An overview of plant growth promoting rhizobacteria and their influence on essential oils of medicinal plants

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Abstract

One of the important and necessary practices for improving nutrients availability in sustainable agriculture is using microorganisms. Beside the negative effects of chemical fertilizers on the soil and human health, plant growth promoting rhizobacteria are known as an alternative to supply the organic nutrients of plants during the past decades. Enriching soil fertility by eco-friendly methods in medicinal plants could well-support plants growth and production. Most studies found that bio-fertilizers such as Plant Growth Promoting Rhizobacteria (PGPR) could promote physio-morphological characteristics and yield of medicinal plants. The mechanisms of plant growth promoting rhizobacteria could be summarized in symbiotic and associative nitrogen fixation, solubilization and mineralization of nutrients, production of phytohormones, vitamins, and antagonistic components against pathogens which enhance plant resistance to the stress and non-stress conditions. This paper also concluded that the soil type, environmental variables, soil management practices, microbial interactions and plant species could affect bacterial diversity and composition of the rhizosphere. Three major secondary metabolites of medicinal plants such as Terpenoids, phenolics and alkaloids were also increased due to the impact of microorganisms in metabolic pathway of plants such as Jasmonic acid signaling pathway. Thereby, significant increases in growth and yield of medicinal plants in response to inoculation with PGPR could be one of the promising approaches in sustainable agriculture.

Keywords: Bio-fertilizer, essential oils, mycorrhiza, N-fixation bacteria, P- solubilizing bacteria


Introduction

Essential oils due to the therapeutic activities have a great importance in the cultivation of medicinal plants while yield quantity comes in the second order of importance. For some medicinal plants, sustainable agricultural approaches are the best method to achieve better performance on the account of the harmony with nature; therefore, global approach is more focused on eco-friendly production of medicinal plants using sustainable agriculture.
agricultural systems (Sharifi Ashorabadi et al., 2002). The term bio-fertilizer refers to the microbial inoculants that contain one or more beneficial soil organisms, such as nitrogen fixing, phosphate solubilizing or cellulolytic microorganisms that provide the plant nutrient needs in a form which could be assimilated by the plant (Mohammadi et al., 2012). Since chemical fertilizers could not supply crop nutrients directly, organic fertilizers are applied with special bacteria and fungi. In fact, bio-fertilizers could be introduced as a good alternative to chemical fertilizers eliminating several negative impacts of the chemical fertilizers on the environment and sustainable agriculture (Wu et al., 2005). Nitrogen fixation bacteria such as *Rhizobium* and *cyanobacteria*, bio-inoculants namely, *Azotobacter*, *Azospirillum*, Phosphorus Solubilizing Bacteria (PSB), siderophores, and Vesicular Arbuscular Mycorrhiza (VAM) could be regarded as a broad spectrum of bio-fertilizers (Gupta, 2004).

Recently, environmentally-friendly agricultural practices have attracted a lot of attention. A considerable number of bacterial species could handle a beneficial effect on plant growth. Application of these bacteria and crop production have been the focus of many studies in agriculture. Microbial populations are key components of the soil–plant continuum where they are involved in interactions affecting plant development (Vassilev et al., 2006). Plant growth promoting rhizobacteria (PGPR) or root-colonizing bacteria are known as effective factors for plant growth. In fact, most of the effective colonizers are from species of *Azospirillum*, *Alcaligenes*, *Arthrobacter*, *Acinetobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Pseudomonas*, and *Rhizobium*, *Serratia* (Krishnamurthy et al., 1998 and Tilak et al., 2005). PGPR plays an important role in many of ecosystem processes such as those involved in the biological control of plant pathogens, N fixation, solubilizing of nutrients, and phytohormone synthesis. In general, the beneficial effects of these rhizobacteria on plant growth can be categorized into direct or indirect mechanisms (Lugtenberg and Kamilova, 2009).

**Direct mechanisms**

**Nitrogen fixation**

The process of micro-organisms fixing atmospheric nitrogen is called Biological Nitrogen Fixation (BNF) where using a complex enzyme system known as nitrogenase, N₂ in the atmosphere changes to ammonia (Fig. I). This is mostly done within subsoil plant nodules making the nitrogen available for assimilation by plants (Odame, 1997).

