VOL. 7, NO. 5, MAY 2012 ISSN 1990-6145
ARPN Journal of Agricultural and Biological Science


www.arpnjournals.com

BACTERIAL BIOFERTILIZERS FOR SUSTAINABLE CROP PRODUCTION: A REVIEW

Khosro Mohammadi[1] and Yousef Sohrabi[2]
[1]Department of Agronomy, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran
[2]Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Kurdistan, Sanandaj, Iran
E-Mail: Khosromohammadi60@yahoo.com

ABSTRACT

The most important constraint limiting crop yield in developing nations worldwide, and especially among resource-poor farmers, is soil infertility. Therefore, maintaining soil quality can reduce the problems of land degradation, decreasing soil fertility and rapidly declining production levels that occur in large parts of the world needing the basic principles of good farming practice. Minerals, organic components and microorganisms are three major solid components of the soil. They profoundly affect the physical, chemical, and biological properties and processes of terrestrial systems. Biofertilizer are the products containing cell of different types of beneficial microorganisms. Thus, biofertilizers can be important components of integrated nutrients management. Organisms that are commonly used as biofertilizers component are nitrogen fixers (N-fixer), solubilizer (K-solubilizer) and phosphorus solubilizer (P- solubilizer), or with the combination of molds or fungi. These potential biological fertilizers would play key role in productivity and sustainability of soil and also protect the environment as eco-friendly and cost effective inputs for the farmers. With using the biological and organic fertilizers, a low input system can be carried out and it can be help achieving sustainability of farms.

Keywords: biofertilizer, microorganism, phosphorus, potassium solubilizer, crop production.

INTRODUCTION

For optimum plant growth, nutrients must be available in sufficient and balanced quantities (Chen, 2006). The most important constraint limiting crop yield in developing nations worldwide, and especially among resource-poor farmers, is soil infertility. Unless the fertility is restored in these areas, farmers will gain little benefit from the use of improved varieties and more productive cultural practices. Soil fertility can be restored effectively through adopting the concept of integrated soil fertility management (ISFM) encompassing a strategy for nutrient management-based on natural resource conservation, biological nitrogen fixation (BNF) and increased efficiency of the inputs (Vlek and Vielhauer, 1994).

Biofertilizers are important components of integrated nutrients management. These potential biological fertilizers would play key role in productivity and sustainability of soil and also protect the environment as ecofriendly and cost effective inputs for the farmers. They are cost effective, ecofriendly and renewable source of plant nutrients to supplement chemical fertilizers in sustainable agricultural system.

Biofertilizers are products containing living cells of different types of microorganisms which when, applied to seed, plant surface or soil, colonize the rhizosphere or the interior of the plant and promotes growth by converting nutritionally important elements (nitrogen, phosphorus) from unavailable to available form through biological process such as nitrogen fixation and solubilization of rock phosphate (Rokhzadi *et al*., 2008). Beneficial microorganisms in biofertilizers accelerate and improve plant growth and protect plants from pests and diseases (El-yazeid *et al*., 2007). The role of soil microorganisms in sustainable development of agriculture has been reviewed (Lee and Pankhurst, 1992; Wani *et al*., 1995).

What is the biofertilizer?

The term biofertilizer or called 'microbial inoculants' can be generally defined as a preparation containing live or latent cells of efficient strains of nitrogen fixing, phosphate solubilizing or cellulytic microorganisms used for application of seed, soil or composting areas with the objective of increasing the numbers of such microorganisms and accelerate certain microbial process to augment the extent of the availability of nutrients in a form which can assimilated by plant (NIIR Board, 2004). In large sense, the term may be used to include all organic resources (manure) forplant growth which are rendered in an available form for plant absorption through microorganisms or plant associations or interactions (NIIR Board, 2004).

The knowledge of applied microbial inoculums is long history which passes from generation to generation of farmers. It started with culture of small scale compost production that has evidently proved the ability of biofertilizer. This is recognize when the cultures accelerate the decomposition of organics residues and agricultural by-products through various processes and gives healthy harvest of crops (Abdul Halim, 2009). In Malaysia, industrial scale microbial inoculants are started in the late 1940's and peaking up in 1970's taking guide by *Brady rhizobium* inoculation on legumes. Government research institute, the Malaysian Rubber Board (MRB) had been conducting research on *Rhizobium* inoculums for leguminous cover crops in the inter rows of young rubber trees in the large plantations. Besides, University Putra Malaysia (UPM) also has conducted many researches since 1980's on *Mycorrhiza* and initiated the research to

evaluate the contribution of nitrogen from *Azospirillum* to oil palm seedlings (Abdul Halim, 2009).

Mycorrhiza inoculums are the biofertilizer that is increasingly being utilized and accepted in agriculture industry of Malaysia Large scale productions of biofertilizer are produced mainly for supplying nutrient, amelioration of toxic effect in soils, root pest and disease control, improved water usage and soil fertility (Abdul Halim, 2009). Since the substrate for inoculate are abundant such as the mine sands and agricultural wastes, the production cost is cheaper and environmentally safe.

There are lot of perception is lay on biofertilizer. It is often perceived to be more expensive than the chemical fertilizers due to the lack of skills and technology to produce biofertilizer products from abundant wastes. Besides, the effect on the crops is slow, compared to chemical fertilizers. Special care such as storage or mixing with powders is also needed to handle microbial inocula to make they remain effective for extended use. As biofertilizers contain living organisms, their performance therefore depends on environment surrounding. Hence, outcomes are bound to be inconsistent (Rahim, 2002). Short shelf life, lack of suitable carrier materials, susceptibility to high temperature, problems in transportation and storage are biofertilizer bottlenecks that still need to be solved in order to obtain effective inoculation.

BIOFERTILIZER MAKING

There are several things need to be considered in biofertilizer making such as microbes' growth profile, types and optimum condition of organism, and formulation of inoculum. The formulation of inocula, method of application and storage of the product are all critical to the success of a biological product. In general, there are 6 major steps in making biofertilizer. These includes choosing active organisms, isolation and selection of target microbes, selection of method and carrier material, selection of propagation method, prototype testing and large scale testing. First of all, active organisms must be decided. For example, we must decide to use whether organic acid bacteria or nitrogen fixer or the combination of some organisms. Then, isolation is made to separate target microbes from their habitation. Usually organism are isolate from plants root or by luring it using decoy such as putting cool rice underground of bamboo plants.

