

Chapter 2

Rhizosphere Microbes Interactions in Medicinal Plants

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2.1 Introduction

The diversity and functions of microbes in the rhizosphere, a narrow region around the root, are related to the root exudates (proteins and sugars), biogeochemical reactions and respiration (Narula et al. 2009). The rhizosphere contains abundant bacteria, fungi, protozoa and nematodes. Some nematodes are feeding on bacteria and fungi. The root exudates in the rhizosphere may control disease suppression and help in nutrient cycling. The different compounds secreted by plant roots into the rhizosphere perform multiple functions. For example, strigolactones stimulate the colonisation of the mycorrhiza fungi and germination of the parasitic plant such as *Striga*. The flavonoids secreted by the roots of leguminous plants increase the growth of symbiotic and non-symbiotic nitrogen-fixing bacteria, root nodules and nitrogen uptake by plants. Allelochemicals can inhibit the growth of other micro-organisms in the rhizosphere, and therefore interactions are complex.

In the mycorrhizosphere around the mycorrhiza-colonised roots, most of the actively absorbing rootlets are extended to the surrounding soil for nutrient uptake (Johansson et al. 2004). Since mycorrhizal fungi stimulated by some root exudates may modify root morphology and metabolic functions, the volume of the mycorrhizosphere soil is larger than the rhizosphere soil (Linderman 1988), and root exudates in the mycorrhizosphere is quantitatively and qualitatively different from that in the rhizosphere (Leyval and Berthelin 1993; Rygielwicz and Andersen 1994) producing the ‘mycorrhizosphere effect’ (Linderman 1988). In addition, mycorrhizal fungi can produce antibiotics that may reduce bacterial activity in sandy soil (Olsson et al. 1996).

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A wide range of organic compounds secreted by plant roots in the rhizosphere provide a food source for microorganisms increasing microbial density and activity in the rhizosphere than in the bulk soil (the soil away from the rhizosphere is known as bulk soil). Most of the microorganisms in the rhizosphere are related to plant species that can efficiently solubilise poorly soluble inorganic P and mineralise organic P sources (unaccessible to plants) and markedly increase plant growth in soils with low P availability. However, the contribution of the plant-specific microorganisms to plant P uptake in soils with low P availability is poorly understood. The arbuscular mycorrhizal (AM) fungi form symbioses with more than 80 % of all land plant species and can help for plant P acquisition via the fungal hyphae (Jasper et al. 1989; Smith and Read 2008).

Medicinal plants are a rich source of bioactive compounds (Toussaint et al. 2007), and these are thought to be safe to human beings and the environment compared to the synthetic medicines for the treatment of cancer and many other diseases (Nema et al. 2013). The use of medicines of plant origin has a long tradition in Europe and Asia such as traditional Chinese medicine, Indian Ayurvedic medicine and herbal medicine. More than 600 medicinal plants, comprising more than 30 % of known plant species, are recorded in the Chinese *Materia Medica*, citing the first use of medicinal herbals in China as early as 1100 BC (Cragg et al. 1997; Joy et al. 1998). With the increased population pressure, costs and side effects and the development of resistance to allopathic drugs for infectious diseases, the uses of medicines of plant sources for a wide variety of human ailments are increasing. So, large-scale productions of medicinal plants using modern cultivation technologies are being practised across Asian countries, to meet the demand of medicinal plants. The pests and diseases of plant are hampering the growth and quality of medicinal plants. In addition, excessive use of pesticides may degrade the quality of medicinal plant products. Therefore, the development of innovative technologies for cultivation of medicinal plants is required.