Nitrogen fixing organisms are generally categorized as (a) symbiotic N₂ fixing bacteria including members of the family rhizobiaceae which forms symbiosis with leguminous plants (e.g. *rhizobia*) (Zahran, 2001) and non-leguminous trees (e.g. *Frankia*) and (b) non-symbiotic (free living, associative and endophytes) N₂ fixing bacteria.

Examples of free living nitrogen fixing bacteria are classified into obligate anaerobes (*Clostridium pasteurianum*), obligate aerobes (*Azotobacter*), facultative anaerobes, Oxygenic photosynthetic bacteria (*Nostoc commune* belonging to species of *cyanobacterium*), Anoxygenic photosynthetic bacteria, (*Rhodobacter*), and some methanogens (Bhattacharyya and Jha, 2012; Mohammadi and Sohrabi, 2012). Generally, rhizobacteria could affect plant in two ways: some rhizobacteria fix atmospheric nitrogen, making it available to the plant and thereby promoting plant growth in nitrogen-deficient soils. Other rhizobacteria directly impress plant growth by production of hormones. These beneficial root-interactive

![Fig. I. Nitrogen fixation cycle](image-url)
Phosphate solubilization

Soil P is mainly found in insoluble forms which is not available for plants, while the plants absorb it only in two soluble forms, the monobasic ($H_2PO_4^-$) and the dibasic ($HPO_4^{2-}$) ions (Bhattacharyya and Jha, 2012). The phospho-microorganisms which are mainly bacteria and fungi, make insoluble phosphorus available to the plants (Gupta, 2004) (Fig. II). Some of the soil bacteria and a few species of fungi by secreting organic acids can bring insoluble phosphate into soluble forms (Gupta, 2004). Examples of P-Solubilizing Bacteria (PSB) are Bacillus, Beijerinckia, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Microbacterium, Pseudomonas and Serratia (Bhattacharyya and Jha, 2012).

A universal and important symbiosis phenomenon in the nature is Mycorrhiza, and Arbuscular Mycoriza (AM) is the most widespread mycorrhiza type developed from the terrestrial plant roots and Zygomycete fungus (Lin et al., 2010). AM is one of the essential factors in low-input sustainable agriculture so that, production of many agricultural and horticultural crops in soil is dependent on it (Bethlenfalvay and Linderman, 1992). Most studies show that in the presence of Mycorrhiza increase in absorption of mineral nutrition and plants growth, tolerability to the drought, and toxic pollution could be seen (Fig. III).

Indirect mechanisms

The application of PGPR could indirectly control plant diseases and keep them from negative effects of environmental stress conditions and in some ways, could promote plant characteristics (Kamilova and Lugtenberg, 2009; Vacheron et al., 2013). Then, indirect effects of PGPRs could be linked to the production of phytohormones and biocontrol agents.

