

## Biological fertilization

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With respect to the adverse effects of chemical fertilization on the environment and their related expenses, especially when overused, alternative methods of fertilization have been suggested and tested. For example, the combined use of chemical fertilization with organic fertilization and/or biological fertilization is among such methods. It has been indicated that use of organic fertilization with chemical fertilization is a suitable method of providing crop plants with adequate amount of nutrients, while environmentally and economically appropriate. In this article the importance of soil microbes to the ecosystem is reviewed, with particular emphasis on the role of PGPR in providing necessary nutrients for plant growth and yield production. Although there has been extensive research work regarding the use of microbes as a method of fertilizing plants; it is yet a question how the efficiency of such microbial fertilization to the plants can be determined and increased. In other words, how the right combination of chemical and biological fertilization can be specified. In this article also the most recent advances regarding the effects of microbial fertilization on plant growth and yield production in their combined use with chemical fertilization is reviewed.

**Key words:** Arbuscular mycorrhiza, biological fertilization, chemical fertilization, organic fertilization, PGPR, soil microbes

### Introduction

Plant growth and yield production is affected by different parameters including soil, plant and climate properties. Altering soil properties including physical, chemical and biological ones can influence plant growth and yield production. There are different methods of adjusting soil properties, resulting in enhanced soil production. For example, addition of soil organic matter is a favorable way of improving soil properties (Bohme and Bohme, 2006) by providing a favorable soil structure, enhancing soil cation exchange capacity, increasing the quantity and availability of plant nutrients and providing the substrate for microbial activities. However, the availability of plant nutrients is a determining factor for plant growth and yield production.

Plants require macro- and micro-nutrients for their optimal growth and production. Among the different methods of enhancing nutrients quantity and availability for plant utilization is the use of chemical fertilization, which is a fast way of providing plants with necessary macro- and micro-nutrients. With the rapid growth of world population the use of chemical fertilization has tremendously increased and hence the probability of environmental pollution. Such unfavorable effects include leaching, runoff, emission and eutrophication of aquatic ecosystems resulted by excess amounts of chemical fertilization (Flessa et al., 2002; Vessey et al., 2003; Ma et al., 2007; Adesemoye and Kloepper, 2009a, b; Yang et al., 2009). Hence, it is important to optimize the use of chemical fertilization to fulfill crop nutrient requirements and to minimize the risk of environmental pollution.

With respect to the effects of different parameters affecting nutrients amount and availability, it is likely to predict the optimal amounts of nutrients for yield production (Miransari and Mackenzie, 2010a, b, c). Nutrient availability is a function of nutrient chemical properties, soil and climate properties and plant species. Some nutrients like nitrogen (N) and potassium (K) have higher mobility relative to the others such as calcium (Ca), phosphorous (P), magnesium (Mg) and micronutrients. Such a property can definitely influence nutrient availability and hence plant growth and yield production. Although the higher mobility and availability of nutrients in soil make them more available to crop plants, such a property can also make them more vulnerable to leaching, especially in humid areas. Nutrients with less solubility can precipitate immediately after adding to the soil. For example, due to the little availability of P compounds, after fertilizing soil with P products in the first year, on average only 20% of the P will be available to the plant and the remaining part would precipitate.

Furthermore, the environmental issues regarding the use of chemical fertilization is also of significance as excess amount of chemical fertilization results in the pollution of the environment. Chemical fertilization can also decrease the enzyme activities of soil microbes, soil pH, and soil structure (Bohme and Bohme, 2006). It is therefore pertinent to apply the optimum amounts of fertilization in the field. Accordingly, it can be favorable that other methods of fertilization be also tested and used to provide necessary nutrients for plant growth and yield production, while keeping the soil structure in good shape and the environment clean.

The alternative methods of soil fertilization are organic fertilization, including the addition of manure and plant residue, use of legumes as green fertilization, and use of soil microbes. The advantages of adding plant residues are improved soil structure, enhanced nutrient availability, and benefits for environment. However, relative to chemical fertilization ( $\text{kg ha}^{-1}$ ) the efficiency of organic fertilization is less, because much higher amounts of organic fertilization ( $\text{ton ha}^{-1}$ ) is necessary to supply the adequate amounts of plant nutrients.