Many recent research works have indicated that mycorrhizal colonisation is common in most of the medicinal plants in Fiji Island and Hawaii, America (Taber and Trappe 1982), Pakistan (Waheed 1982; Gorski 2002; Haq and Hussain 1995; Iqbal and Nasim 1986), China (Wei and Wang 1989), Japan (Udea et al. 1992) and many other areas that play many significant roles in increasing soil structure, nutrient uptake by plants, plant growth, productivity and biodiversity in the diverse agroecosystems (Smith and Read 2008). The AM fungi are the most widely distributed symbioses out of all types of mycorrhizas such as arbuscular mycorrhiza, ectomycorrhiza, ectoendomycorrhiza, ericoid, orchid, arbutoid and monotropoid mycorrhiza (Smith and Read 2008). Many researches have focused on the AM fungal community and diversity in the rhizosphere of medicinal plants (e.g. Kumar et al. 2010; Wubet et al. 2003; Zeng et al. 2013) and improved plant growth (Karthikeyan et al. 2009; Chandra et al. 2010) and medicinal values by AM fungal colonisation (e.g. Copetta et al. 2006; Yuan et al. 2007; Morone-Fortunato and Avato 2008; Toussaint et al. 2008; Sasanelli et al. 2009; Koeberl et al. 2013). However, the microbes in the rhizosphere of medicinal plants are largely unexplored. Therefore, further research is recommended to provide the novel

insights on (1) the microbiome of medicinal plants, (2) plant- and microbe-derived ingredients of medicinal plants and (3) plant growth promotion and plant protection for pests and diseases.

2.2 Microbial Diversity in the Rhizosphere of Medicinal Plants

2.2.1 Bacterial Diversity

The study of rhizosphere bacteria from the important medicinal plants is very crucial, as they are well known to have impact on plant growth and also produce industrially important metabolites and improve quality of medicinal product (Bafana and Lohiya 2013). A significant number of bacteria produce the phytotherapeutic compounds (Koeberl et al. 2013) and increase the growth of the medicinal plants when they are associated with rhizosphere of plants that are listed in Table 2.1. This information will be useful in developing a biofertiliser consortium for commercially grown medicinal plants.

Gram-negative, nonmotile, catalase-positive and oxidase-negative short rods and exopolysaccharide-producing bacterium, designated as strain DRP 35 (T) (Whang et al. 2014) and DR-9(T) (Lee et al. 2013), were isolated from the rhizosphere soil of a medicinal herb, *Angelica sinensis*. The phylogenetic analyses based on 16S rRNA gene sequences indicated that strain DRP 35(T) belongs to the genus *Terriglobus* in the phylum *Acidobacteria* with a similarity to *Terriglobus saanensis* SP1PR4(T) and *Terriglobus roseus* KBS63(T), while strain DR-9(T) formed a lineage within the genus *Mucilaginibacter* and was closely related to *Mucilaginibacter polysacchareus* DRP28(T), *Mucilaginibacter myungsuensis* HMD1056(T), *Mucilaginibacter ximonensis* XM-003(T) and *Mucilaginibacter boryungensis* BDR-9(T).

The soil microbes in the rhizosphere of three medicinal plants (*Matricaria chamomilla* L., *Calendula officinalis* L. and *Solanum distichum* Schumach. & Thonn.) grown on the desert ecosystem had a high abundance of Gram-positive bacteria of prime importance for pathogen suppression (Koeberl et al. 2013). For all three plants, a plant-specific selection of the microbes as well as highly specific diazotrophic communities was found. The results identified that the plant species were important drivers in structural and functional diversity. Furthermore, the native *Bacillus* strains promoted the plant growth and elevated the plants' flavonoid production. Among 28 endophytic bacterial isolates from different organs of *Plectranthus tenuiflorus* medicinal plant, 8 isolates were *Bacillus* sp., *Bacillus megaterium*, *Bacillus pumilus*, *Bacillus licheniformis*, *Micrococcus luteus*, *Paenibacillus* sp., *Pseudomonas* sp. and *Acinetobacter calcoaceticus* (El-Deeb et al. 2013). Li et al. (2013) found the great differences in the endophytic bacterial diversity in the three medicinal plant species of *Codonopsis pilosula*, *Ephedra*