Phytohormones

Several PGPR strains like Azospirillum brasilense are able to produce NO which is involved in the auxin signaling pathway controlling lateral root formation (Molina-Favero...
et al., 2008). DAPG (2,4-diacetylphloroglucinol) is a well-known antimicrobial compound produced by biocontrol fluorescent pseudomonads (Couillerot et al., 2009) and at lower concentrations involved in systemic resistance (Bakker et al., 2007), stimulating root exudation (Phillips et al., 2004) and enhancing root branching (Walker et al., 2011). Cytokinin production (especially Zeatin) has been documented in various PGPR like Arthrobacter giacomelloi, Azospirillum brasilense, Bradyrhizobium japonicum, Bacillus licheniformis, Pseudomonas fluorescens and Paenibacillus polymyxa (Vacheron et al., 2013). Cytokinins stimulate plant cell division, control root meristem differentiation and induce proliferation of root hairs while inhibiting lateral root formation and primary root elongation (Riefler et al., 2006). Ethylene is another key phytohormone, which inhibits root elongation and auxin transport, promotes senescence and abscission of various organs and leads to fruit ripening (Glick et al., 2007). The ability of Azospirillum brasilense to produce ethylene presumably promotes root hair development in tomato plants (Ribaudo et al., 2006). Several reports have revealed that ABA produced by PGPRs is involved in drought stress by closing stomata and limiting water loss (Bauer et al., 2013). Production of gibberellins has been documented in several PGPR belonging to Achromobacter xylosox-idsans, Acinetobacter calcoaceticus, Azospirillum spp., Azotobacter spp., Bacillus spp., Herbaspirillum seropedicae, Gluconobacter dia-zotrophicus and rhizobia (Gutiérrez-Mañero, 2001, Bottini et al., 2004, Dodd et al., 2010). Gibberellins promote primary root elongation and lateral root extension (Yaxley et al., 2001). Although the production of hormones by PGPR has been well described, the genetic determinants involved in their biosynthesis remain largely unknown and bacterial mutants affected in hormone biosynthesis are mostly lacking (Vacheron et al., 2013) (Fig. III).
Table 1
Effects of PGPR strains on medicinal plants growth characteristics and essential oil yield

<table>
<thead>
<tr>
<th>PGPR</th>
<th>Plant species</th>
<th>Results of addition of bacteria to plants</th>
<th>References</th>
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<tbody>
<tr>
<td><strong>G. moseae and B. subtilis</strong></td>
<td>Thymus daenensis</td>
<td>75% increase in shoot/root dry weight, 117% in plant yield and stimulated essential oil yield by 93% compared to non-inoculated controls or to plants single inoculated.</td>
<td>(Bahadori et al., 2013)</td>
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<tr>
<td><em>Arbuscular Mycoriza</em></td>
<td>Lemon grass (Symbopogon martini)</td>
<td>Percentage of essential oil and essential yield increased by mycorrhizal inoculation in comparison with non-inoculated</td>
<td>(Gupta, 1990; Khalil, 1997)</td>
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<tr>
<td><em>Glomus macrocarpum</em> and</td>
<td>Foeniculum vulgare.</td>
<td>Growth characteristics and essential oil concentration significantly improved</td>
<td>(Kapoor et al., 2004)</td>
</tr>
<tr>
<td><em>Glomus fasciculatum</em></td>
<td>Palmarosa (Cymbopogon martini)</td>
<td>Biomass and phosphorus content maximized</td>
<td>(Ratti et al., 2001)</td>
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<tr>
<td><em>Polymyxa</em> and <em>Azospirillum</em></td>
<td>Fennel (Foeniculum vulgare)</td>
<td>Essential oil and phenolic content by single inoculation and co-inoculation of <em>Pseudomonas fluorescens</em> and <em>Azospirillum brasilense</em> had been significantly increased</td>
<td>(Cappellari et al., 2013)</td>
</tr>
<tr>
<td><em>Azotobacter Chroococcum</em> +</td>
<td>Rosmarinus officinalis circulateae</td>
<td>Plant height; number of branches; plant fresh and dry weights, oil percentage and yield in fresh herb and total carbohydrates were increased compared to other biofertilizers treatments</td>
<td>(Abdullah et al., 2012)</td>
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<tr>
<td><em>Bacillus megaterium</em> +</td>
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<td><em>Bacillus circulanse</em></td>
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<tr>
<td><em>G. moseae and B. subtilis</em></td>
<td>Not specific plant</td>
<td>Plant P uptake improved and enhanced essential oil content</td>
<td>(Artursson et al., 2006)</td>
</tr>
<tr>
<td><em>G. fasciculatum</em></td>
<td>Basil (Ocimum basilicum),</td>
<td>Inoculation significantly increased essential oil content and yield</td>
<td>(Rasouli-Sadaghiani et al., 2010)</td>
</tr>
<tr>
<td><em>Arbuscular Mycoriza</em></td>
<td>Basil (Ocimum basilicum),</td>
<td>Linalool formed the highest relative abundance of the main compounds in leaf essential oils</td>
<td>(Kumar et al., 2002; Mahfouz and Sharaf-Eldin, 2007; Velmurugan and Chezihiyan, 2008; Kumar et al. 2009 and Darzi et al., 2012) (Shafagh-Kolvanagh and Shokati, 2010; Shokati and Ghassemi-Golezani, 2013 and Shokati and Zehtab-Salmasi, 2014)</td>
</tr>
<tr>
<td><em>Azotobacter chroococcum</em> and</td>
<td>Coriander, Fennel,</td>
<td>Increased yield and essential oil</td>
<td></td>
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<tr>
<td><em>Azospirillum lipoferum</em></td>
<td>Davana turmeric and Dill</td>
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<td><em>Rhizobium bacteria</em></td>
<td>fenugreek (Trigonella foenum-graecum)</td>
<td>promote dill (Anethum graveolens L.) fresh and dry weight, height and umbel number, essential oil and yield components</td>
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<tr>
<td><em>Pseudomonas fluorescens</em>,</td>
<td>Origanum majorana L.</td>
<td>P. fluorescens and Bradyrhizobium sp. showed significant increases in shoot length, shoot weight, number of leaf, number of node, and root dry weight</td>
<td>(Banchio et al., 2008)</td>
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<tr>
<td><em>Bacillus subtilis</em>, <em>Sinorhizobium melloti</em>, and <em>Bradyrhizobium</em></td>
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Biocontrol agents