The other alternative method of providing nutrients for plant growth and yield production is use of soil microbes, which have been proved to be advantageous (Adesemoye et al., 2008; 2009a, b; Berg, 2009). There are a wide range of microbes in the soil, which are able to act in symbiosis or non-symbiosis association with their host plant (Gray and Smith, 2005). Soil microbes, are a necessary part of soil ecosystem and can handle the following important functions in the soil (Emmerling et al., 2002; Bohme and Bohme, 2006; Daei et al., 2009; Jalili et al., 2009; Abbaszadeh et al., 2010; Arzanesh et al., 2010) : 1) recycling soil nutrients available in organic form, 2) enhancing soil nutrient availability and hence uptake by plant, 3) improving soil structure by producing different biochemicals, 4) controlling the adverse effect of pathogens on plant growth (Haas and Defago, 2005), 5) and alleviating soil stresses on plant growth and yield production.

Plant growth promoting rhizobacteria (PGPR) are among such soil microbes greatly contributing to enhanced plant growth and yield production. However, it is yet a question that how complementary can be the use of organic or microbial fertilization to chemical fertilization. In other words how the efficiency of chemical fertilization can be increased by using the alternative methods of fertilization, with respect to the environmental and economical points of views.

### **Plant Growth Promoting Rhizobacteria (PGPR)**

Among the most effective soil bacteria, which can promote plant growth, are plant growth promoting rhizobacteria (PGPR). Such bacteria are able to colonize root surface, as a result of some signal communications between the host plant and the bacteria (Bianciotto et al., 2000). PGPR include a wide range of soil microbes including the microbes, which are in symbiosis with their host plant like rhizobiums, fixing atmospheric N<sub>2</sub>, and the ones, which are not in symbiosis association with their host plant such as *Pseudomonas* spp., *Bacillus* spp., *Azospirillum* spp. and *Burkholderia* spp. (Glick and Penrose, 1998).

In their association with their host plant PGPR may differently colonize their host plant. They may colonize the rhizosphere, the root surface or the intercellular spaces of the host plant. The colonizing ability of PGPR is determined by utilizing organic acids rather than sugars, their chemotaxis response and mobility, and production of lypopolysaccharides and proteins (Lugtenberg and Bloemberg, 2004).

The benefit of the host plant to PGPR includes the production of organic products by the plant roots (rhizodeposition), utilized by PGPR in the rhizosphere, on plant roots or the in the intercellular places called nodules (rhizobium). Nodules are the intimate structure of the differentiated root cells for the fixation of atmospheric N<sub>2</sub> by rhizobium. Soil rhizobium in the genera *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Azorhizobium*, *Mezorhizobium* and *Allorhizobium* belong to the family Rhizobiaceae and are collectively called rhizobia invading the host plant root and fixing atmospheric N<sub>2</sub> (Martinez-Romero and Wang, 2000).

Rhizosphere is the soil environment, for root growth and is specified by the high volume of root products and hence microbial population. Soil microbes are usually distributed in a 50 µm distance from the plant root and at the 10 µm distance their population may increase up to 10<sup>9</sup> - 10<sup>12</sup> /g soil. However, interestingly although a large microbial population is present in the rhizosphere only 7-15% of plant roots are colonized by soil microbes (Pinton et al., 2001).

As previously mentioned different nutrients are necessary for plant growth and yield production. Chemical fertilization is a fast way of providing plants with their necessary nutrients. However, because of the following deleterious effects on the environment they are not recommendable at high rates: 1) the increased concentrations of NO<sub>3</sub>, which is not recommendable at concentrations higher than 50 mg/l, in different water sources (surface and ground) by WHO, 2) their unfavorable effects on the soil structure and soil pH, and 3) the emission of greenhouse gases such as N<sub>2</sub>O. Accordingly researchers have tested and proved the methods by which the efficiency of rhizobium-plant symbiosis can be enhanced under different conditions including stress (Gray and Smith, 2005).