Table 2.1 Medicinal plants and rhizosphere-associated bacteria

Plant species	Microorganisms	References
<i>Angelica sinensis</i>	<i>Terriglobus saanensis</i> <i>Mucilaginibacter polysacchareus</i> <i>Mucilaginibacter myungsuensis</i> , <i>Mucilaginibacter ximonensis</i>	Whang et al. (2014) Lee et al. (2013)
<i>Matricaria chamomilla</i> <i>Calendula officinalis</i> , <i>Solanum distichum</i>	<i>Bacillus</i> sp.	Koeberl et al. (2013)
<i>Rumex patientia</i>	<i>Proteobacterium</i> <i>Bacteroidetes</i> <i>Acidobacteria</i> <i>Gemmatimonadetes</i> <i>Verrucomicrobia</i> <i>Planctomycetes</i> <i>Actinobacteria</i> <i>Firmicutes</i> <i>Chloroflexi</i>	Qi et al. (2013)
<i>Atractylodes lancea</i>	Gram-negative bacteria	Dai et al. (2013)
<i>Plectranthus tenuiflorus</i>	<i>Bacillus</i> sp. <i>Bacillus megaterium</i> <i>Bacillus pumilus</i> <i>Bacillus licheniformis</i> <i>Micrococcus luteus</i> <i>Paenibacillus</i> sp. <i>Pseudomonas</i> sp. <i>Acinetobacter calcoaceticus</i>	El-Deeb et al. (2013)
<i>Origanum vulgare</i>	<i>Pseudomonas</i> , <i>Stenotrophomonas</i>	Bafana and Lohiya (2013)
<i>Typhonium giganteum</i>	<i>Kribbella flavida</i> <i>K. karoensis</i> <i>K. alba</i>	Xu et al. (2012)
Ginseng plants	Actinomycetes	Zhang et al. (2013)
<i>Hypericum silenoides</i>	<i>Acinetobacter</i> <i>Enterobacter</i> <i>Pseudomonas</i> <i>Sphingobium</i> <i>Stenotrophomonas</i> <i>Agrobacterium</i> <i>Pantoea</i> <i>Serratia</i>	Lopez-Fuentes et al. (2012)
<i>Ajuga bracteosa</i>	<i>Pseudomonas</i>	Kumar et al. (2012)
<i>Nerium indicum</i>	<i>Pontibacter</i>	Raichand et al. (2011)
<i>Fritillaria thunbergii</i>	<i>Proteobacteria</i> <i>Acidobacteria</i> <i>Actinobacteria</i> <i>Bacteroidetes</i>	Shi et al. (2011)

(continued)

Table 2.1 (continued)

Plant species	Microorganisms	References
<i>Astragalus membranaceus</i>	<i>Geodermatophilus obscurus</i>	Zhang et al. (2011a)
<i>Phytolacca acinosa</i>	<i>Aspergillus fumigatus</i>	Guo et al. (2010)
<i>Agathosma betulina</i>	<i>Cryptococcus laurentii</i>	Cloete et al. (2010)
<i>Ocimum sanctum</i> , <i>Coleus forskohlii</i> , <i>Catharanthus roseus</i> , <i>Aloe vera</i>	<i>Azospirillum</i> <i>Azotobacter</i> <i>Pseudomonas</i>	Karthikeyan et al. (2008)
<i>Annona squamosa</i> <i>Eclipta alba</i> <i>Cassia auriculata</i>	<i>Bacillus</i> <i>Pseudomonas</i> <i>Enterobacter</i> , <i>Corynebacterium</i> , <i>Micrococcus</i> <i>Serratia</i>	Tamilarasi et al. (2008)

sinica and *Lamiophlomis rotata*. Zhao et al. (2013) explored the microbial diversity from the rhizosphere soils of some medicinal plants and found a total of 50 strains identified into 7 genera, *Myxococcus* (18), *Corallococcus* (11), *Cystobacter* (7), *Archangium* (8), *Stigmatella* (1), *Chondromyces* (4) and *Pyxidicoccus* (1) with the dominant genera of *Myxococcus* and *Corallococcus*.

