimnez-salgado, T.; Caballero-Mellado, J.; Fuentez-Ramirez, L.E.: 'Natural endophytic occurrence of *Acetobacter* diazotrophicus in pineapple plants'. *Microbial Ecology.* 39, pp. 49–55, (2000).
37. Loganathan. P. and Nair. S.: 'Crop-specific endophytic colonization by a novel, salt tolerant , nitrogen fixing and phosphate solubilizing Gluconacetobacter sp from wild rice'. *Biotechnology letter.* 25, pp. 497–501, (2003).
38. Saravanan, V.S.; Madhaiyan, M.; Osborne, Jabez.; Thangaraju, M.; Sa, T.M.: 'Ecological Occurrence of *Gluconacetobacter diazotrophicus* and Nitrogen-fixing Acetobacteraceae Members: Their Possible Role in Plant Growth Promotion'. *Microboial Ecology.* 55, pp. 130–140, (2008).
39. Dobereiner, J.: 'Biological Nitrogen fixation in tropics: Social and economic contributions'. *Soil Biology and Biochemistry*. 29(5, pp. 6), pp. 771–774, (1997).
40. Meenakshisundaram, M.; Karrupagnaniar Santhaguru.: 'Isolation and nitrogen fixing efficiency of a novel endophytic diazotroph gluconacetobacter diazotrophicus associated with *Saccharum officinarum* from southern districts of Tamilnadu'. *International Journal of Biology and Medical Research. 1*(4), pp. 298–300, (2010).
41. Goyal, S.K.; Venkataraman, G.S.: 'Effects of algalization on high yielding rice varieties.II. Response of soil types'. *Phykos.* 10, pp. 32–38, (1971).
42. Venkataraman, G.S.: 'Blue green algae for rice production—A manual for its production'. F.A.O. *Soil Bulletin.* 46, pp. 102, (1981).
43. Kaushik, B.D.; Subhasini, D.: 'Amelioration of salt affected soils with BGA. II. Improvement of soil properties'. *Proceding of Natural Science Academy.* 51B, pp. 386–389, (1995).
44. Rath, B.; adhikary, S.P.: 'Toxicity of Furadan to several nitrogen fixing cynobacteria from rice field of coastal Orissa, India'. *Tropical Agriculture Trinidad.* 72, pp. 80–84, (1995).
45. Padhi, H.; Rath, B.; Adhikary,S.P.: 'Tolerance of nitrogen fixing cynobacteria to NaCl'. *Biology of Plant.* 40, pp. 262–268, (1997).
46. Adhikary, S.P.; Sahu, J.K.: 'Studies on the establishment and nitrogenase activity of inoculated cynobacteria on the field and their effect on yield of rice'. *Oryza.* 37, pp. 39–43, (2000).
47. Goldstein, A.H.: 'Bacterial solubilization of mineral phosphates: historical perspectives and future prospects'. *American Journal of Alternate Agriculture.* 1, pp. 57–65, (1986).
48. Kucey, R.M.N.: 'Phosphate-solubilizing bacteria and fungi in various cultivated and virgin Alberta soils'. *Canadian Journal of Soil Science.* 63, pp. 671–678, (1983).
49. Goldstein, A.H.: 'Recent progress in understanding the molecular genetics and biochemistry of calcium phosphate solubilization by Gram negative bacteria'. *Biology, Agriculture and Horticulture.* 12, pp. 185–193, (1995).
50. Kim, K.Y.; Jordan, D.; Krishnan, H.B.: '*Rahnella aquali-tis*, a bacterim isolated from soybean rhizosphere, can solubilize hydroxyapatite'. *FEMS Microbiology Letter.* 153, pp. 273–277, (1997).
51. Rodríguez, H.; R. Fraga.: 'Phosphate solubilizing bacteria and their role in plant growth promotion'. *Advances in Biotechnology.* 17, pp. 319–339, (1999).
52. Suh, J.S.; S.K. Lee, K.S. Kim, and K.Y. Seong.: 'Solubilization of Insoluble Phosphates by Pseudomonas putida, Penicillium sp.; Aspergillus niger Isolated from Korean Soils'. *Journal of Korean Society of Soil Science and Fertilizer.* 28:3, pp. 278–286, (1995).
53. Whitelaw M.A.; Harden, R.J.; Helyar, K.R.: 'Phosphate solubilisation in culture by the soil fungus *Penicillium radicum*'. *Soil biology and biochemistry.* 31, pp. 655–665. (1999).