Next, the isolated organisms will be grown on Petri plate, shake flask and then glasshouse to select the best candidates. It is also important to decide form of our biofertilizer product wisely so that the right carrier material can be determined. If it is desired to produce biofertilizer in powder form, then tapioca flour or peat are the right carrier materials. Selection of propagation method is mainly to find out the optimum condition of organism. This can be achieved by obtaining growth profile at different parameter and conditions. After that, prototype (usually in different forms) is made and tested. Lastly, biofertilizer is testing on large scale at different environment to analyze its effectiveness and limitability at different surrounding.

Biofertilizers are usually prepared as carrier-based inoculants containing effective microorganisms. Incorporation of microorganisms in carrier material enables easy-handling, long-term storage and high effectiveness of biofertilizers. Sterilization of carrier material is essential to keep high number of inoculant bacteria on carrier for long storage period. Gamma-irradiation or autoclaving can be used as method for sterilization.

Various types of material can be used as carrier for seed or soil inoculation. The properties of a good carrier material for seed inoculation are inexpensive and available in adequate amounts. It must non-toxic to inoculants bacterial strain and non-toxic to plant itself. Because it acts as carrier for seed inoculation, it should have good moisture absorption capacity and good adhesion to seeds. Last but not the least; carrier should have good pH buffering capacity, easy to process and sterilized by either autoclaving or gamma radiation.

MOST IMPORTANT MICROORGANISMS USED IN BIOFERTILIZER

Organisms that are commonly used as biofertilizers component are nitrogen fixers (N-fixer), potassium solubilizer (K-solubilizer) and phosphorus solubilizer (P- solubilizer), or with the combination of molds or fungi. Most of the bacteria included in biofertilizer have close relationship with plant roots. *Rhizobium* has symbiotic interaction with legume roots, and Rhizobacteria inhabit on root surface or in rhizosphere soil.

The phospho-microorganism mainly bacteria and fungi make insoluble phosphorus available to the plants (Gupta, 2004). Several soil bacteria and a few species of fungi possess the ability to bring insoluble phosphate in soil into soluble forms by secreting organic acids (Gupta, 2004). These acids lower the soil pH and bring about the dissolution of bound forms of phosphate.

While *Rhizobium*, Blue Green Algae (BGA) and *Azolla*are crop specific, bio-inoculants like *Azotobacter, Azospirillum,* Phosphorus Solubilizing Bacteria (PSB), Vesicular Arbuscular Mycorrhiza (VAM) could be regarded as broad spectrum biofertilizers (Gupta, 2004). VAM is fungi that are found associated with majority of agriculture crops and enhanced accumulation of plant nutrients (Gupta, 2004). It has also been suggested that VAM stimulate plant growth by physiological effects or by reducing the severity of diseases caused by the soil pathogens (Gupta, 2004). Examples of free living nitrogen fixing bacteria are obligate anaerobes (*Clostridium pasteurianum*), obligate aerobes (*Azotobacter*), facultative anaerobes, photosynthetic bacteria (*Rhodobacter*), cyanobacteria and some methanogens. The example of K-solubilizer is *Bacillus mucilaginous* while for P-solubilizer are *Bacillus megaterium*, *Bacillus circulans*, *Bacillus subtilis* and *Pseudomonas straita.*

Nitrogen

Nitrogen is one of the major important nutrients very essential for crop growth. Atmosphere contains about 80 percent of nitrogen volume in Free State. The major part of the elemental nitrogen that finds its way into the soil is entirely due to its fixation by certain specialized group of microorganisms. Biological Nitrogen Fixation (BNF) is considered to be an important process which determines nitrogen balance in soil ecosystem. Nitrogen inputs through BNF support sustainable environmentally sound agricultural production. The value of nitrogen fixing legumes in improving and higher yield of legumes and other crops can be achieved by the application of biofertilizers (Kannaiyan, 2002).

Biological nitrogen fixation is one way of converting elemental nitrogen into plant usable form (Gothwal *et al*., 2007). Nitrogen-fixing bacteria (NFB) that function transform inert atmospheric N2 to organic compounds (Bakulin *et al*., 2007). Nitrogen fixer or N-fixers organism are used in biofertilizer as a living fertilizer composed of microbial inoculants or groups of microorganisms which are able to fix atmospheric nitrogen. They are grouped into free-living bacteria (*Azotobacter* and *Azospirillium*) and the blue green algae and symbionts such as *Rhizobium*, *Frankia* and *Azolla* (Gupta, 2004).

The list of N_2-fixing bacteria associated with non-legumes includes species of *Achromobacter*, *Alcaligenes*, *Arthrobacter*, *Acetobacter*, *Azomonas*, *Beijerinckia*, *Bacillus*, *Clostridium*, *Enterobacter*, *Erwinia*, *Derxia*, *Desulfovibrio*, *Corynebacterium*, *campylobacter*, *Herbaspirillum*, *Klebsiella*, *Lignobacter*, *Mycobacterium*, *Rhodospirillum*, *Rhodo-pseudomonas*, *Xanthobacter*, *Mycobacterium* and *Methylosinus* (Wani, 1990). Although many genera and species of N_2-fixing bacteria are isolated from the rhizosphere of various cereals, mainly members of *Azotobacter* and *Azospirillum* genera have been widely tested to increase yield of cereals and legumes under field conditions.

Rhizobium inoculation is well known agronomic practice to ensure adequate nitrogen of legumes instead of N-fertilizer (Gupta, 2004). In root nodules the O_2 level is regulated by special hemoglobin called leg-hemoglobin. This globin protein is encoded by plant genes but the heme cofactor is made by the symbiotic bacteria. This is only produced when the plant is infected with *Rhizobium*. The plant root cells convert sugar to organic acids which they supply to the bacteroids. In exchange, the plant will receive amino-acids rather than free ammonia.

Azolla biofertilizer is used for rice cultivation in different countries such as Vietnam, China, Thailand and Philippines. Field trial indicated that rice yields are increased by 0.5-2 t/ha due to *Azolla* application (Gupta, 2004). *Azobacter* and *Azospirillum* can fix atmospheric nitrogen in cereal crops without any symbiosis while blue-green algae have been found to be very effective on the rice and banana plantation (Gupta, 2004). El-Komy (2005) demonstrated the beneficial influence of co-inoculation of *Azospirillum lipoferum* and *Bacillus megaterium* for providing balanced nitrogen and phosphorus nutrition of wheat plants. The inoculation with bacterial mixtures provided a more balanced nutrition for the plants and the improvement in root uptake of nitrogen and phosphorus was the major mechanism of interaction between plants and bacteria.