The continuous cropping of *Rehmannia glutinosa*, an important medicinal plant, on the same land decreases its productivity (Qi et al. 2009). An alteration of soil microbial community following *R. glutinosa* cropping might be an important reason for the constraints associated with continuous cropping. There were several characteristic differences in the microbial community composition and activities in the rhizosphere following *Rehmannia glutinosa* monoculture (Qi et al. 2009; Wu et al. 2013). However, the interactions among plant, soil and microflora are crucial for the productivity and quality of *Rehmannia glutinosa* in consecutive monoculture system (Wu et al. 2011). The relative proportion of bacterial communities in rhizosphere soils of the wild medicinal plant *Rumex patientia* was similar to non-rhizosphere soils in five phylogenetic groups (*Acidobacteria*, *Actinobacteria*, *Chloroflexi*, *Planctomycetes* and *Proteobacteria*), but there were differences in five other phylogenetic groups (*Bacteroidetes*, *Firmicutes*, *Gemmatimonadetes*, *Verrucomicrobia* and unclassified bacteria) (Qi et al. 2012). Qi et al. (2013) identified a total of 83 unique phylotypes classified as *Proteobacterium* (43.37 %), *Bacteroidetes* (13.25 %), *Acidobacteria* (10.84 %), unclassified bacteria (9.64 %), *Gemmatimonadetes* (7.23 %), *Verrucomicrobia* (4.82 %), *Planctomycetes* (4.82 %), *Actinobacteria* (3.61 %), *Firmicutes* (1.20 %) and *Chloroflexi* (1.20 %) in the rhizosphere soil of *Rumex patientia*.

The peanut production in continuous monocrop farming system is affected by various environmental factors that deteriorate soil microbial communities, especially decrease in fungal diversity and increase in fungal pathogens. Whereas, the peanut production was increased by the improved soil microcosm environment and

the fungal diversity and decreased fungal pathogens such as *Fusarium* sp. and *Verticillium* sp. when peanut was intercropped with *Atractylodes lancea* and *Euphorbia pekinensis*, traditional Chinese medicinal plants (Dai et al. 2009, 2013). The increase in the Gram-negative bacterial population and the decrease of phenolic allelochemicals resulted in the promotion of peanut growth and increased peanut yield in the intercropping treatments.

The *Origanum vulgare* is a perennial medicinal aromatic plant rich in phenolic antioxidants. Bafana and Lohiya (2013) isolated both root endophytes and rhizospheric soil bacteria with a total of 120 morphologically different isolates grouped into 21 phylotypes. Majority of the isolates belonged to *Firmicutes* and gamma-*Proteobacteria*. *Pseudomonas* and *Stenotrophomonas* were the most dominant species and together constituted 27.5 % of the total isolates. Lopez-Fuentes et al. (2012) isolated and identified the 103 bacterial communities in the rhizosphere and roots of *Hypericum silenoides* Juss, mostly belonging to the genera *Acinetobacter*, *Agrobacterium*, *Enterobacter*, *Pseudomonas*, *Sphingobium*, *Stenotrophomonas*, *Pantoea* and *Serratia*. In order to determine their plant growth-promoting and biotechnological potential, Kumar et al. (2012) isolated a total of 123 morphologically different bacteria from the rhizospheric soil and roots of the medicinal plant *Ajuga bracteosa* that belonged to alpha- and gamma-*Proteobacteria*, with *Pseudomonas* constituting the most dominant species. The endophytic bacterial community consisted almost exclusively of *Firmicutes*.

Raichand et al. (2011) isolated a Gram-negative, pink pigmented bacterium strain from the rhizosphere of an Indian medicinal plant, *Nerium indicum* (*Chuvanna arali*), that matched with most of the phenotypic and chemotaxonomic properties of the genus *Pontibacter* and represents a novel species. The main bacterial population found in the rhizosphere of medicinal plant *Fritillaria thunbergii* was *Proteobacteria* (55 %), *Acidobacteria* (12 %), *Actinobacteria* (12 %) and *Bacteroidetes* (18 %) (Shi et al. 2011). The bacterial diversity of *Indigofera tinctoria* and *Pueraria mirifica* rhizospheres was significantly different from that of *Derris elliptica* Benth rhizosphere (Nimnoi et al. 2011). The microbial population is more in the rhizosphere soil compared to non-rhizosphere soil of the medicinal plants *Ocimum sanctum* L., *Coleus forskohlii* Briq., *Catharanthus roseus* (L.) G. Don and *Aloe vera*. The diazotrophic bacterial population studied includes *Azospirillum*, *Azotobacter* and *Pseudomonas* (Karthikeyan et al. 2008).