### **Effects of soil microbes on the soil ecosystem**

#### **Enhancing nutrient uptake**

Soil microbes including arbuscular mycorrhizal (AM) fungi and PGPR are able to enhance the availability of different nutrients by utilizing different mechanisms including the production of different enzymes. AM fungi can enhance the solubility and availability of different nutrients including P and micronutrients. AM fungi produce phosphatases, which can enhance P availability to the plant under different conditions including soil stresses. AM hypha are able to grow in parts of the soil, which plant roots are not able to grow. It is because, AM hypha are finer than even the finest plant root hairs and hence grow into the smallest soil micropores absorbing water and nutrients. Such a character can significantly enhance plant roots absorbing surface and hence the uptake of water and nutrients (Hodge et al., 2001; Harrier et al., 2001; Tanaka and Yano, 2005; Miransari et al., 2009a, b).

PGPR are also able to enhance the availability of different nutrients including N, P and micronutrients. For example, *Rhizobium* spp., in symbiosis with their legume host plant, and *Azospirillum* in non-symbiotic association with their

host plant, can fix atmospheric N<sub>2</sub> (Miransari and Smith, 2007; 2008; 2009; Abbaszadeh et al., 2010; Arzanesh et al., 2010). PGPR including *Bacillus* spp. *Pseudomonas fluorescense* and *P. putida* are able to enhance P availability, by production of organic acids and phosphatase enzymes. Through producing siderophores, PGPR can also increase Fe solubility and hence uptake by plant (Glick et al., 1998; Jalili et al. 2009; Abbaszadeh, et al., 2010; Zabihi et al., 2010).

#### Recycling organic nutrients

Some of the soil microbes are able to mineralize soil organic matter in the soil. Although usually are not among soil PGPR, however, their presence in the soil is important because they make nutrients available to plants and microbes by recycling them in the soil. For example, in areas with acceptable level of soil organic matter, soil nitrifying bacteria are able to mineralize organic N to nitrite and then to nitrate, which can be absorbed by plant. The other nutrients in soil organic matter can also become available to plants and microbes by soil microbes. It is because soil microbes have the necessary enzymes for nutrients turnover. Soil microbes can act specifically in that regard, because they have some of enzymes for the mineralization of soil organic matter (Bohme and Bohme, 2006).

Soil organic matter is a more efficient and useful source of energy for soil microbes as determined by parameters including qCO<sub>2</sub> (CO<sub>2</sub> – microbial C). Chemical fertilization results in higher qCO<sub>2</sub> values indicating that under such conditions soil microbes are more stressed relative to the use of organic fertilization, or soil microbes must use higher rate of energy to compensate for the adverse effects of stress. Soil fungi have a lower rate of qCO<sub>2</sub> than soil bacteria, indicating that soil fungi are more efficient than soil bacteria (Bohme and Bohme, 2006).

#### Improving soil structure

Soil particles are bound by organic chemicals including compounds produced by soil microbes. A wide range of biochemicals are produced by soil microbes among which poly saccharides are the ones with the highest impact on binding soil particles. In addition, AM fungi are able to produce a glycoprotein called glomalin, binding soil aggregates and hence improving soil structure. Similar to plant roots, AM hypha by itself can also bind soil particles and hence results in the production of soil aggregates. The mineralizing effects of soil microbes on organic matter can also influence soil structure. The enhanced growth of plant growth by soil microbes and hence the increased amount of root exudates and rhizodeposition can also affect soil structure directly or by increasing the microbial population and activities, indirectly (Rillig and Mummey, 2006).

#### Controlling pathogens

Soil microbes produce a wide range of biochemicals, affecting soil environment. Among which there are the products, adversely affecting the growth and activities of soil pathogens including soil bacteria and soil fungi. For example, PGPR produce hydrogen cyanide (HCN), which can have unfavorable effects on the growth of soil pathogens. In addition through stimulating plant systemic resistance, soil microbes can enhance plant resistance to pathogens (Principe et al., 2007). The presence of soil microbes in the rhizosphere and production of different compounds can stimulate plant growth and systemic resistance. This is through the expression of plant genes, which can in turn produce some products affecting plant resistance to pathogens or the production of root exudates, which can affect soil microbial population and activities in the rhizosphere (Principe et al., 2007). For example, Ryu et al. (2003) indicated that the induction of systemic resistance (ISR) in plant is stimulated in the presence of *Bacillus* spp., through the production of volatiles including butanediol and acetoin. Generally, the adverse effects of PGPR, which is mostly related to *Pseudomonas* spp. and *Bacillus* spp. on soil pathogens is through the following: 1) production of antibiotic compounds (HCN, pyrrolnitrin, phloroglucinols, phenazines, pyoluteorin, and cyclic lipopeptides), which for example can inhibit electron transport 2) plant induced systemic resistance, and 3) interfering with pathogens ability to suppress plant growth, for example through degrading the unfavourable products produced by the pathogen (Haas and Defago, 2005). It has been indicated that the pathway resulting in the production of jasmonate/ethylene production is more important than the salicylate related pathway for plant ISR (Ton et al., 2002; Verhagen et al., 2003).