Co-inoculation of some *Pseudomonas* and *Bacillus* strains along with effective *Rhizobium* spp. is shown to stimulate chickpea growth, nodulation and nitrogen fixation. Findings of Mohammadi *et al*. (2010) showed that the highest sugar, protein, starch contents, nodule weight and seed nitrogen, potassium, phosphorus of chickpea were obtained from combined application of phosphate solubilizing bacteria, *Rhizobium* and *Trichoderma* fungus. Shanmugam and Veeraputhran (2000) stated that application of green manure and biofertilizer stimulated the growth of plants with more number of tillers and broader leaves in rice that could be the possible reason for the increased leaf area. Application of biofertilizer increased the number of leaves in betlevine and this could be due to properly colonized roots, increased mineral and water uptake from the soil and biological nitrogen fixation (Okon, 1984). It could be also attributed to the production of the IAA, gibberellins and cytokinins like substances produced by the bacterium as evident from the findings in banana by Jeeva (1987).

Phosphorus

The fixed phosphorus in the soil can be solubilized by phosphate solubilizing bacteria (PSB), which have the capacity to convert inorganic unavailable phosphorus form to soluble forms HPO_4^{2-} and $H_2PO_4^-$ through the process of organic acid production, chelation and ion exchange reactions and make them available to plants. Therefore, the use of PSB in agricultural practice would not only offset the high cost of manufacturing phosphate fertilizers but would also mobilize insoluble in the fertilizers and soils to which they are applied (Chang and Yang, 2009; Banerjee *et al*., 2010). Evidence of naturally occurring rhizospheric phosphorus solubilizing microorganism (PSM) dates back to 1903 (Khan *et al*., 2007). Bacteria are more effective in phosphorus solubilization than fungi (Alam *et al*., 2002). Among the whole microbial population in soil, phosphate solubilizing bacteria (PSB) constitute 1 to 50%, while phosphorus solubilizing fungi (PSF) are only 0.1 to 0.5% in P solubilization potential (Chen *et al*., 2006). Number of PSB among total PSM in north Iranian soil was around 88 % (Fallah, 2006). Microorganisms involved in phosphorus acquisition include mycorrhizal fungi and PSMs (Fankem *et al*., 2006). Among the soil bacterial communities, ecto-rhizospheric strains from *Pseudomonas* and *Bacilli*, and endosymbiotic rhizobia have been described as effective phosphate solubilizers (Igual *et al*., 2001). Strains from bacterial genera *Pseudomonas*, *Bacillus*, *Rhizobium* and *Enterobacter* along with *Penicillium* and *Aspergillus* fungi are the most powerful P solubilizers (Whitelaw, 2000). *Bacillus megaterium*, *B. circulans*, *B.subtilis*, *B. polymyxa*, *B. sircalmous*, *Pseudomonas striata*, and *Enterobacter*

could be referred as the most important strains (Subbarao, 1988; Kucey *et al.*, 1989). A nemato fungus *Arthrobotrys oligospora* also has the ability to solubilize the phosphate rocks (Duponnois *et al.*, 2006).

High proportion of PSM is concentrated in the rhizosphere, and they are metabolically more active than from other sources (Vazquez *et al*., 2000). Usually, one gram of fertile soil contains 101 to 1010 bacteria, and their live weight may exceed 2,000 kg ha-1. Soil bacteria are in cocci (sphere, 0.5 µm), bacilli (rod, 0.5-0.3 µm) or spiral (1-100 µm) shapes. Bacilli are common in soil, where as spirilli are very rare in natural environments (Baudoin *et al*., 2002). The PSB are ubiquitous with variation in forms and population in different soils. Population of PSB depends on different soil properties (physical and chemical properties, organic matter, and P content) and cultural activities (Kim *et al.*, 1998). Larger populations of PSB are found in agricultural and rangeland soils (Yahya and Azawi, 1998). In north of Iran, the PSB count ranged from 0 to 107 cells g-1 soil, with 3.98% population of PSB among total bacteria (Fallah, 2006).

Some bacterial species have mineralization and solubilization potential for organic and inorganic phosphorus, respectively (Hilda and Fraga, 2000; Khiari and Parent, 2005). Phosphorus solubilizing activity is determined by the ability of microbes to release metabolites such as organic acids, which through their hydroxyl and carboxyl groups chelate the cation bound to phosphate, the latter being converted to soluble forms (Sagoe *et al.*, 1998). Phosphate solubilization takes place through various microbial processes / mechanisms including organic acid production and proton extrusion (Surange, 1995; Dutton and Evans, 1996; Nahas, 1996). General sketch of P solubilization in soil is shown in Figure-1. A wide range of microbial P solubilization mechanisms exist in nature and much of the global cycling of insoluble organic and inorganic soil phosphates is attributed to bacteria and fungi (Banik and Dey, 1982). Phosphorus solubilization is carried out by a large number of saprophytic bacteria and fungi acting on sparingly soluble soil phosphates, mainly by chelation-mediated mechanisms (Whitelaw, 2000). Phosphate solubilizing microorganism's secret organic acids and enzymes that act on insoluble phosphates and convert it into soluble form, thus, proving P to plants (Ponmurugan and Gopi, 2006). Inorganic P is solubilized by the action of organic and inorganic acids secreted by PSB in which hydroxyl and carboxyl groups of acids chelate cations (Al, Fe, Ca) and decrease the pH in basic soils (Kpomblekou and Tabatabai, 1994; Stevenson, 2005). The PSB dissolve the soil P through production of low molecular weight organic acids mainly gluconic and ketogluconic acids (Goldstein, 1995; Deubel *et al.*, 2000), in addition to lowering the pH of rhizosphere. The pH of rhizosphere is lowered through biotical production of proton / bicarbonate release (anion / cation balance) and gaseous (O_2/CO_2) exchanges. Phosphorus solubilization ability of PSB has direct correlation with pH of the medium.

