



## 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

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### 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

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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). N 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,  $N_2$  in the atmosphere changes to ammonia (Fig. 1). 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  $N_2$  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)  $N_2$  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*), 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

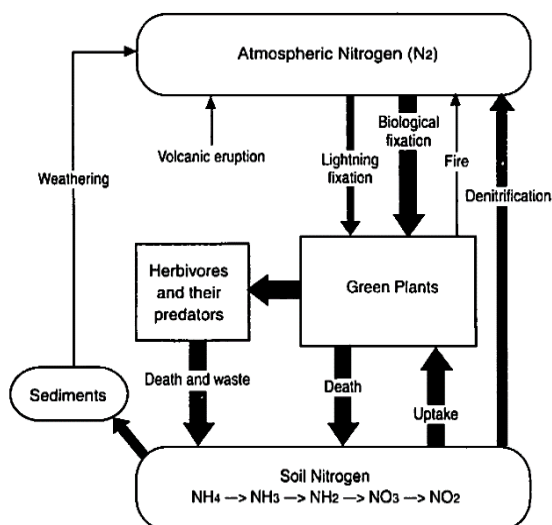


Fig. 1. Nitrogen fixation cycle

microbes are complex and cumulative because of their ability in interactions with plants, pathogens, antagonists, and environmental factors (Babalola, 2010).

### 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 ( $\text{H}_2\text{PO}_4^-$ ) and the dibasic ( $\text{HPO}_4^{2-}$ ) 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 (Bethlenfalvai 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).

### Siderophore production

Iron is a vital nutrient for almost all forms of life. In the aerobic environment, iron occurs principally as  $\text{Fe}^{3+}$  and is likely to form insoluble hydroxides and oxyhydroxides, thus making it generally inaccessible to both plants and microorganisms (Rajkumar et al., 2010). The best implications to free-living rhizobia are siderophore production and cross-utilization as compared to siderophore non-producing strains for being able to survive better in soil (Raaijmakers et al., 1995). Most of the siderophores are water-soluble and can be divided into extracellular siderophores and intracellular siderophores (Ahemad and Kibret, 2014). In addition to being able to use their own ferri-siderophore complexes, *S. meliloti* and *Bradyrhizobium japonicum* can also utilize iron complexed to siderophores produced by other rhizospheric microorganisms (Loper and Henkels, 1999). Besides iron, siderophores also form stable complexes with other heavy metals that are of environmental concern, such as Al, Cu, Cd, Ga, P and Zn, as well as with radionuclides including U and Np (Neubauer et al., 2000). Hence, bacterial siderophores help to reduce the stresses imposed on plants by high soil levels of heavy metals (Ahemad and Kibret, 2014).

### 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

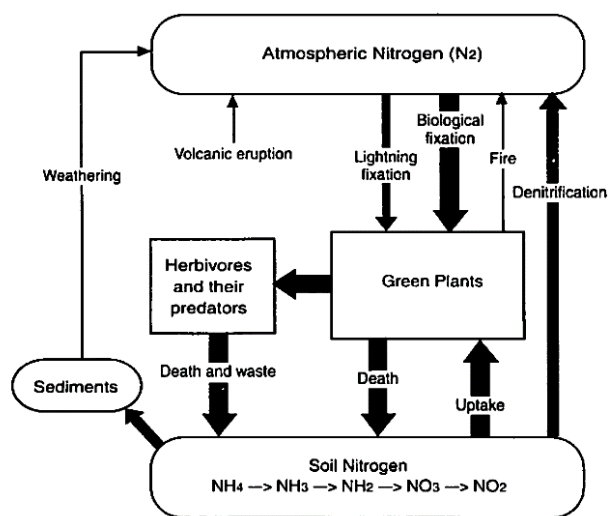


Fig. II: Phosphorous fixation cycle.