54. Chen Y.P.; Rekha, P.D.; Arun, A.B.; Shen, F.T. Lai, W.; Young, C.C.: 'Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities'. *Applied Soil Ecology.* 34, pp. 33–41, (2006).
55. Saber, M.S.M.; Yousry, M.; Kabesh, M.O.: 'Effect of manganese application on the activity of phosphate-dissolving bacteria in a calcareous soil cultivated with pea plants'. *Plant and Soil.* 47, pp. 335–339, (1977).
56. Raj. J.; Bagyaraj D.J.; Manjunath. A.: 'Influence of soil inoculation with vesicular-arbuscular mycorrhiza and a phosphate

dissolving bacterium on plant growth and ^{32}P-uptake'. *Soil Biology and Biochemistry.* 13, pp. 105–108, (1981).

57. Gaind, S.; Gaur, A.C.: 'Thermotolerant phosphate solubilizing microorganisms and their interactions in mungbean'. *Plant and Soil.* 133, pp. 141–149, (1991).
58. Verma, L.N.: 'Biofertiliser in agriculture'. In: *Organics in soil health and crop production* (ed. P.K. Thampan). Peekay Tree Crops Development Foundation, Cochin, India. pp. 152–183, (1993).
59. Goenadi, D.H.; Siswanto and Sugiarto,Y.: 'Bioactivation of poorly soluble phosphate rocks with a phosphate solubilizing fungus'. *Soil Science Society of America Journal*. 64, pp. 927–932, (2000).
60. Cabello, M.; Irrazabal, G.; Bucsinszky A.M.; Saparrat M.; and Schalamuck. S.: 'Effect of an arbuscular mycorrhizal fungus, *G. mosseae* and a rock-phosphate-solubilizing fungus, *P. thomii* in *Mentha piperita* growth in a soil less medium'. *Journal of Basic Microbiology.* 45, pp. 182–189, (2005).
61. Kucey, R.M.N.; H.H. Janzen and Legget, M.E.: 'Microbial mediated increase in plant available phosphorus'. *Advances in Agronomy.* 42, pp. 199–228, (1989).
62. Ponmurugan, P.; Gopi, C.: 'Distribution pattern and screening of phosphate solubilizing bacteria isolated from different food and forage crops'. *Journal of Agronomy.* 5, pp. 600–604, (2006).
63. Kumara swamy, C.A.; Raghunandan, B.L.; Chandrashekhar, M.; Brahmaprakash, G.P.: 'Bio-activation of rock phosphate vis- a- vis seed treatment with phosphorus solubilizing microbes (PSM) in enhancing P nutrition in cowpea and ragi'. *Indian Journal of Science and Technology.* 3(7), pp. 689–692, (2010).
64. Gaur, A.C.: 'Phosphate solubilizing microorganisms as bio-fertilizers'.Omega scientific publishers, New Delhi. pp. 176, (1990).
65. Hebbara, H.; Suseeladevi, L.: 'Effect of phosphate solubilizing bacteria on P availability to groundnut from rock phosphate'. *Current Research. 19*, pp. 56–57, (1990).
66. Kole, S.C.; Hajra, J.N.M.: 'Mineral phosphate solubilization with reference to horticultural crops'. *Indian Agriculturist.* 43, pp. 97–105, (1999).
67. Tanwar, S.P.S.; Sharma, G.L.; Chahar, M.S.: (2003). 'Effect of phosphorus and bio-fertilizers on yield, nutrient content and uptake by black gram (Vigna mungo (L.) Hepper). '*Legume Research* 35, pp. 67–76.
68. Olsson, P.A.; Thingstrup, I.; Jakobsen, I.; Baath, E.: 'Estimation of the biomass of arbuscular mycorrhizal fungi in a linseed field'. *Soil Biology and Biochem*istry. 31, pp. 1879–1887, (1999).
69. Zhu, Y.G.; Miller, R.M.; 'Carbon cycling by arbuscular mycorrhizal fungi in soil—plant systems'. *Trends Plant Sciences.* 8, pp. 407–409, (2003).
70. Lovelock, C.E.; Wright, S.F.; Clark, D.A.; Ruess, R.W.: 'Soil stocks of glomalin produce by arbuscular mycorrhizal fungi across a tropical rain forest landscape'. *Journal of Ecol*ogy. 92, pp. 278–287, (2004).
71. Rillig, M.C.; Wright, S.F.; Nichols, K.A.; Schmidt, W.F.; Torn, M.S.: 'Large contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils'. *Plant and Soil.*233, pp. 167–177, (2001).