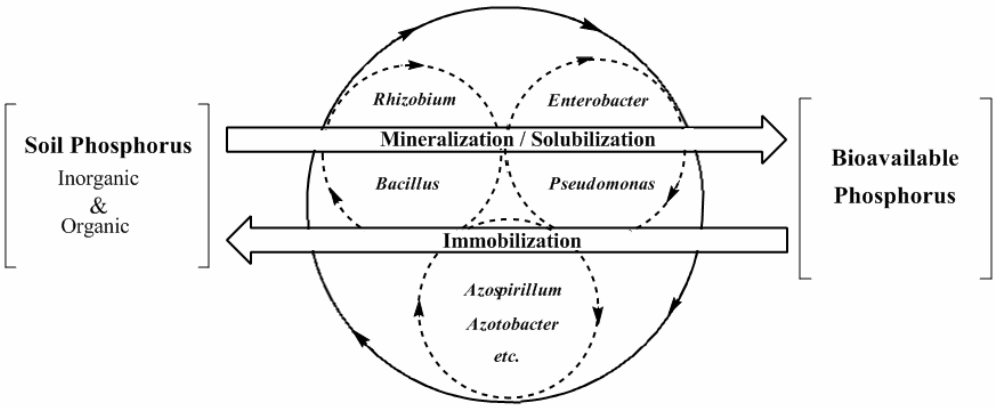

Figure-1. Schematic diagram of soil phosphorus mobilization and immobilization by bacteria.

Release of root exudates such as organic ligands can also alter the concentration of P in the soil solution (Hinsinger, 2001). Organic acids produced by PSB solubilize insoluble phosphates by lowering the pH, chelation of cations and competing with phosphate for adsorption sites in the soil (Nahas, 1996). Inorganic acids e.g. hydrochloric acid can also solubilize phosphate but they are less effective compared to organic acids at the same pH (Kim *et al.*, 1997). In addition, the microorganisms involved in P solubilization as well as can enhance plant growth by enhancing the availability of other trace element such as iron (Fe), zinc (Zn), etc. (Ngoc *et al*., 2006), synthesize enzymes that can modulated plant hormone level, may limit the available iron via siderophore production and can also kill the pathogen with antibiotic (Akhtar and Siddiqui, 2009).

The PSB solubilize the fixed soil P and applied phosphates resulting in higher crop yields (Gull *et al*., 2004). Direct application of phosphate rock is often ineffective in the short time period of most annual crops (Goenadi *et al*., 2000). Acid producing microorganisms are able to enhance the solubilization of phosphatic rock (Gyaneshwar *et al*., 2002). The PSB strains exhibit inorganic P-solubilizing abilities ranging between 25-42

μg P mL-1 and organic P mineralizing abilities between 8-18 μg P mL-1 (Tao *et al*., 2008). The PSB in conjunction with single super phosphate and rock phosphate reduce the P dose by 25 and 50%, respectively (Sundara *et al*., 2002). *Pseudomonas putida*, *P. fluorescens* Chao and *P. fluorescens* Tabriz released 51, 29 and 62% P, respectively; with highest value of 0.74 mg P/50 mL from Fe_2O_3 (Ghaderi *et al*., 2008). *Pseudomonas striata* and *Bacillus polymyxa* solubilized 156 and 116 mg P L-1, respectively (Rodríguez and Fraga, 1999). *Pseudomonas fluorescens* solubilized 100 mg P L-1 containing $Ca_3(PO_4)_2$ or 92 and 51 mg P L-1 containing $AlPO_4$ and $FePO_4$, respectively (Henri *et al*., 2008).

PLANT GROWTH PROMOTING RHIZOBACTERIA

A group of rhizosphere bacteria (rhizobacteria) that exerts a beneficial effect on plant growth is referred to as plant growth promoting rhizobacteria or PGPR (Schroth and Hacock, 1981). PGPR belong to several genera, e.g. Agrobacterium, Alcaligenes, Arthrobacter, Actinoplanes, Azotobacter, *Bacillus*, *Pseudomonas* sp., *Rhizobium*, *Bradyrhizobium*, *Erwinia*, *Enterobacter*, *Amorpho sporangium*, *Cellulomonas*, *Flavobacterium*, *Streptomyces* and *Xanthomonas* (Weller, 1988).

PGPR increased recently as a result of the numerous studies covering a wider range of plant species and because of the advances made in bacterial taxonomy and the progress in our understanding of the different mechanisms of action of PGPR. In all successful plant-microbe interactions, the competence to colonize plant habitats is important. Single bacterial cells can attach to surfaces and, after cell division and proliferation, form dense aggregates commonly referred to as macro colonies or biofilms. Steps of colonization include attraction, recognition, adherence, invasion (only endophytes and pathogens), colonization and growth, and several strategies to establish interactions (Nihorimbere *et al*., 2011). Plant roots initiate crosstalk with soil microbes by producing signals that are recognized by the microbes, which in turn produce signals that initiate colonization (Berg, 2009). PGPR reach root surfaces by active motility facilitated by flagella and are guided by chemotactic responses. This implies that PGPR competence highly depends either on their abilities to take advantage of a specific environment or on their abilities to adapt to changing conditions or plant species (Nihorimbere *et al*., 2011).

CROP RESPONSES TO INOCULATION

Symbiotic nitrogen fixer and phosphate solubilizing microorganisms play an important role in supplementing nitrogen and phosphorus to the plant, allowing a sustainable use of nitrogen and phosphate fertilizers (Tambekar *et al*., 2009). Zaddy *et al*. (1993) studied the promoting of plant growth by inoculation with aggregated and single cell suspensions of *A. brasilense*. They reported that inoculation of single cell suspensions of *Azospirillum* (prepared with fructose) significantly increased the root surface area, root and foliage dry weight of the maize seedling as compared to plants inoculated with malate grown *Azospirillum* or the controls. Fulchieri and Frioni (1994) observed that maize inoculated with *Azospirillum* had enhanced dry weight of seed by 59 percent and also the yield which was similar to 60 kg urea N ha^{-1}. A significant positive effect on grain yield was obtained due to combined inoculation of *Azospirillum* and *Pseudomonas striata* in *Zea mays* (Prabakaran and Ravi, 1991) and cotton (Radhakrishnan, 1996). Crops inoculated with *Azotobacter* and *Azospirilla* reviewed by Wani (1990) indicated that Pearl millet and Sorghum, which are grown as dryland crops showed 11-12% increased yields due to inoculations. Maize, Wheat and Rice which receive better management and inputs than Pearl millet and Sorghum showed 15-20% increased yields due to inoculation.