In addition, PGPR are able to alleviate the adverse effects of stresses on plant growth through different mechanisms. The alleviating effects of PGPR on plant growth enhances if the strains are isolated from the soils under stress. In other words if the strains are isolated from soils, which are subjected to salinity or acidity or drought they can more effectively alleviate the stress (Daei et al., 2009; Jalili et al., 2009). Furthermore, there are also other mechanisms by which PGPR can enhance plant growth under stress including: 1) enhanced production of osmolytes under drought or salinity stress, 2) enhanced activities of plant antioxidants, which can catabolize the stress products, 3) production of different metabolites by PGPR or by plant as a result of the symbiosis association, 4) production of ACC deaminase, which can help plant survive under stress, 5) the solubilization of insoluble P products, 6) siderophore production, 7) inhibition of pathogens (Chebotar et al., 2009), and 8) adjustment of ions ratio for example K/Na<sup>+</sup> under stress.

### Alleviating soil stresses

In the recent years there have been some interesting research work regarding the use of PGPR under stress. For example, Glick et al. (1998) indicated that under different stresses PGPR such as *Pseudomonas fluorescense* and *P. putida* can alleviate the adverse effects of stress on plant growth through the production of the bacterial enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase. ACC is the prerequisite for the production of ethylene, which is increased under stress adversely affecting plant growth and yield production. Hence, ethylene is one of the stress hormones regulating plant growth under different conditions including stress. Production of ACC deaminase by PGPR can turn ACC, which is the prerequisite for ethylene production into  $\text{NH}_3$  and  $\alpha$ -ketobutyrate and hence control the stress. ACC deaminase is also able to increase root growth under stress, affecting plant growth. In addition, the alleviating effects of AM fungi on plant growth under different stresses including salinity (Daei et al., 2009) and soil compaction (Miransari et al., 2007; 2008; 2009a, b) have been indicated.

There are also other soil stressors affecting plant growth and yield production including soil pH, suboptimal root zone temperature, and heavy metals. Interestingly, researchers have found that the adverse effects of different stressors on the *Rhizobium*-legume N fixation can be alleviated by the use of signal molecule genistein. Genistein is produced by the specific legume host plant like soybean (*Glycine max* L.) activating the nodulation (nod) genes in *Bradyrhizobium japonicum* (Miransari and Smith, 2007; 2008; 2009).

Under humid or dry climate conditions soil pH can fluctuate much. The high amount of rain in the humid area reduce soil pH significantly through: 1) leaching alkaline cations such as calcium (Ca) and magnesium (Mg) or 2) enhancing the weathering processes, which eventually result in the production of iron and aluminum oxides with a high affinity for the exchange of protons with the soil solution. Under dry climate conditions there is a high concentration of alkaline cations in the soil including Ca and Mg, considerably increasing soil pH. Soil acidity can affect soil efficiency through affecting: 1) plant growth and yield production, 2) microbial population and activities, 3) soil nutrient availability, and 4) pathogens activities. Plants can grow and produce optimum yield under optimal soil pH, however, deviations from favorable pH can adversely affect plant growth and yield production. It is because different physiological mechanisms in the plant are catalyzed by different enzymes, which can act at a favorable pH. Unfavorable pH can decrease plant growth by adversely influencing the enzyme activities in the plant and the cellular pH, whose optimum level is necessary for appropriate cell activities. Plants can adjust their cellular and rhizosphere pH by different mechanisms. For example, production of different biochemicals by plant cells can adjust cellular pH as well as the rhizosphere pH, affecting microbial activities and nutrient availability.