72. McGonigle, T.P.; Miller, M.H.: 'Winter survival of extra radical hyphae and spores of arbuscular mycorrhizal fungi in the field'. *Applied Soil Ecology.* 12, pp. 41–50, (1999).
73. Mahdi,S.S.; Hassan, G.I.;Samoon S.A.; Rather, H.A.; Dar, Showkat A.; Zehra, B.: 'Bio-Fertilizers In Organic Agriculture'. *Journal of Phytology.* 2(10), pp. 42–54. (2010).
74. Kasiamdar, R.S.; Smith, S.E.; Smith, F.A.; Scott, E.S.: 'Influence of the mycorrhizal fungus, *Glomus coronatum*, and soil phosphorus on infection and disease caused by binucleate Rhizoctonia and *Rhizoctonia solani* on mung bean (*Vigna radiata*)'. *Plant and Soil*.238, pp. 235–244, (2001).
75. Kloepper, J.W.: 'Plant growth promoting rhizobacteria as biological control agents'. In: *Soil Microbial Ecology*. *Applications in Agricultural and Environmental Management* (ed. F.B. Metting Jr.). 'Marcel Dekker, New York, USA, pp. 255–274, (1992).
76. Mishra, M.; Umesh Kumar.; Mishra,P.K.; Veeru Prakash.: 'Efficiency of plant growth promoting rhizobacteria for the enhancement of *Cicer arietinum L*. growth and germination under salinity'. *Advances in Biological Research. 4*(2), pp. 92–96, (2010).
77. Gholami, A.; Shahsavani, S.; Nezarat. S.: 'The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth and yield of maize'. *World Academy of Science, Engineering and Technology.* 49, (2009).
78. Suslov, T.V.: 'Role of root-colonizing bacteria in plant growth. In: *Phytopathogenic Prokariotes*' (eds. M.S. London Mount and G.H. Lacy). Academic Press. pp. 187–223, (1982).
79. Kloepper, J.W.: 'Plant growth promoting bacteria (other systems). 'In: *Azospirillum/Plant Association*' (ed. J. Okon). 'Boca Raton, FL: CRC Press. pp. 137–154, (1994).
80. Kloepper, J.W.: 'Plant growth promoting rhizobacteria as biological control agents'. In: *Soil Microbial Ecology*. *Applications in Agricultural and Environmental Management* (ed. F.B. Metting Jr.). 'Marcel Dekker, New York, USA, pp. 255–274, (1992).
81. Brown, M.E.: 'Seed and root bacterization'. *Annual Review of Phytopathology.* 12, pp. 181–97, (1974).
82. Glick, B.R.: 'The enhancement of plant growth by free-living bacteria'. *Canadian Journal of Microbiology,* 41, pp. 109–117, (1995).
83. Whipps, J.M.: 'Microbial interactions and biocontrol in the rhizosphere'. *Journal of Experimental Botany.* 52 (Suppl 1), 487–511, (2001).
84. Defago, G.: '2,4-Diacetylphloroglucinol, a promising compound in biocontrol'. *Plant Pathology.* 42, pp. 311–312, (1993).
85. Chernin, L.; Chet, I.: 'Microbial enzymes in biocontrol of plant pathogens and pests' In R.G. Burns and R.P. Dick (ed.), Enzymes in the environment: activity, ecology, and applications. Marcel Dekker, New York. pp. 171–225, (2002).

86. Frankowski, J.; Lorito, M.; Scala, F.; Schmidt, R.; Berg, G.; Bahl, H.: 'Purification and properties of two chitinolytic enzymes of *Serratia plymuthica* HRO-C48'. *Archives of Microbiology.* 176, pp. 421–426, (2001).
87. Bloemberg, G.V.; Lugtenberg, B.J.J.: 'Molecular basis of plant growth promotion and biocontrol by rhizobacteria'. *Current Opinion in Plant Biology.* 4, pp. 343–350, (2001).
88. Peer, Van, R.; Niemann, G.J.; Schippers. B.: 'Induced resistance and phytoalexin accumulation in biological control of *Fusarium* wilt of carnation by *Pseudomonas sp.* strain WCS417r'. *Phytopathology.* 81, pp. 728–734, (1991).
89. Wei, L.; Kloepper, J.W.; Tuzun, S.: 'Induction of systemic resistance of cucumber to *Colletotrichum orbiculare* by selected strains of plant growth-promoting rhizobacteria'. *Phytopathology.* 81, pp. 1508–1512, (1991).
90. Benizri, E.; Baudoin, E.; Guckert, A.: 'Root colonization by inoculated plant growth promoting rhizobacteria'. *Biocontrol Science and Technology.* 11, pp. 557–574, (2001).