The actinobacterial biocontrol strains in medicinal plants are important as they can be a source of potent antibiotics. Zhao et al. (2012) analysed the actinobacterial diversity in the rhizosphere of seven traditional medicinal plant species and found 18 actinobacterial genera. In particular, Diels hosted a diverse selection of *Actinobacteria*. Xu et al. (2012) isolated an actinomycete, designated XMU 198 (T), from the rhizosphere soil of a pharmaceutical plant, *Typhonium giganteum* Engl., exhibiting highest sequence similarities with *Kribbella flavida*, *K. keroonensis* and *K. alba*. Zhang et al. (2011a) isolated a novel actinobacterial strain, CPCC 201356(T), from a rhizosphere soil sample of the medicinal plant *Astragalus membranaceus* that belonged to the family *Geodermatophilaceae*.

Table 2.2 Medicinal plants and rhizosphere-associated fungi

Plant species	Microorganisms	References
<i>Atractylodes lancea</i> <i>Dioscorea zingiberensis</i> , <i>Euphorbia pekinensis</i> <i>Ophiopogon platyphyllum</i> , <i>Pinellia ternata</i>	<i>Fusarium</i> sp. <i>Verticillium</i> sp.	Dai et al. (2009)
<i>Andrographis paniculata</i>	<i>Acaulospora scrobiculata</i> , <i>Glomus aggregatum</i>	Radhika and Rodrigues (2010)
<i>Hemidesmus indicus</i>	<i>Ambispora leptoticha</i> <i>G. maculosum</i> <i>G. geosporum</i> <i>G. multicaule</i> <i>G. fasciculatum</i>	Radhika and Rodrigues (2010)
<i>Aloe vera</i>	<i>G. maculosum</i> <i>G. multicaule</i> <i>G. geosporum</i>	Radhika and Rodrigues (2010)
<i>Azadirachta indica</i>	<i>A. scrobiculata</i> <i>G. fasciculatum</i> <i>Gi. albida</i> <i>S. calospora</i>	Radhika and Rodrigues (2010)
<i>Naregamia alata</i>	<i>A. scrobiculata</i> <i>Am. Leptoticha</i> <i>A. nicolsonii</i> <i>G. rubiforme</i> <i>G. maculosum</i> <i>G. fasciculatum</i> <i>S. verrucosa</i>	Radhika and Rodrigues (2010)
<i>Physalis minima</i>	<i>A. rehmi</i> <i>G. fasciculatum</i> <i>G. multicaule</i> <i>G. maculosum</i> <i>G. geosporum</i> <i>G. rubiforme</i>	Radhika and Rodrigues (2010)
<i>Centella asiatica</i>	<i>G. multicaule</i> , <i>G. clarum</i> , <i>G. fasciculatum</i> , <i>A. delicate</i> , <i>S. scutata</i>	Radhika and Rodrigues (2010)
<i>Panax ginseng</i>	<i>A. cavernata</i> , <i>A. spinosa</i> , <i>G. fasciculatum</i> , <i>G. geosporum</i> , <i>G. macrocarpum</i> , <i>G. microaggregatum</i> , <i>G. mosseae</i>	Cho et al. (2009)
<i>Panax notoginseng</i>	<i>G. versiforme</i> , <i>G. monosporum</i> , <i>G. mosseae</i> , <i>G. constrictum</i> , <i>G. claroideum</i>	Zhang et al. (2011b)
<i>Arnica montana</i>	<i>G. geosporum</i> , <i>G. constrictum</i> , <i>G. intraradices</i> , <i>G. mosseae</i> , <i>G. macrocarpum</i> , <i>G. fasciculatum</i> , <i>G. versiforme</i>	Jurkiewicz et al. (2010)
<i>Echinacea purpurea</i>	<i>G. intraradices</i>	Araim et al. (2009)

(continued)

Table 2.2 (continued)