A wide range of organic products are produced by plant roots due to the process of rhizo-deposition including carbohydrates, organic and carboxylic acids significantly influencing plant rhizosphere and its microbes. Such products are a significant carbon source for soil microbes. They can affect microbial activities for example as secondary metabolites such as signal molecules activating the microbial genes in symbiosis or non-symbiosis association with their host plant, adjust soil pH by producing  $\text{H}^+$  and hence the availability of soil nutrients (Benizri and Amiaud, 2005). In addition, plant growth stage and root properties can influence the rhizodeposition properties. There are different parameters affecting the quantity and quality of rhizodeposition including temperature, light,  $\text{CO}_2$ , and agricultural practices (Rovira et al., 1959; Cheng and Johnson, 1998; Nicol et al., 2003).

Furthermore, the genetic combination of soil microbes is influenced by agricultural practices. It is because agricultural practices and soil and climate properties influence plant diversity and accordingly the quality of plant rhizodeposition and soil organic matter and hence the genetic combination of soil microbes is affected (Quideau et al., 2001; Warembourg and Estelrich, 2001).

Different soil microbes including AM fungi and PGPR are able to alleviate salinity stress on plant growth. AM fungi, as previously mentioned, can significantly increase plant water and nutrient uptake through their extensive hyphal network. This can be advantageous under different conditions including salinity. AM fungi can also alleviate the salinity stress on plant growth and hence yield production by adjusting the ratio of  $\text{Na}^+$  and  $\text{Cl}^-$  in the plant. Mycorrhizal plants are able to absorb higher rate of K under salinity, adjusting the K/Na ratio in the plant and hence alleviating the stress (Daei et al., 2009). Under stress plant allocate more C to their roots, which can be beneficial for the plant under stress. In addition, AM fungi can also intensify such a process by enhancing plant P uptake, which can significantly increase root and hence plant growth (Miransari and Smith, 2007; Miransari et al., 2007; 2008).

PGPR can also alleviate salinity stress on plant growth. They are able to produce the important bacterial enzyme ACC-deaminase under different conditions including stress, which can effectively control the stress. This is because, as previously mentioned, ACC-deaminase can catalyze the ACC, which is the prerequisite for the production of the stress hormone, ethylene. Increased level of ethylene in the plant can adversely affect plant growth and yield production (Glick et al., 1998; Jalili et al., 2009).

Plant physiological and morphological characters are also important in the alleviation of stress in symbiosis of non-symbiosis association with their associative soil microbes. The more resistant plant species can perform more efficiently under stress and their symbiosis with their associative soil microbes also can intensify such abilities. However, selecting the right combination of soil microbes such as AM species and host plant to achieve the highest likely efficiency under stress is also important (Daei et al., 2009). It has also been indicated that some PGPR are able to produce

polysaccharide products, binding  $\text{Na}^+$  in the root zone and hence alleviating the stress of salt on plant and microbial growth and activities (Han and Lee, 2005).

PGPR can also enhance P solubility and hence availability by producing different enzymes including phosphatase and also by increasing plant root growth. For example this can be achieved by controlling the activities of stress hormones such as ethylene or by altering plant root rhizodeposition. ACC-deaminase can catabolize ACC, which is the prerequisite for ethylene production in the plant to  $\text{NH}_3$  and  $\alpha$ -ketobutyrate and hence controlling the adverse effects of ethylene on plant growth (Glick et al., 1998; Jalili et al., 2009; Abbas Zadeh et al., 2010; Zabihi et al., 2010).

Plants are also able to produce extracellular phosphatase, as the molecular pathways for the production of phosphatase have been indicated (Miller et al., 2001; George et al., 2008; Richardson et al., 2009). Plants use different strategies to improve their P uptake efficiency as indicated in the following:

1) Adjustment of their root morphology, for example by producing more lateral roots, which can be used as a suitable trait for plant selection for breeding (Lynch, 2005; Liao et al., 2008). 2) The ability of plant species for the production of organic acids; there are some plant species such as chick pea and pigeon pea, which are efficient in producing organic acids. This can be advantageous, especially under rotation, because cropping plants with higher efficiency to produce organic acids, prior to the less efficient plants, can enhance P availability in the soil for a longer time for the following crop (Wouterlood et al., 2004a; b). 3) The altered expression of genes, producing organic anions, can be effective in plants, with the low ability to produce organic anions. For example citrate synthase, which is the important enzyme for the production of citrate, as well as phosphoenol pyruvate carboxylase and malate dehydrogenase have been tested and shown to be promising in some cases. In addition, the alteration of other genes, including the ones responsible for the production of citrate permeable and malate channels, transferring the anions from the roots to the rhizosphere can also be used as the alternative (Sasaki et al., 2004). 4) The ability of plants to produce phosphatase, which is another approach for breeding selection (Richardson et al., 2009).

### Interaction between soil microbes

There is a wide range of microbes in the soil, contributing to some of the most important processes in the soil necessary for efficient soil production. In addition, soil microbes are interactive antagonistically or synergistically affecting their efficiency in the soil. Such a property can also influence the production and use of bioinoculants. Among the most influential interactions between different soil microbes are the interactions between AM fungi and soil bacteria (Artursson et al., 2006).

AM fungi are able to alter soil bacterial combination by affecting root growth and hence rhizodepositions, which are important sources of nutrients and may also, act as secondary metabolites for soil bacteria in the mycorrhizosphere (Gryndler, 2000). In addition, through the competition for nutrients and the highly specific responses of some bacteria to certain AM species, due to the production of some AM products including polysaccharide products (Artursson and Jansson, 2003; Toljander et al., 2005), the interactions between AM fungi and soil bacteria can be significant.

Different researchers have indicated the positive interactions between AM fungi and PGPR including *Pseudomonas* spp., *Bacillus* spp., *Paenibacillus* spp., *Rhizobium* spp., and *Enterobacter* spp. Gram-positive bacteria may be more associative with AM relative to Gram-negative bacteria. PGPR may also be able to colonize AM hypha (Hilderbrandt et al., 2002; Artursson et al., 2006).

### PGPR and fertilization

PGPR can be used both under stress for alleviating the stress on plant growth and used singly or in combination with other forms of fertilization including chemical and organic to increase plant growth and yield production. The selection of the appropriate strains for the enhanced efficiency of PGPR under different conditions is of significance. It indicates that PGPR must be exactly screened for their growth promoting characters, and be tested under different conditions including stress for the selection of the most efficient strains.

As previously mentioned, different strains of PGPR can enhance the availability of different nutrients. *Rhizobium* spp. and *Azospirillum* spp. can fix N and hence are important in combination with N fertilization. In other words, at the time of inoculating plants with *Rhizobium* or/and *Azospirillum* it must be exactly indicated that if the bacteria alone is able to supply the host plant with adequate amount of N or N fertilization must also be utilized to provide the plant with N requirement.

Usually for legumes the rhizobium is able to fix N at high amount so that additional fertilization would not be necessary, though N fertilizer may be used as starter. However, in the case of PGPR including *Azospirillum* although atmospheric N is fixed by the bacteria, it is not adequate for the plant requirements. Hence, N chemical fertilization would also be necessary to supply N for optimal plant growth and yield production. For P there are also some similarities, although the P solubilizing microbes usually cannot provide the complete P requirement for optimal plant growth (Zabihi et al., 2010).

Soil P solubilizing microbes including AM fungi and bacteria can enhance P solubility by producing enzymes such as phosphatase. The synergistic interactions between AM fungi and PGPR including N-fixing *Rhizobium* can enhance the efficiency of soil microbes. Especially, in tripartite symbiosis AM hypha in the soil can result in the movement of soil bacteria through different parts of the soil (Bonfante, 2003; Arthurson et al., 2006). This is of importance for the production of biofertilizers, because the right combination of soil microbes can enhance the biofertilizer efficiency (Adesemoye et al., 2008; Yang et al., 2009).

An interesting and important point about the combined use of soil microbes and chemical or/and organic fertilization is to determine their appropriate rates. This is of environmental and economical significance. There are several important key issues about this: 1) the microbial potential for providing nutrients under certain conditions for plant utilization, 2) plant species, 3) soil properties, and 4) climate properties. Soil microbial potential for enhanced nutrient availability differs under different conditions. Such abilities must be tested under different conditions to determine their capacity for providing nutrients for plant use. Plant species are also of importance as there are high variations among different plant species for their nutrient requirements. Soil properties can affect both plant and microbial growth. Climate properties affect microbial activities and plant growth and hence the related fertilization rates.