91. Fages, J.: 'An industrial view of *Azospirillum* inoculants: Formulation and application technology'. *Symbiosis.*13, pp. 15–26, (1992).
92. Bashan, Y.: 'Inoculants for plant growth promoting bacteria for use in agriculture'. *Advances in Biotechnol*ogy. 16, pp. 729–770, (1998).
93. Xavier, I.J.; Holloway, G.; Leggett, M.: 'Development of rhizobial inoculant formulations'. In *Proceedings of The Great Plains Inoculant Forum.* Plant Management Network, Saskatoon, Saskatchewan. (2004).
94. Wani, S.P.; Lee, K.K.: 'Biofertilizers for sustaining cereal crop production. In *Biotechnology of Biofertilizers*' (ed. S. Kannaiyan). 'Narosa publishing House, New Delhi. pp. 50–64, (2002).
95. Wani, S.P.; Lee, K.K.: 'Role of biofertilizers in upland crop production'. In *Fertilizers of organic manures, Recycle waste and biofertilizers*, (ed. H.L.S. Tandon). 'Fertilizer development and Consultaion Organization, New Delhi, pp. 91–112, (1991)
96. Smith, R.S.: 'Legume inoculant formulation and application'. *Canadian Journal of Microbiology*. 38, pp. 485–492, (1992).
97. Chao, W.L.; Alexander, M.: 'Mineral soils as carriers for *Rhizobium* inoculants'. *Applied and Environmental Microbiology*. 47, pp. 94–97, (1984).
98. Dube, J.N.; Mahere, D.P.; Bawat, A.F.: 'Development of coal as a carrier for rhizobial inoculants'. *Science and Culture*, 46, pp. 304, (1980).
99. Kandasamy, R.; Prasad, N.N.: 'Lignite as a carrier of rhizobia'. *Current Science.* 40, pp. 496, (1971)
100. Iswaran, V.; Sen, A.; Apte, R.: 'Plant compost as a substitute for peat for legume inoculants'. *Current Science.* 41, pp. 299, (1972).
101. Jackson, A.M.; Whipps, J.M.; Lynch, J.M.: 'Production, delivery systems and survival in soil of four fungi with disease biocontrol potential'. *Enzyme Microbiology and Technology.* 13, pp. 636–642, (1991).
102. Philip, K.; Jauhri, K.S.: 'Pressmud: a potential carrier for *Rhizobium* and *Azotobacter* 1. Comparative analytical studies of various carrier materials'. *Z. Mikrobiol.* 139, pp. 5–4, (1984).
103. Sadasivam, K.V.; Tyagi, R.K.; Ramarethinam, S.: 'Evaluation of some agricultural wastes as carriers for bacterial inoculants'. *Agricultural Wastes.* 17, pp. 301–306, (1986).
104. Bahl, N.; Jauhri, S.: 'Spent mushroom compost as a carrier for bacterial inoculant production'. In *Proceedings of the International symposium on scientific and technological aspects of cultivating edible fungi.* The Pennsylvania State University, University Park, PA. pp. 63–68, (1986).
105. Paau, A.S.; Graham, L.L.; Bennett, M.: 'Progress in formulation research for PGPR and biocontrol inoculants'. *In Plant Growth-Promoting Rhizobacteria-Progress and Prospects* (C. Keel, B. Koller, and G. Défago Eds.). 'IOBC/WPRS Bulletin, Zurich, Switzerland, pp. 399–403, (1991).
106. Sparrow, S.D.; Ham, G.E.: 'Survival of *Rhizobium phaseoli* in six carrier materials'. *Journal of Agronomy.* 75, pp. 181–184, (1983).
107. Dommergues, Y.R.; Diem, H.G.; Divies, C.: 'Polyacrylamide entrapped *Rhizobium* as an inoculant for legumes'. *Applied and Environmental Microbiology*. 37, pp. 779–981, (1979).
108. Bashan, Y.: 'Alginate beads as synthetic inoculant carriers for the slow release of bacteria that affect plant growth'. *Applied and Environmental Microbiology*. 51, pp. 1089–1098, (1986).
109. Jung, G.; Mugnier, J.; Diem, H.G.; Dommergues, Y.R.: 'Polymer-entrapped *Rhizobium* as an inoculant for legumes'. *Plant and Soil.* 65, pp. 219–231, (1982).
110. Walter, J.F.; Paau, A.S.: 'Microbial inoculant production and formulation'. In *Soil Microbial Ecology,* (ed. F.B. Metting, Jr.). Marcel Dekker Inc. NY. pp. 579–594, (1993).