Plant species	Microorganisms	References
<i>Cercidiphyllum japonicum</i>	<i>G. aggregatum</i> , <i>G. constrictum</i> , <i>G. dimorphicum</i> , <i>G. fasciculatum</i> , <i>G. flavisporum</i> , <i>G. intraradices</i> , <i>G. mosseae</i> , <i>S. aurigloba</i> , <i>Archaeospora leptoticha</i>	Wang et al. (2008)
<i>Hippophae rhamnoides</i>	<i>G. albidum</i> , <i>G. claroideum</i> , <i>G. constrictum</i> , <i>G. coronatum</i> , <i>G. intraradices</i>	Tang et al. (2004)
<i>Ziziphus jujuba</i> Mill. var. <i>inermis</i>	<i>G. coronatum</i> , <i>G. intraradices</i> , <i>G. monosporum</i> , <i>G. reticulatum</i>	Tang et al. (2004)
<i>Lycium barbarum</i>	<i>Gi. margarita</i> , <i>G. albidum</i>	Tang et al. (2004)
<i>Taxus chinensis</i>	<i>G. aggregatum</i> , <i>G. ambisporum</i> , <i>G. clarum</i> , <i>G. constrictum</i> , <i>G. fasciculatum</i> , <i>G. geosporum</i> , <i>G. magnicaule</i> , <i>G. reticulatum</i> , <i>G. verruculosum</i> , <i>G. viscosum</i> , <i>A. denticulate</i>	Wang et al. (2008)
<i>Euptelea pleiosperma</i>	<i>G. ambisporum</i> , <i>G. constrictum</i> , <i>G. fasciculatum</i> , <i>G. geosporum</i> , <i>G. hyderabadensis</i> , <i>G. intraradices</i> , <i>S. verrucosa</i>	Wang et al. (2008)
<i>Cassia alata</i> <i>C. occidentalis</i> <i>C. sophera</i>	<i>Glomus</i> spp.	Chatterjee et al. (2010)
<i>Curcuma mangga</i>	<i>Alternaria brassicicola</i> , <i>Colletotrichum gloeosporioides</i> , <i>Fusarium oxysporum</i> , <i>Penicillium digitatum</i> , <i>Sclerotium rolfsii</i>	Khamna et al. (2009)
<i>Centella asiatica</i> and <i>Ocimum sanctum</i>	AM and endophytic fungi	Sagar and Kumari (2009)
<i>Paeonia suffruticosa</i>	<i>Glomus</i> <i>Acaulospora</i> <i>Scutellospora</i>	Shi et al. (2013)
<i>Artemisia annua</i>	<i>Glomus mosseae</i> <i>G. aggregatum</i> <i>G. fasciculatum</i> <i>G. intraradices</i>	Awasthi et al. (2011)
<i>Magnolia cylindrica</i>	<i>Acaulospora</i> <i>Glomus</i> <i>Gigaspora</i> <i>Scutellospora</i>	Yang et al. (2011)
<i>Bacopa monnieri</i>	<i>Glomus mosseae</i> <i>Glomus intraradices</i>	Khaliei et al. (2011)
<i>Leptadenia reticulata</i> <i>Mitragyna parvifolia</i> <i>Withania coagulans</i>	<i>G. constrictum</i> <i>G. fasciculatum</i> <i>G. geosporum</i> <i>G. intraradices</i> <i>G. mosseae</i> <i>G. rubiforme</i>	Panwar and Tarafdar (2006)

(continued)

Table 2.2 (continued)

Plant species	Microorganisms	References
<i>Sorghum bicolor</i>	<i>G. mosseae</i> <i>G. intraradices</i>	Sun and Tang (2013)
<i>Curculigo orchoides</i>	<i>G. geosporum</i> <i>G. microcarpum</i>	Sharma et al. (2008)
Ginseng plants	Soil fungi	Zhang et al. (2013)

Zhang et al. (2010) determined that allelochemicals released by the medicinal plant *Scutellaria baicalensis* Georgi negatively affected *S. baicalensis* directly by inducing autotoxicity and indirectly by increasing pathogen activity in the soil.

2.2.2 Fungal Diversity

AM fungal colonisations in the medicinal plants have been reported widely. However, the diversity of AM fungal species and the extent of colonisation in the rhizosphere of medicinal plants may vary depending on host plant species, growing season, soil properties, local climate and environmental factors. Various informations of medicinal plants and rhizosphere-associated fungi are stated in Table 2.2.