For example, under humid conditions mineral nutrients in different form of fertilization are subjected to leaching, which can definitely affect the fertilization rates. For example it has been indicated that with respect to the higher levels of soil organic matter under humid conditions and the subsequent mineralized nutrients, such amount of nutrients must also be noticed, when developing fertilization recommendations (Miransari and Mackenzie, 2010a, b, c). This can have similarities to the combined use of soil microbes and chemical or/and organic fertilization.

PGPR efficiency to increase plant growth is dependent on the level of nutrients in the soil. According to Carlier et al. (2008), Adesemoye et al. (2008; 2009a) and Zabihi et al. (2010) increased level of NPK fertilization decreased the efficiency of *Pseudomonas* spp. They attributed such observations to the production of stress level ethylene under low levels of NPK, whose prerequisite is catabolized by ACC deaminase to  $\text{NH}_3$  and  $\alpha$ -ketobutyrate. In addition root growth and efficiency is of importance under nutrient deficiency and higher root growth results in higher nutrient uptake and hence plant growth. Accordingly, PGPR, with higher efficiency under nutrient efficient conditions, are able to increase plant growth by enhancing root growth and hence nutrient uptake (Glick et al., 1998; Jalili et al., 2009; Zabihi et al., 2010).

Determining the efficiency of chemical and biological fertilization is of significance as the contribution and hence the optimum rate of each would be clearly indicated. It has been indicated that PGPR are not a complete replacement for chemical fertilization with higher efficiency relative to PGPR (Zabihi et al., 2010; Salimpour et al., 2010). For example, Canbalt et al. (2006) examined the effects of chemical and biological fertilization on plant growth and nutrient uptake and indicated that chemical fertilization resulted in higher NPK uptake by plants. However, on the other hand, Elcoka et al. (2008) indicated that the efficiency of biological fertilization is a matter of the microbial combination as double and triple inoculation with PGPR may significantly enhance the bio fertilizer efficiency. Shaharoon et al. (2008) and Cruz et al. (2009) indicated that the performance of *Pseudomonas* spp. in enhancing plant growth is fertilizer dependent. However, more research is necessary to clearly indicate the contributing role of chemical and biological fertilization in providing necessary nutrients for plant growth and yield production.

According to the research, conducted so far, use of chemical fertilization is necessary as biological fertilization has not yet been proven to be good enough for completely providing plant nutrient requirement. However, what matters about chemical fertilization is to enhance its efficiency environmentally and economically using biofertilizers. It has been indicated that about less than 50% of chemical fertilizers is absorbed by plant and the rest would not be accessible by plant as it is subjected to leaching, run-off and emission from the soil (Adesemoye et al., 2009). Hence, use of biological fertilizers as supplementary fertilization to chemical fertilization is necessary with the above mentioned advantages. Accordingly, the right and proper application of chemical and biological fertilization is much dependent on realizing the interactions between soil, plant and microorganisms. Soil microbes are a big help to plant and the environment as they own some abilities that collectively enhance plant growth. Among such abilities enhanced nutrient uptake by plant is also of importance; in the presence of soil microbes, plant absorb higher amounts of nutrients and less risk of environmental pollution is likely.

## Conclusion

Some of the most important functions of soil microbes were reviewed in this article. However, the particular emphasis has been on the use of soil microbes including AM fungi, *rhizobium* and other PGPR for biological fertilization. Chemical fertilization is the common method of providing plants with their necessary nutrients because of its rapid effects on plant growth and yield production. However, there are important issues regarding the use of chemical fertilizers, as their improper and excess use can adversely affect the environment. Accordingly, it is important to indicate the contribution of chemical and biological fertilization to the plant growth. This can be used for the development of proper methods of fertilization. For the efficient development of biofertilizers the microbes must be properly selected, combined and formulated with respect to the present conditions. The appropriate use of fertilization,

which is a combination of chemical and biological fertilization can much contribute to the enhanced food production in the world, while economically and environmentally recommendable.

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