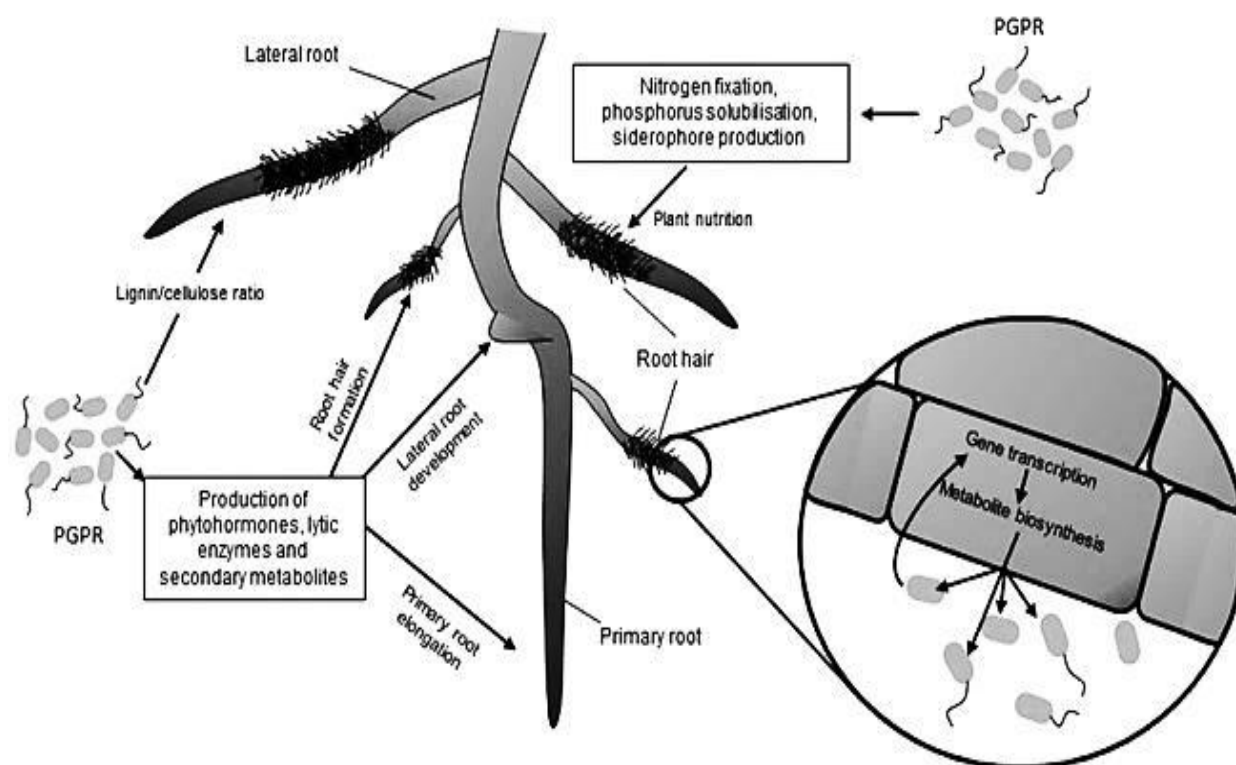


Fig. III. Impact of phyto-stimulating PGPR on root system architecture (RSA), nutrient acquisition, and root functioning

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 (Ribaud 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 xylosoxidans*, *Acinetobacter calcoaceticus*, *Azospirillum* spp., *Azotobacter* spp., *Bacillus* spp., *Herbaspirillum seropedicae*, *Gluconobacter diazotrophicus* 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