111. Van Elsas, J.D.; Heijnen, C.E.: 'Methods for the introduction of bacteria into soil: A review'. *Biology and Fertility of Soils.* 10, pp. 127–133, (1990).
112. Huber, D.M.; El-Nasshar, L.; Moore, H.W.; Mathre, D.E.; Wagner, J.E.: 'Interaction between a peat carrier and bacterial seed treatments evaluated for biological control of the take –all diseases of wheat (*Triticum aestivum* L.)'. *Biology and Fertility of Soils.* 8, pp. 166–171, (1989).
113. Olsen, P.E.; Rice, W.A.; Bordeleau, L.M.; Biederbeck, V.O.: 'Analysis and regulation of legume inoculants in Canada: the need for an increase in standards'. *Plant and Soil.* 161, pp. 127–134, (1994).
114. Olsen, P.E.; Rice, W.A.; Collins, M.M.: 'Biological contaminants in North American legume inoculants'. *Soil Biology and Biochem*istry. 27, pp. 699–701, (1994).
115. Smith, R.S.: 'Inoculant formulations and applications to meet changing needs'. In *Nitrogen fixation: Fundamentals and Applications* (I.A. Tikhonovich, N.A. Provorov, V.I. Romanov and W.E. Newton Eds). Kluwer Academic Publishers, Dordrecht, The Netherlands, (1995).
116. Tittabutr, P.; Payakapong, W.; Teaumroong, N.; Singleton, P.W.; Boonkerd, N.: 'Growth, survival and

field performance of bradyrhizobial liquid inoculant formulations with polymeric additives'. *Science Asia.* 33, pp. 69–77, (2007).

117. Deaker, R.; Roughley, R.J.; Kennedy, I.R.: 'Legume seed inoculation technology- a review'. *Soil Biology and Biochemistry.* 36, pp. 75–88, (2004).
118. Mugnier, J.; Jung, G.: 'Survival of bacteria and fungi in relation to water activity and solvent properties of water in biopolymer gels'. *Applied and Environmental Microbiology*. 50, pp. 108–114, (1985).
119. Singleton, P.; Keyser, H.; Eve Sande.: 'Development and evaluation of liquid inoculants'. In *Inoculants and nitrogen fixation of legume in Vietnam,* ACIAR Proceedings (D. Herridge Ed). pp. 52–66, (2002).
120. Ali, F.S.; Loynachan, T.E.: 'Inhibition of *Bradyrhizobium japonicum* by diffusates from soybean seed'. *Soil Biology and Biochemistry.* 22(7), pp. 973–976, (1990).
121. Mary, P.; Ochin, D.; Tailliez, R.: 'Rates of drying and survival of *Rhizobium meliloti* during storage at different relative humidities'. *Applied Environmental Microbiology*. 50, pp. 207–211, (1985).
122. Al-Rashidi, R.K.; Loynachan, T.E.; Frederick, L.R.: 'Desiccation tolerance of four strains of *Bradyrhizobium japonicum*'. *Soil Biology and Biochemistry*. 14, pp. 489–493, (1982).
123. Lippert, K.; Galinski, E.A.: 'Enzyme stabilization by ectoine type compatible solutes: protection against heating, freezing and drying'. *Applied Microbiology and Biotechnol*ogy. 37, pp. 61–65, (1992).
124. Streeter, J.G.: 'Accumulation of *alpha*, *alpha*-trehalose by *Rhizobium* bacteria and bacteroids'. *Journal of Bacteriology.* 164, pp. 78–84, (1985).
125. Bushby, H.V.A.; Marshall, K.C.: 'Water status of rhizobia in relation to their susceptibility to desiccation and to their protection by montmorillonite'. *Journal of General Microbiology*. 99, pp. 19–28, (1977).
126. Vincent, J.M.; Thompson, J.A.; Donovan, K.O.: 'Death of root nodule bacteria on drying'. *Australian Journal of Agriculture Research. 1*3, 258, (1962).
127. Girisha, H.C.; Brahmaprakash, G.P.; Mallesha, B.C.: 'Effect of osmoprotectant (PVP-40) on survival of *Rhizobium* in different inoculants formulation and nitrogen fixation in cowpea'. *Geobios*. 33, pp. 151–156, (2006).
128. Dayamani, K.J.: 'Formulation and determination of effectiveness of liquid inoculants of plant growth promoting rhizobacteria'. *PhD Thesis*, University of Agricultural Sciences, Bangalore, India, (2010).