Several soil bacteria and fungi notably species of *Pseudomonas*, *Bacillus*, *Penicillum* and *Aspergillus* etc., secret organic acids and lower the pH in their vicinity to bring about solubilization of bound phosphates in soil (Sundara Rao and Sinha, 1963). Saving of 50 percent of recommended level of P_2O_5 is possible in sugarcane by inoculation with phosphor-bacteria as the cane yield and sugar yield of 50 percent P_2O_5 and phosphor-bacteria treatments are on par with 100 percent P_2O_5 application (Kathiresan *et al*., 1995). Habibi *et al*. (2011) strongly suggested that using biofertilizers (combined strains) plus half a dose of organic and chemical fertilizers have resulted in the greatest grain yield and oil yield in medicinal pumpkin. They revealed that 50% of required nitrogen and phosphorus fertilizers could be replaced by bio and organic fertilizers, because bio and organic fertilizers improved the use efficiency of recommended nitrogen and phosphorus fertilizers and reduced the cost of chemical fertilizers, also prevented the environment pollution from extensive application of chemical fertilizers (Figure-2). Beans with *R. leguminosarum* and *P. putida* R 105 increased the number of nodules and acetylene reduction activity (ARA) significantly (de Freitas *et al*., 1993). A significant positive effect on grain yield and ARA in roots of barley was obtained due to combined inoculation of nitrogen fixer's *A. lipoferum*, *Arthrobacter mysorens* and the phosphate solubilizing strain *Agrobacterium radiobacter* by Belimov *et al*. (1995). Radhakrishnan (1996) reported that inoculation of *Azospirillum* and phosphor-bacteria resulted in higher root biomass and more bolls in cottn. Findings of Mohammadi (2010) showed that inoculation of biofertilizers (PSB+ *Trichoderma* fungi) + application of FYM had a great influence on canola growth, height and grain yield in compared to control treatment.

Findings of Mohammadi *et al*. (2011) showed that application of biofertilizers had a significant effects on nutrient uptake of chickpea (Table-1) combined application of Phosphate solubilizing bacteria and *Trichoderma harzianum* produced the highest leaf P content (0.33%) and grain P content (279 mg 100 g^{-1}). Ability of *Bacillus* sp. to produce organic acid such as

gluconic, citric and fumaric acids under P-limiting conditions may increase the solubility of poorly soluble phosphorus. These findings also showed that chickpea inoculated with biofertilizers have significantly higher grain protein content. Maximum protein content (%15.06) was observed in the treatment that received a combined inoculation of PSB and *T. harzianum* (Table-2).

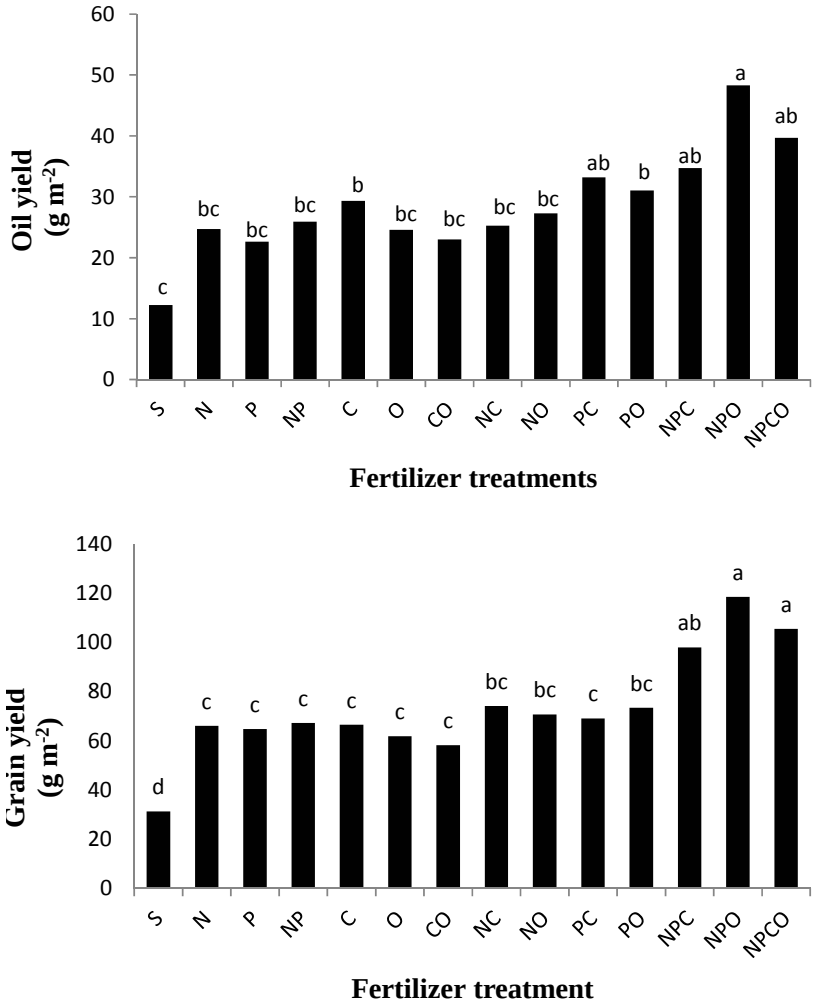

Figure-2. The effect of fertilizer treatments on oil yield of pumpkin. S = Control; N = NFB; P = PSB; NP = NFB + PSB; C = chemical fertilizer; O = organic fertilizer; CO = 50% organic fertilizer + 50% chemical; NC = NFB + 50% chemical fertilizer; NO = NFB + 50% organic fertilizer; PC = PSM + 50% chemical fertilizer; PO = PSB + 50% organic fertilizer; NPC = NFB + PSB + 50% chemical fertilizer; NPO = NFB + PSM + 50% organic fertilizer; NPCO = NFB + PSB + 50% organic fertilizer+ 50% chemical fertilizer.

Table-1. Effect of soil fertility systems on chlorophyll and nutrient accumulation in chickpea seed.