PGPR	Plant species	Results of addition of bacteria to plants	References
<i>G. moseae</i> and <i>B. subtilis</i>	<i>Thymus daenensis</i>	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.	(Bahadori et al., 2013)
<i>Arbuseular Mycoriza</i>	Lemon grass ( <i>Symbopogon martini</i> ) and on mint ( <i>Mentha arvensis</i> )	Percentage of essential oil and essential yield increased by mycorrhizal inoculation in comparison with non-inoculated	(Gupta, 1990; Khaliq, 1997)
<i>Glomus macrocarpum</i> and <i>Glomus fasciculatum</i>	<i>Foeniculum vulgare</i> .	Growth characteristics and essential oil concentration significantly improved	(Kapoor et al., 2004)
<i>Polymyxa</i> and <i>Azospirillum brasilense</i>	Palmarosa ( <i>Cymbopogon martini</i> )	Biomass and phosphorus content maximized	(Ratti et al., 2001)
<i>Glomus macrocarpum</i> and <i>Glomus fasciculatum</i>	Fennel ( <i>Foeniculum vulgare</i> )	Improved properties as follow; number of umbels in plant, seed weight, phosphorus concentration, biomass, percentage of AM root colonization, root and amount of essence (concentration of essential oil).	(Kapoor et al., 2004)
<i>Pseudomonas fluorescens</i> and <i>Azospirillum brasilense</i>	Marigold ( <i>Tagetes minuta</i> )	Essential oil and phenolic content by single inoculation and co-inoculation of <i>Pseudomonas fluorescens</i> and <i>Azospirillum brasilense</i> had been significantly increased	(Cappellari et al., 2013)
<i>Azotobacter Chroococcum</i> + <i>Bacillus megaterium</i> + <i>Bacillus circulanse</i>	<i>Rosmarinus officinalis</i>	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	(Abdullah et al., 2012)
<i>G. moseae</i> and <i>B. subtilis</i>	Not specific plant	Plant P uptake improved and enhanced essential oil content	(Artursson et al., 2006)
<i>G. fasciculatum</i>	Basil ( <i>Ocimum basilicum</i> ),	Inoculation significantly increased essential oil content and yield	(Rasouli-Sadaghiani et al., 2010)
<i>Arbuseular Mycoriza</i>	Basil ( <i>Ocimum basilicum</i> ),	Linalool formed the highest relative abundance of the main compounds in leaf essential oils	(Rasouli-Sadaghiani et al., 2010) (Kumar et al., 2002; Mahfouz and Sharaf-Eldin, 2007; Velmurugan and Chezhiyan, 2008; Kumar et al. 2009 and Darzi et al., 2012)
<i>Azotobacter chroococcum</i> and <i>Azospirillum lipoferum</i>	Coriander, Fennel, Davana turmeric and Dill	Increased yield and essential oil	(Shafagh-Kolvanagh and Shokati, 2010; Shokati and Ghassemi-Golezani, 2013 and Shokati and Zehtab-Salmasi, 2014)
Rhizobium bacteria	fenugreek ( <i>Trigonella foenum-graecum</i> )	promote dill ( <i>Anethum graveolens</i> L.) fresh and dry weight, height and umbel number, essential oil and yield components	
<i>Pseudomonas fluorescens</i> , <i>Bacillus subtilis</i> , <i>Sinorhizobium melloti</i> , and <i>Bradyrhizobium</i>	<i>Origanum majorana</i> L.	<i>P. fluorescens</i> and <i>Bradyrhizobium</i> sp. showed significant increases in shoot length, shoot weight, number of leaf, number of node, and root dry weight	(Banchio et al., 2008)

## 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 monoterpenes (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-inoculated plants. This paper also concluded that the soil type, environmental variables, soil management practices, microbial interactions, and plant species could affect

Table 2  
Efficient biotic elicitors used for the production of secondary metabolites in different plant species (Adapted from Egamberdieva et al., 2015)

PGPRs as elicitors	Plant species	Elicitation of secondary metabolites	Reference
<i>Pseudomonas putida</i> and <i>fluorescens</i>	<i>Hyoscyamus niger</i> L.	Hyoscyamine and scopolamine	(Ghorbanpour et al., 2013)
<i>Pseudomonas putida</i> and <i>fluorescens</i>	<i>Salvia officinalis</i> L.	<i>Cis</i> -thujone, camphor, 1,8-cineole	(Ghorbanpour et al., 2014)
<i>Bacillus polymyxa</i> , <i>Pseudomonas putida</i> , <i>Azotobacter chroococcum</i> , and <i>Glomus intraradices</i>	<i>Stevia rebaudiana</i>	Stevioside	(Vafadar et al., 2013)
Arbuscular mycorrhizal and phosphatesolubilizing bacteria	Rose-scented geranium ( <i>Pelargonium</i> sp.)	Citronellol, geraniol, geraniol, and 10-epi- $\gamma$ eudesmol	(Prasad et al., 2012)
<i>Pseudomonas fluorescens</i> and <i>Azospirillum brasilense</i>	<i>Tagetes minuta</i>	Monoterpenes and phenolic compounds	(Cappellari et al., 2013)
<i>Pseudomonas aeruginosa</i> and <i>Pseudomonas fluorescens</i>	<i>Pisum sativum</i>	Phenolic compounds (gallic, cinnamic, and ferulic acid)	(Bahadur et al., 2007)
<i>Hormonema</i> ssp. <i>homogenates</i>	<i>Brugmansia candida</i>	Hyoscyamine and scopolamine	(Pitta-Alvarez et al., 2000)
<i>Bacillus cereus</i>	<i>Salvia miltiorrhiza</i> Bunge	Tanshinone	(Zhao et al., 2010)

bacterial diversity and composition of the rhizosphere.

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