129. Sridhar, V.; Brahmaprakash, G.P.; Hegde, S.V.: 'Development of a liquid inoculant using osmoprotectants for Phosphate solubilizing bacteria'. *Karnataka Journal of Agriculture Science.* 17, pp. 251–257, (2004).
130. Vithal Navi.: 'Development of Liquid Inoculant Formulations for *Bradyrhizobium* sp.: (*Arachis*), *Azospirillum lipoferum* and *Azotobacter chroococcum*'. Ph.D. Thesis, University of Agricultural Sciences, Bangalore, (2004).
131. Velineni, S.; Brahmaprakash, G.P.: Survival and phosphate solubilizing ability of *Bacillus megaterium* in liquid inoculants under high temperature and desiccation stress'. *Journal of Agricultural Science and Technology.* 13, 795–802, (2011).
132. Brahmaprakash, G.P.; Girisha, H.C.; Vithal Navi.; Laxmipathy, R.; Hegde, S.V.: 'Liquid *Rhizobium* inoculant formulations to enhance biological nitrogen fixation in food legumes'. *Journal of Food Legumes.* 20, pp. 75–79, (2007).
133. Saxena, D.; Mohammed, A.; Khanna, S.: 'Modulation of protein profiles in *Rhizobium* sp under salt stress'. *Canadian Journal of Microbiology.* 42, pp. 617–620, (1996).
134. Apte, S.K.; Bhagwat, A.A.: 'Salinity-stress-induced proteins in two nitrogen fixing *Anabaena* strains differentially tolerant to salt'. *Journal of Bacteriology.* 171, pp. 909–915, (1989).
135. Munchbach, M.; Nocker, A and Narberhaus, F.: 'Multiple small heat shock proteins in rhizobia'. *Journal of Bacteriol*ogy. 181, pp. 83–90, (1999).
136. Hynes, R.K.; Jans, D.C.; Bremer, E.; Lupwayi, N.Z.; Rice, W.A.; Clayton, G.W.; Collins, M.M.: '*Rhizobium* sp. population dynamics in the pea rhizosphere of rhizobial inoculant strain applied in different formulations'. *Canadian Journal of Microbiology*. 47, pp. 595–600, (2001).
137. Jung, G.; Mugnier, J.; Diem, H.G.; Dommergues, Y.R.: 'Polymer-entrapped *Rhizobium* as an inoculant for legumes'. *Plant and Soil.* 65, pp. 219–231, (1982).
138. Bashan, Y.; Carrillo, A.: 'Bacterial inoculants for sustainable agriculture'. In *New horizons in agriculture: Agroecology and sustainable development*. Proceedings of the 2nd international symposium on agroecology, sustainable agriculture and education (J. Pérez-Moreno and R. Ferrera-Cerrato Eds). Colegio de Postgraduados en ciencias agricolas, Montecillo, Mexico. pp. 125–155, (1996).
139. Digat, B.: 'A new encapsulation technology for bacterial inoculants and seed bacterization. In *Plant Growth-Promoting Rhizobacteria- Progress and Prospects* (C. Keel, B. Koller, and G. Défago Eds). 'IOBC/WPRS Bulletin, Zurich, Switzerland, pp. 383–391, (1991).
140. DeLucca, A.J.; Connick, W.J. Jr.; Fravel, D.R.; Lewis, J.A.; Bland, J.M.: 'The use of bacterial alginates to prepare biocontrol formulations'. *Journal of Industrial Microbiology*. 6, pp. 129–134, (1990).
141. Smidsrod, O.; Skjak-Braek, G.: 'Alginate as immobilization matrix for cells'. *Trends in Biotechnology*. 8, pp. 71–78, (1990).
142. Kitamikado, M.; Yamaguchi, K.; Tseng, C.H.; Okabe, B.: 'Methods designed to detect alginate-degrading bacteria'. *Applied and Environmental Microbiology*. 56, pp. 2939–2940, (1990).
143. Bashan, Y.; Levanony, H.; Ziv-Vecht O.: 'The fate of field-inoculated *Azospirillum brasilense* Cd in wheat rhizosphere during the growing season'. *Canadian Journal of Microbiology*. 33, pp. 107, (1987).

144. Hegde, S.V.; Brahmaprakash, G.P.: 'A dry granular inoculant of *Rhizobium* for soil application'. *Plant and Soil.* 144, pp. 309–311, (1992).
145. Ganry, F.; Diem, H.G.; Dommergues, Y.R.: 'Effect of inoculation with *Glomus mosseae on* nitrogen fixation by field grown soybeans'. *Plant and Soil.* 68, pp. 321–329, (1982).