One of the environmentally friendly approaches in bio-controlling of diseases is using PGPRs. In this sense, interaction of some rhizobacteria with the plant roots can result in plant resistance against some pathogenic bacteria, fungi, and viruses. This phenomenon is called Induced Systemic Resistance (ISR) (Lugtenberg and Kamilova, 2009). In this process, rhizobacteria could produce antifungal metabolites like, HCN, phenazines, pyrrolnitrin, 2,4-diacetylphloroglucinol, pyoluteorin, viscosinamide, and tensin (Bhattacharyya and Jha, 2001). Moreover, ISR involves jasmonate and ethylene signaling within the plant and these hormones stimulate the host plant defense responses against a variety of plant pathogens (Glick et al., 2007). More results of PGPR strains on medicinal plants growth characteristics and essential oil contents are shown in Table 1.

Beside the positive effects of PGPRs on medicinal plants shown in Table 1, it should be mentioned that there are significant differences between the effectiveness of PGPRs. In a study to evaluate PGPR strains Pseudomonas fluorescens, Bacillus subtilis, Sinorhizobium meliloti, and Bradyrhizobium, it was found that only P. fluorescens and Bradyrhizobium sp. showed significant increases in shoot weight, shoot length, number of nodes, number of leaves, and root dry weight of Origanum majorana L. (Sweet marjoram) in comparison with control plants or plants treated with other PGPRs (Banchio et al., 2008). On the other hand, another important point to establish a strong relationship between medicinal plants and PGPRs is the genus of plant which had a meaningful effect on microbial population. Ahmed Eman et al. (2014) reported a significant difference in densities of microbial count in the rhizosphere of eleven medicinal plants viz., Ocimum basilicum, Marrubium vulgare, Melissa officinalis, Origanum syriacum, Quisqualis indica, Solidago virgaurea, Melilotus officinalis, Cymbopogon citratus, Matricaria chamomilla, Thymus vulgaris, and Majorana hortensis where the lowest populations were found in the rhizosphere of M. chamomilla and M. hortensis. Similar results have been reported showing that beside the soil type, environmental variables, soil management practices and microbial interactions, plant species could affect the diversity and composition of bacterial taxa in the rhizosphere (Backman et al., 1997, Bashan et al., 2008, Chet and Chernin, 2002, Khalid et al., 2004).