Treatment	Chlorophyll (spad reading)	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium	Manganese	Iron
		(mg/100g)						
Biofertilizers								
PSB (B_1)	43.41 [b]	2269 [b]	273.5 [b]	1201.1 [b]	184.3 [a]	4.3 [a]	2.63 [a]	4.42 [a]
Trichoderma (B_2)	43.35 [b]	2295 [b]	266.2 [c]	1176.3 [c]	183.7 [ab]	4.2 [b]	2.56 [b]	4.35 [c]
PSB + fungi (B_3)	44.12 [a]	2315 [a]	279.8 [a]	1232.1 [a]	183.2 [b]	4.3 [a]	2.62 [a]	4.47 [a]
Control (B_4)	43.22 [b]	2167 [c]	264.9 [c]	1199.8 [b]	184.4 [a]	4.2 [b]	2.57 [b]	4.36 [c]

Mean values in each column with the same superscript(s) do not differ significantly by DMRT (P = 0.05).

Table-2. Effect of soil fertility systems on grain yield and yield components of chickpea.

Treatment	Grain yield (kg ha^{-1})	Pod number per plant	Fertile pods per plant	Grain number per pod	100 grain weight (g)
Biofertilizer					
PSB (B_1)	1756.1 [c]	39.72 [b]	25.84 [c]	1.083 [b]	20.79 [a]
Trichoderma fungi (B_2)	1866.2 [b]	40.79 [b]	27.41 [b]	1.072 [b]	21.15 [a]
PSB + fungi (B_3)	2560.3 [b]	57.66 [a]	35.07 [a]	1.144 [a]	21.19 [a]
Control (B_4)	1310.7 [d]	30.83 [c]	20.73 [d]	1.028 [c]	19.52 [b]

Mean values in each column with the same superscript(s) do not differ significantly by DMRT (P = 0.05).

CONCLUSIONS

Biofertilizer help in increasing crop productivity by way of increased BNF, increased availability or uptake of nutrients through solubilization or increased absorption stimulation of plant growth through hormonal action or antibiosis, or by decomposition of organic residues. Furthermore, biofertilizer as to replace part of the use of chemical fertilizers reduces amount and cost of chemical fertilizers and thus prevents the environment pollution from extensive application of chemical fertilizers. With using the biological and organic fertilizers, a low input system can be carried out, and it can be helped achieving sustainability of farms.

REFERENCES

Abdul Halim N.B. 2009. Effects of using enhanced biofertilizer containing N-fixer bacteria on patchouli growth. Thesis. Faculty of Chemical and Natural Resources Engineering University Malaysia Pahang. p. 145.

Akhtar M.S. and Siddiqui Z.A. 2009. Effect of phosphate solubilizing microorganisms and Rizobium sp. on the growth, nodulation, yield and root-rot disease complex of chickpea under field condition. Afr. J. Biotech. 8(15): 3489-3496.

Alam S., Khalil S., Ayub N. and Rashid M. 2002. In *vitro* solubilization of inorganic phosphate by phosphate solubilizing microorganism (PSM) from maize rhizosphere. Intl. J. Agric. Biol. 4: 454-458.

Bakulin M.K., Grudtsyna A.S. and Pletneva A. 2007. Biological fixation of nitrogen and growth of bacteria of the genus Azotobacter in liquid media in the presence of Perfluoro carbons. Appl. Biochem. Microbiol. 4: 399-402.

Banerjee S., Palit R., Sengupta C. and Standing D. 2010. Stress induced phosphate solubilization by Arthrobacter sp. and Bacillus sp. Isolated from tomato rhizosphere. Aust. J. Crop Sci. 4(6): 378-383.

Banik S., Dey B.K. 1982. Available phosphate content of an alluvial soil as influenced by inoculation of some isolated phosphate solubilizing microorganisms. Plant Soil. 69: 353-364.

Bashan Y. and Holguin G. 1997. *Azospirillum*-plant relationships: environmental and physiological advances (1990-1996). Can J. Microbiol. 43: 103-121.

Baudoin E., Benizri E. and Guckert A. 2002. Impact of growth stages on bacterial community structure along maize roots by metabolic and genetic finger printing. Appl. Soil Ecol. 19: 135-145.

Belimov A.A., Kojemiakov A.P. and Chuvarliyeva C.V. 1995. Interaction between barley and mixed cultures of nitrogen fixing and phosphate-solubilizing bacteria. Plant Soil. 173: 29-37.

Berg G. 2009. Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl. Microbiol Biotech. 84: 11-18.

Broeckling C.D. 2008. Root exudates regulate soil fungal community composition and diversity. Appl. Environ Microbiol. 74: 738-744.

Chang C.H. and Yang S.S. 2009. Thermo-tolerant phosphate-solubilizing microbes for multi-functional biofertilizer preparation. Biores .Technol. 100: 1648-1658.

Chen J. 2006. The combined use of chemical and organic fertilizer and or biofertilizer for crop growth and soil fertility. International Workshop on Sustained Management of the Soil-Rhizosphere System for Efficient Crop Production and Fertilizer Use. October, Thailand. pp . 16-20.

Chen Y.P., Rekha P.D., Arunshen A.B., Lai W.A. and Young C.C. 2006. Phosphate solubilizing bacteria from subtropical soil and their tri-calcium phosphate solubilizing abilities. Appl. Soil Ecol. 34: 33-41.

De Freitas J.R., Gupta V.V.S. and Germida J.J. 1993. Influence of *Pseudomonas syringae* R 25 and *P. putida* R 105 on the growth and N_2 fixation (ARA) of pea (*Pisum*

sativum L.) and field bean (*Phaseolus vulgaris* L.). Biol Fertil Soils. 16: 215-220.

Deubel A., Gransee G. and Merbach W. 2000. Transformation of organic rhizodeposits by rhizoplane bacteria and its influence on the availability of tertiary calcium phosphate. J. Plant Nutr. Soil Sci. 163: 387-392.

Duponnois R., Kisa M. and Plenchette C. 2006. Phosphate solubilizing potential of the nemato fungus *Arthrobotrys oligospora*. J. Plant Nutr. Soil Sci. 169: 280-282.

Dutton V.M. and Evans C.S. 1996. Oxalate production by fungi: its role in pathogenicity and ecology in the soil environment. Can J. Microbiol. 42: 881-895.

El-Komy H.M.A. 2005. Co-immobilization of *A. lipoferum* and *B. megaterium* for plant nutrition. Food Technol Biotech. 43(1): 19-27.