146. Marx, D.H.; Kenney, D.S.: 'Production of ectomycorrhizal fungus inoculum'. *In Methods and Principles of Mycorrhizal Research,* (N.C. Schenck Ed). The American Phytopathological Society, St. Paul, Minn, pp. 131–146, (1982).
147. Le Tacon, F.; Jung, G.; Mugnier, J.; Michelot, P.; Mauperin, C.: 'Efficiency in a forest nursery of an ectomycorrhizal fungus inoculum produced in a fermentor and entrapped in polymeric gels'. *Canadian Journal of Botany.* 63, pp. 1664–1668, (1985).
148. Sougoufara, B.; Diem, H.G.; Dommergues, Y.R.: 'Response of field -grown *Casuarina equisetifolia* to inoculation with *Frankia* strain ORS 021001 entrapped in alginate beads'. *Plant and Soil.* 118, pp. 133–137, (1989).
149. Fravel, D.R.; Marois, J.J.; Lumsden, R.D.; Connick, W.J.: 'Encapsulation of potential biocontrol agents in an alginate-clay matrix'. *Phytopathology.* 75, pp. 774–777, (1985).
150. Lewis, J.A.; Papavizas, G.C.: 'Characteristics of alginate pellets formulated with *Trichoderma* and *Gliocladium* and their effect on the proliferation of the fungi in the soil'. *Plant Pathology.* 34, pp. 571–577, (1985).
151. Nethravathi, C.S.; Brahmaprakash,G.P.: 'Alginate based composite biofertilizer of *Bradyrhizobium japonicum* and *Bacillus megaterium* for soybean (*Glycine max* (L) Merrill)'. *Journal of Soil Biology and Ecol*ogy. 25, pp. 1–13, (2005).
152. Bagyaraj, D.J.; Mehrotra, V.S.; Suresh, C.K.: 'Vesicular arbuscular mycorrhizal biofertilizer for tropical forest plants'. In *Biotechnology of Biofertilizers,* (ed. S. Kannaiyan). Narosa publishing House, New Delhi, pp. 299–311, (2002).
153. Napamornbodi, O.; Rajanasiriwong, W.; Thamsurakul, S.: 'Production of VAM fungi, *Glomus intraradices* and *G. mosseae* in tissue culture'. In *mycorrhiza for green Asia.* Univeristy of Madras, Tamil Nadu, pp. 351–376, (1988).
154. Cooper, A.J.: 'Crop production recirculating nutrient solution'. *Scientia Horticulturae.* 3, pp. 35–38, (1985).
155. Yadav, A.K.: 'Glimpses of Fertilizer (control) order for biofertilizers (amendment, November 2009)'. *Biofertilizer Newsletter.* 17(2), pp. 11–14, (2009).
156. Bashan, Y.; Holguin, G.: '*Azospirillum*-plant relationships: environmental and physiological advances (1990–1996)'. *Canadian Journal of Microbiology*. 43, pp. 103–121, (1997).
157. Alagawadi, A.R.; Gaur, A.C.: 'Inoculation of *Azospirillum brasilense* and phosphate solubilizing bacteria on yield of sorghum [*Sorghum bicolor* (L.) Moench] in dry land'. *Tropical Agriculture.* 69, pp. 347–350, (1992).
158. Belimov, A.A.; Kojemiakov, A.P.; Chuvarliyeva, C.V.: 'Interaction between barley and mixed cultures of nitrogen fixing and phosphate-solubilizing bacteria'. *Plant and Soil* 173, pp. 29–37, (1995).
159. Andreeva, I.N.; Red'kina, T.V.; Ismailov, S.F.: 'The involvement of indole acetic acid in the stimulation of *Rhizobium*-legume symbiosis by *Azospirillum brasilense*'. *Russian Journal of Plant Physiology.* 40, pp. 901–906, (1993).
160. Itzigsohn, R.; Kapulnik, Y.; Okon, Y.; Dovrat, A.: 'Physiological and morphological aspects of interactions between *Rhizobium meliloti* and alfalfa (*Medicago saliva*) in association with *Azospirillum brasilense*'. *Canadian Journal of Microbiology.* 39, pp. 610–615, (1993).
161. Bagyaraj, D.J.: 'Biological interaction with VA mycorrhizal fungi'. In *VA Mycorrhiza* (eds. C.L. Powell and D.J. Bagyaraj). CRC Press, USA, pp. 131–153, (1984).
162. Garbaye, L.: 'Helper bacteria: A new dimension to the mycorrhizal symbiosis'. *New Phytology.* 128, pp. 197–210, (1994).