PGPRs in addition to increasing essential oil yield, biomass, and absorption of nutrients are associated with activation of octadecanoid, shikimate, jasmonate, and terpenoid pathways. In fact, one of the benefits of replacing PGPRs is developing stable formulation of antagonistic PGPR (Ghorbanpour et al., 2015). The Jasmonic Acid (JA) signaling pathway is generally regarded as an integral signal for the biosynthesis of many plant secondary products including terpenoids, flavonoids, alkaloids, and phenylpropanoids. Many elicitors (like pathogens and PGPRs) stimulate endogenous JA biosynthesis in plants, so the JA signaling pathway functions as a transducer or mediator for elicitor signaling pathways, leading to the accumulation of secondary metabolites in plants (Mueller et al. 1993). Application of methyl-jasmonate (0.5 mM) significantly increased the quantity of monoterpenes in basil (Ocimum basilicum) via increasing the number of transcripts of the enzymes linked to metabolic pathways of monoterpene (Kim et al. 2003). It should be mentioned that, terpenoids, phenolics and alkaloids are the three major groups of secondary plant metabolites used for pharmacological and therapeutical purposes (Ghorbanpour et al., 2015). Biosynthesis of terpenoids depends on the primary metabolism, e.g., photosynthesis, and oxidative pathways for carbon and energy supply (Singh et al. 1990). Accordingly, Copetta et al. (2006) suggested that increases in total essential oils yield of basil (O. basilicum) in response to inoculation were not merely due to increased biomass, and might have resulted from increased biosynthesis of terpenes. Some of the PGPRs proved to be biotic elicitors for the production of secondary metabolites in medicinal and aromatic plants are presented in Table 2.

According to Table 2, infection by microorganisms as well as physiological and genetic factors and environmental conditions are the main agents affecting the accumulation and composition of secondary metabolites in plants.
As an environmentally friendly strategy, PGPRs should be considered to achieve sustainable high yields of industrially important secondary metabolites in plants using minimum chemical inputs (Ghorbanpour and Hatami, 2014).

**Conclusion**

The trade and cultivation of medicinal and aromatic plants is an important sector in agriculture in many countries. Medicinal and aromatic plants are the main source of the well-known drugs. Increases in the prices of chemical fertilizers, avoidance of soil pollution, and the need for finding methods for increasing essential oil contents, led scientists to use bio-fertilizers like plant growth promoting rhizobacteria which would be an environmentally friendly approach. This paper by reviewing the necessity of PGPR application also indicated that PGPRs such as N fixation bacteria, Phosphorus Solubilizing Bacteria (PSB), Vesicular Arbuscular Mycorrhiza (VAM) and siderophores could improve essential oil of medicinal plant contents compared to chemical fertilizers or non-inoculate plants. This paper also concluded that the soil type, environmental variables, soil management practices, microbial interactions, and plant species could affect

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<th>Table 2</th>
<th>Efficient biotic elicitors used for the production of secondary metabolites in different plant species (Adapted from Egamberdieva et al., 2015)</th>
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<td><strong>PGPRs as elicitors</strong></td>
<td><strong>Plant species</strong></td>
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<tr>
<td><em>Pseudomonas putida</em> and <em>fluorescens</em></td>
<td><em>Hyoscyamus niger L.</em></td>
</tr>
<tr>
<td><em>Pseudomonas putida</em> and <em>fluorescens</em></td>
<td><em>Salvia officinalis L.</em></td>
</tr>
<tr>
<td><em>Bacillus polymyxa,</em> <em>Pseudomonas putida,</em> <em>Azotobacter chroococcum,</em> and <em>Glomus intraradices</em></td>
<td><em>Stevia rebaudiana</em></td>
</tr>
<tr>
<td><em>Arbuscular mycorrhizal</em> and <em>phosphatesolubilizing bacteria</em></td>
<td><em>Rose-scented geranium</em> (<em>Pelargonium sp.)</em></td>
</tr>
<tr>
<td><em>Pseudomonas fluorescens</em> and <em>Azospirillum brasilense</em></td>
<td><em>Tagetes minuta</em></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> and <em>Pseudomonas fluorescens</em></td>
<td><em>Pisum sativum</em></td>
</tr>
<tr>
<td><em>Hormonema ssp.</em> homogenates</td>
<td><em>Brugmansia candida</em></td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td><em>Salvia miltiorrhiza Bunge</em></td>
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</table>
bacterial diversity and composition of the rhizosphere.

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