El-Yazeid A.A., Abou-Aly H.A., Mady M.A. and Moussa S.A.M. 2007. Enhancing growth, productivity and quality of squash plants using phosphate dissolving microorganisms (bio phosphor) combined with boron foliar spray. Res. J. Agric. Biol. Sci. 3(4): 274-286.

Fallah A. 2006. Abundance and distribution of phosphate solubilizing bacteria and fungi in somesoil samples from north of Iran. 18th World Congress of Soil Science, July 9-15, Philadelphia, Pennsylvania, USA.

Fankem H., Nwaga D., Deubel A., Dieng L., Merbach W. and Etoa F.S. 2006. Occurrence and functioning of phosphate solubilizing microorganisms from oil palm tree rhizosphere in Cameroon. Afr J. Biotech. 5: 2450-2460.

Fulchieri M and Frioni L. 1994. *Azospirillum* inoculation on maize (*Zea mays*): Effect of yield in a field experiment in Central Argentina. Soil Biol. Biochem. 26: 921-924.

Ghaderi A., Aliasgharzad N., Oustan S. and Olsson P.A. 2008. Efficiency of three *Pseudomonas* isolates in releasing phosphate from an artificial variable-charge mineral (iron III hydroxide). Soil Environ. 27: 71-76.

Goenadi D.H., Siswanto G. and Sugiarto Y. 2000. Bioactivation of poorly soluble phosphate rocks with a phosphorus-solubilizing fungus. Soil Sci Soc Am J. 64: 927-932.

Goldstein A.H. 1995. Recent progress in understanding the molecular genetics and biochemistry of calcium phosphate solubilization by Gram-negative bacteria. Biol. Agri Hort. 12: 185-193.

Gothwal R.K., Nigam V.K., Mohan M.K., Sasmal D. and Ghosh P. 2007. Screening of nitrogen fixers from rhizospheric bacterial isolates associated with important desert plants. Appl. Ecol. Environ. Res. 6(2): 101-109.

Gull M., Hafeez F.E., Saleem M. and Malik K.A. 2004. Phosphorus uptake and growth promotion of chickpea by co-inoculation of mineral phosphate solubilizing bacteria and a mixed rhizobial culture. Aust J. Exp Agric. 44: 623-628.

Gupta A.K. 2004. The complete technology book on biofertilizers and organic farming. National Institute of Industrial Research Press. India.

Gyaneshwar P., Kumar G.N., Parekh L.J and Poole P.S. 2002. Role of soil microorganisms in improving P nutrition of plants. Plant Soil. 245: 83-93.

Gyaneshwar P., Parekh L.J., Archana G., Podle P.S., Collins M.D., Hutson R.A. and Naresh H. 1999. Involvement of a phosphate starvation inducible glucose dehydrogenase in soil phosphate solubilization by *Enterobacter asburiae*. Microbiol Lett. 171: 223-229.

Habibi A., Heidari G., Sohrabi Y., Badakhshan H. and Mohammadi K. 2011. Influence of bio, organic and chemical fertilizers on medicinal pumpkin traits. Journal of Medicinal Plants Research. 5(23): 5590-5597.

Henri F., Laurette N.N., Annette A., John Q., Wolfgang M., François-Xavier E. and Dieudonné E. 2008. Solubilization of inorganic phosphates and plant growth promotion by strains of *Pseudomonas fluorescens* isolated from acidic soils of Cameroon. Afri J. Microbiol Res. 2: 171-178.

Hilda R. and Fraga R. 2000. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotech Adv. 17: 319-359.

Hinsinger P. 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root induced chemical changes: a review. Plant Soil. 237: 173-195.

Igual J.M., Valverde A., Cervantes E. and Velázquez E. 2001. Phosphate-solubilizing bacteria asinoculants for agriculture: use of updated molecular techniques in their study. Agronomie. 21: 561-568.

Jeeva S. 1987. Studies on the effect of *Azospirillum* on the growth and development of banana cv. Poovan (AAB). M.Sc. (Hort.) Thesis, TNAU, Coimbatore, Tamil Nadu, India.

Kannaiyan S. 2002. Biofertilizers for sustainable crop production. Biotechnology of biofertilizers. Narosa Publishing House, New Delhi, India. p. 377.

Kathiresan G., Manickam G. and Parameswaran P. 1995. Efficiency of phosphobacteria addition on cane yield and quality. Cooperative Sugar. 26: 629-631.

Khan M.S., Zaidi A. and Wani P.A. 2007. Role of phosphate-solubilizing microorganisms in sustainable agriculture - A review. Agron Sustain Dev. 27: 29-43.

Khiari L. and Parent L.E. 2005. Phosphorus transformations in acid light-textured soils treated with dry swine manure. Can J. Soil Sci. 85: 75-87.

Kim K.Y., Jordan D. and Mc Donald G.A. 1998. Effect of phosphate-solubilizing bacteria and vesicular-arbuscular mycorrhizae on tomato growth and soil microbial activity. Biol. Fertil Soils. 26: 79-87.

Kpomblekou K. and Tabatabai M.A. 1994. Effect of organic acids on release of phosphorus from phosphate rocks. Soil Sci. 158: 442-453.

Lee K.E. and Pankhurst C.E. 1992. Soil organisms and sustainable productivity. Australian J. Soil Res. 30: 855-92.

Mohammadi K., Ghalavand A., Aghaalikhani M., Sohrabi Y. and Heidari G.R. 2010. Impressibility of chickpea seed quality from different systems of increasing soil fertility. Electron J. Crop Prod. 3(1): 103-119.

Mohammadi K. 2010. Ecophysiological response of canola (*Brassica napus* L.) to different fertility systems in crop rotation. Ph.D thesis. Agronomy Department. Tarbiat Modares University, Tehran, Iran. p. 354.

Mohammadi K., Ghalavand A., Aghaalikhani M., Heidari G.R. and Sohrabi Y. 2011. Introducing the sustainable soil fertility system for chickpea (*Cicer arietinum* L.). African Journal of Biotechnology. 10(32): 6011-6020.

Nahas E. 1996. Factors determining rock phosphate solubilization by microorganism isolated from soil. World J. Microb Biotech. 12: 18-23.

Ngoc S.T.T., Ngoc D.C.M. and Giang T.T. 2006. Effect of Bradyrhizobia and phosphate solubilizing bacteria application on soybean in rotational system in the Mekong delta. J. Omonrice. 14: 48-57.