163. Li, C.Y.; Huang, L.L.: 'Nitrogen fixing (acetylene reducing) bacteria associated with ectomycorrhizas of Douglas –fir'. *Plant and Soil.* 98, pp. 425–428, (1987).
164. Li, C.Y.; Massicote, H.B.; Moore, L.V.H.: 'Nitrogen-fixing *Bacillus* sp. associated with Douglas -fir tuberculate ectomycorrhizae'. *Plant and Soil.* 140, pp. 35–40, (1992).
165. Singh, C.S.; Amawate, J.S.; Tyagi, S.P.; Kapoor, A.: 'Interaction effect of *Glomus fasciculatum* and *Azospirillum brasilense* on yields of various genotypes of wheat (*Triticum aestivum*) in pots'. *Zentralblatt fur Mikrobiologie*. 145, pp. 203–208, (1990).
166. Woomer, P.; Bennet, J.; Yost, R.: 'Overcoming the inflexibility of most probable number procedures'. *Journal of Agronomy.* 82, pp. 349–353, (1990).
167. Olsen, P.E.; Rice, W.A.: '*Rhizobium* strain identification and quantification in commercial inoculants by immunoblot analysis'. *Applied and Environmental Microbiology*. 55, pp. 520–522, (1989).
168. Olsen, P.E.; Rice, W.A.; Steinke, G.W.; Page, W.J.: 'Strain-specific serological techniques for the identification of *Rhizobium meliloti* in commercial alfalfa inoculants'. *Canadian Journal of Microbiology*. 29, pp. 225–230, (1983).
169. Rice, W.A.; Olsen, P.E.: 'Soil inoculants for alfalfa grown on moderately acid soil'. *Communication in Soil Science and Plant Analysis.* 19, pp. 947–956, (1988).
170. Thompson, J.A.: 'Australian quality control and standards'. In *Report of the Expert Consultation on Legume Inoculant Production and Quality Control*, (ed. J.A. Thompson). 'Food and Agriculture Organization of the United Nations, Rome, pp. 107–111, (1991).
171. Scaglia, J.A.: 'Production and quality control of inoculants in Rwanda'. In *Report of the Expert Consultation on Legume Inoculant Production and Quality control, (*ed. J.A.Thampson). 'FAO, Rome, pp. 61–69, (1991).
172. Brockwell, J.; Gault, R.R.; Herridge, D.F.; Morthorpe, L.J.: 'Studies on alternative means of legume inoculation: microbiological and agronomic appraisals of commercial producers for inoculating soybeans with *Bradyrhizobium*

japonicum'. *Australian Journal Agricultural Research.* 39, pp. 965–972, (1988).

173. Shanker, S.; G.P. Brahmaprakash.: 'development of composite biofertilizer containing *Bradyrhizobium*+ *Bacillus megaterium* in different formulations'. *Journal of Soil Biology.* 24(1 & 2), pp. 63–70, (2004).
174. Polonenko, D.R.: 'Commercial opportunities for multi-organism inoculants'. *In Proceedings of the BIOREM 4th Annual Ge neral Meeting* (J.J. Germida ed). 'BIOREM, Montreal, Quebec, Canada, pp. 12–14, (1994).
175. Archana, D.S.: 'Development and evaluation of alginate based microbial consortium for plant growth promotion'. Ph.D. Thesis, university of Agriculture Sciences, Bangalore. (2011).
176. Swapna, G.: 'Utilization of selected materials for development of granular formulation of microbial consortium of agriculturally important microorganisms (AIM)'. 'Ph.D. Thesis, University of Agricultural Sciences, Bangalore. (2011).

G.P. Brahmaprakash is a Professor of Agricultural Microbiology at the University of Agricultural Sciences GKVK, Bangalore. He obtained B.Sc. (Agri) and M.Sc. (Agri) from UAS Bangalore and Ph.D. from Utkal University Bhubaneswar. He conducted his dissertation research on pesticide-microflora interaction at Central Rice Research Institute, Cuttack. He worked as a Research Associate in the department of Metallurgy, Indian Institute of Science, Bangalore1986–89. His area of research interest is legume—*Rhizobium* symbiosis and currently focusing on development of Consortium of agriculturally beneficial microorganisms for agriculture.

Pramod Kumar Sahu obtained B.Sc. (Agri) from Indira Gandhi Agricultural University, Raipur, Chhattisgarh in 2010. He is an ICAR fellow pursuing his post graduation at the Department of Agricultural Microbiology, UAS, GKVK, Bangalore. He is currently working on development of consortium of agriculturally beneficial microorganisms through FBD.