The Egyptian henbane (*Hyoscyamus muticus* L.), a medicinal plant of family Solanaceae native to the desert producing pharmaceutically important compounds (tropane alkaloids) as secondary metabolites, is colonised by a higher number of fungal species and endophytic fungi (El-Zayat et al. 2008). Rhizosphere soil of the medicinal plants (*Centella asiatica* and *Ocimum sanctum*) revealed the presence of 16–17 species of fungi (Sagar and Kumari 2009). The endophytic fungi were also isolated from the roots and leaves of *Centella asiatica* and *Ocimum sanctum*. There was a massive variation in the AM fungi spore population and root colonisation in the rhizosphere of ten medicinal plant species (*Aloe barbadensis*, *Centella asiatica*, *Embllica officinalis*, *Euphorbia longan*, *Mimosa pudica*, *Rauwolfia tetraphylla*, *Rauwolfia serpentina*, *Sapindus trifoliatus*, *Smilax* sp. and *Trachyspermum copticum*) in spite of their growth in similar climatic conditions (Hussain and Srinivas 2013). Chatterjee et al. (2010) surveyed the mycorrhizal status in three different species of *Cassia* plants such as *C. alata*, *C. occidentalis* and *C. sophora*. *Cassia alata* possesses maximum root colonisation by the AM fungus that belongs mostly to the *Glomus* species followed by *C. occidentalis* and *C. sophora*. It seems that *C. alata* is the most potent species for having significant antimicrobial activity.

Mycorrhizal plants (colonised by *Glomus mosseae* or *Glomus intraradices*) of Sorghum (*Sorghum bicolor*) compared with non-mycorrhizal plants contained more alcohols, alkenes, ethers and acids (Sun and Tang 2013). The AM fungi can alter the profile of volatile organic carbon released by roots as well as the root morphology of sorghum plants to adapt to the soil environments. The rhizosphere of

14 common cultivars of tree peony (*Paeonia suffruticosa*) was colonised by AM fungi (Shi et al. 2013). A total of 31 AM fungi species belonging to 3 genera were identified in the rhizospheric soil. *Glomus* (21) was the dominant genus, followed by *Acaulospora* (7) and *Scutellospora* (3). The Paris-type, 17 species of AM fungi and fungal colonisation structures (hyphae, hyphal coils and vesicles) were present in roots of medicinal plant Huangshan magnolia (*Magnolia cylindrica*) (Yang et al. 2011). The species were from the genera *Acaulospora* (6 species), *Glomus* (8 species), *Gigaspora* (1 species) and *Scutellospora* (2 species).

AM fungi (colonised by *Glomus mosseae* and *Glomus intraradices*) have increased plant growth and salinity tolerance by various mechanisms in *B. monnieri*, an important medicinal plant (Khaliel et al. 2011). Sundar et al. (2011) identified 21 AM fungal species in roots of the medicinal plants such as *Eclipta prostrata*, *Indigofera aspalathoides* and *I. tinctoria*. The mean AM fungi colonisation and diversity pattern was dependant on edaphic factors and type of vegetation. Panwar and Tarafdar (2006) identified 5 genera of AM fungi in the rhizosphere of 3 medicinal plant species (*Leptadenia reticulata*, *Mitragyna parvifolia*, *Withania coagulans*). The association with AM fungi of these plant species native to the extreme environmental conditions of the Indian Thar Desert may play a significant role in the re-establishment and conservation of these medicinal plants.

The *Artemisia annua* L. (Asteraceae) is an important medicinal plant whose secondary metabolite artemisinin is used for the treatment of cerebral malaria. Awasthi et al. (2011) found the compatibility and synergy between AM fungus *Glomus mosseae* and *Bacillus subtilis* bacteria and suggested the use of this microbial consortium in *Artemisia annua* L. (Asteraceae) for enhancing growth and the content and yield of artemisinin. Zubek and Blaszkowski (2009) and Zubek et al. (2011) studied AM fungi and dark septate endophyte (DSE) associations in 36 medicinal plant species from 33 genera and 17 families. AM was found in 34 of 36 plant species, and the abundance of AM fungi hyphae in roots varied with particular species, ranging from 2.5 % (*Helianthus tuberosus*) to 77.9 % (*Convallaria majalis*). The mycelium of DSE was observed in 13 plant species; however, the percentage of root colonisation by these fungi was low.