Nihorimbere V., Ongena M., Smargiassi M. and Thonart P. 2011. Beneficial effect of the rhizosphere microbial community for plant growth and health. Biotechnol Agron Soc Environ. 15(2): 327-337.

Okon Y. 1984. Response of cereal and forage grasses to inoculation with N2 fixing bacteria. In: Veeger C, Newton WE, (Ed.). Advances in the Nitrogen Fixation Research Nijoff/Junk. The Hague. pp. 30-39.

Pierik R. 2006. The Janus face of ethylene: growth inhibition and stimulation. Trends Plant Sci. 11: 176-183.

Ponmurugan P. and Gopi G. 2006. Distribution pattern and screening of phosphate solubilizing bacteria isolated from different food and forage crops. J. Agron. 5(4): 600-604.

Prabakaran J. and Ravi K.B. 1991. Interaction effect of *A. Brasilense* and *Pseudomonas* sp. A phosphate solubilizer on the growth of *Zea mays*. In: Microbiology Abstracts, XXXI Annual Conference of the Association of Microbiologists of India, TNAU, Coimbatore, Jan, 23-26. p. 109.

Radhakrishnan K.C. 1996. Role of biofertilizers in cotton productivity. National Seminar Biofertilizer Production Problem and Constraints, TNAU, Coimbatore, Jan 24-25. p. 17.

Rodrguez H. and Fraga R. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotech Adv. 17: 319-339.

Rokhzadi A., Asgharzadeh A., Darvish F., Nour-mohammadi G. and Majidi E. 2008. Influence of plant growth-promoting rhizobacteria on dry matter accumulation and yield of chickpea (*Cicer arietinum* L.) under field condition. Am-Euras. J. Agric. Environ. Sci. 3(2): 253-257.

Rokhzadi A. and Toashih V. 2011. Nutrient uptake and yield of chickpea (*Cicer arietinum* L.) inoculated with plant growth promoting rhizobacteria. Aust J. Crop Sci. 5(1): 44-48.

Rosas S.B., Andre´ s J.A., Rovera M. and Correa N.S. 2006. Phosphate-solubilizing *Pseudomonas putida* can influence the rhizobia-legume symbiosis. Soil BiolBiochem. 38: 3502-3505.

Sagoe C., Ando T., Kouno K. and Nagaoka T. 1998. Relative importance of protons and solution calcium concentration in phosphate rock dissolution by organic acids. Soil Sci. Plant Nutr. 44: 617-625.

Saikia SP and Jain V. 2007. Biological nitrogen fixation with non- legumes:an achievable able target or a dogma. Curr. Sci. 92(3): 317-322.

Schroth M.N and Hancock J.G. 1981. Selected topics in biological control. Ann Rev Microbiol. 35: 453-476.

Shanmugam P.M. and Veeraputhran R. 2000. Effect of organic manure, biofertilizers, inorganic nitrogen and zinc on growth and yield of rabi rice. Madras Agric J. 2: 87-90.

Shehata M.M. and El-khawas S.A. 2003. Effect of biofertilizers on growth parameters, yield characters, nitrogenous components, nucleic acids content, minerals, oil content, protein profiles and DNA banding pattern of sunflower (*Helianthus annus* L. cv. Vedock) yield. Pak. J . Biol. Sci. 6(14): 1257-1268.

Subbarao N.S. 1988. Phosphate solubilizing micro-organism. In: Biofertilizer in agriculture and forestry. Regional Biofert. Dev. Centre, Hissar, India. pp. 133-142.

Sundara B., Natarajan V. and Hari K. 2002. Influence of phosphorus solubilizing bacteria on the changes in soil available phosphorus and sugarcane yields. Field Crops Res. 77: 43-49.

SundraRao W.V.B and Sinha M.K. 1963. Phosphate dissolving organisms in soil and rhizosphere. Indian J. Agric Sci. 33: 272-278.

Tambekar D.H, Gulhane S.R, Somkuwar D.O, Ingle K.B and Kanchalwar S.P. 2009. Potential Rhizobium and phosphate solubilizers as a biofertilizers from saline belt of Akola and Buldhana district, India. Res. J. Agric. Biol. Sci. 5(4): 578-582.

Tao G., Tian S., Cai M. and Xie G. 2008. Phosphate solubilizing and mineralizing abilities of bacteria isolated from soils. Pedosphere. 18: 515-523.

Vazquez P., Holguin G., Puente M., Cortes AE. and Bashan Y. 2000. Phosphate solubilizing microorganisms associated with the rhizosphere of mangroves in a semi arid coastal lagoon. Biol Fertil Soils. 30: 460-468.

Vlek P.L.G. and Vielhauer K. 1994. Nutrient management strategies in stressed environments. In: Stressed ecosystems and sustainable agriculture. Virmani SM, Katyal JC, Eswaran H, Abrol IP (Eds.). Oxford and IBH Publishing Co., New Delhi, India. pp. 203-229.

Wani S.P. 1990. Inoculation with associative nitrogen-fixing bacteria: Role in cereal grain production improvement. Indian J. Microbiol. 30: 363-393.

Wani S.P., Rego T.G., Rajeshwari S. and Lee K.K. 1995. Effect of legume–based cropping systems on nitrogen mineralization potential of Vertisol. Plant Soil. 175(2): 265-274.

Weller D.M. 1988. Biological control of soil borne plant pathogens in the rhizosphere with bacteria. Ann Rev Phytopathol. 26: 379-407.

Whitelaw M.A. 2000. Growth promotion of plants inoculated with phosphate solubilizing fungi. Adv Agron. 69: 99-151.

Xiao C.Q., Chi R.A., Huang X.H. and Zhang W.X. 2008. Optimization for rock phosphate solubilization by phosphate-solubilizing fungi isolated from phosphate mines. Ecol. Eng. 33: 187-193.

Yahya A. and Azawi S.K.A. 1998. Occurrence of phosphate solubilizing bacteria in some Iranian soils. Plant Soil. 117: 135-141.

Zaddy E. and Perevolosky A. 1995. Enhancement of growth and establishment of oak seedling by inoculation with *Azospirillum brasilense*. Forest Eco Manage. 72: 81-83.

Zaddy E., Perevolosky A. and Okon Y. 1993. Promotion of plant growth by inoculation with aggregated and single cell suspension by *Azospirillum brasilense*. Soil Biol. Biochem. 25: 819-823.