Khamna et al. (2009) obtained a total of 445 actinomycete isolates from 16 medicinal plant rhizosphere soils. Among them, 23 *Streptomyces* isolates showed activity against phytopathogenic fungi. The consecutive monoculture of *Rehmannia glutinosa* L. could be a causative agent to decrease the diversity of fungal community in the rhizosphere soil (Zhang et al. 2011b). Sharma et al. (2008) suggest the use of mixed consortium of AM fungi (*Glomus geosporum*, *G. microcarpum* and one crude consortium of AM fungal spores) over monospecific cultures for the sustainable cultivation and conservation of endangered medicinal plant such as *Curculigo orchoides*.

The 76 medicinal plants were reported to have AM fungi in Pakistan (Gorsi 2002). Radhika and Rodrigues (2010) found that 30 out of 36 medicinal plant species were mycorrhizal in Goa region, India. The molecular diversity of AM fungi associated with *Prunus africana* revealed that 109 sequences obtained belong

to the members of the *Glomeromycota* (Wubet et al. 2003), and subsequent 5.8S/ITS2 rDNA sequence analysis indicated high AM fungal diversity and dominance of *Glomus* species. Appoloni et al. (2008) analysed AM fungi community in roots of *Dichanthelium lanuginosum* and found that 18S rDNA phylotypes belong to the genera *Acaulospora*, *Archaeospora*, *Glomus*, *Paraglomus* and *Scutellospora*. The most diverse and abundant AM fungi were from the genera *Glomus*, with the most frequent phylotype corresponding to *Glomus intraradices*. The AM fungal community in the rhizosphere of *Phellodendron amurense* showed three general groups of *Glomus*, *Scutellospora* and *Hyponectria*, respectively (Cai et al. 2009).

2.3 Effect of Microbial Inoculation on the Growth of Medicinal Plants

More than 24 genera of nonpathogenic rhizobacteria have been identified till today. Plant growth-promoting rhizobacteria, first defined by Kloepper and Schroth (1978), after being inoculated on seeds, could successfully colonise plant roots and positively enhance plant growth. Besides this, the plant root-secreted growth-promoting compounds (e.g. auxins or cytokinins) and improvement in mineral nutrient uptake (e.g. siderophore) can increase the plant growth. The synthesis of antibiotics or secondary metabolite-mediated induced systemic resistance can control the pathogens (biocontrol) and promote the plant growth (van Loon 2007).

AM could promote nutrient uptake, improve the functional diversity and activity of microbes in the rhizosphere of *Attractylodes lancea* medicinal plant and influence the composition of the organic matter leading to the growth of *A. lancea*, but not to the quality (Guo et al. 2006). The root-nodulating bacterium, *Rhizobium meliloti*, isolated from the medicinal plant, *Mucuna pruriens*, produced siderophores and thus promotes the plant growth (Arora et al. 2001). The medicinal sclerophyll, *Agathosma betulina* (Berg.) Pillans, grown under nutrient-poor conditions was colonised by *Cryptococcus laurentii* soil yeast as a plant nutrient-scavenging microsymbiont (Cloete et al. 2010). Guo et al. (2010) screened and exploited molluscicidal microorganisms against *Oncomelania hupensis* from the rhizosphere of medicinal plant *Phytolacca acinosa* Roxb. that had a higher similarity to *Aspergillus fumigatus*. The symbiotic interaction between the common soil yeast, *Cryptococcus laurentii*, and medicinal plant *Agathosma betulina* (Berg) Pillans helped the plant growth on nutrient-poor soils (Cloete et al. 2009). The addition of *Streptomyces pactum* (Act12) could improve the soil microbial activity which, eventually, enhances the resistance and root activity of ginseng plant and could increase yield and its quality (Zhang et al. 2013). The medicinal plants forming association with various microorganisms can be formulated as biofertiliser and biocontrol tools. Therefore, it is very important to identify, characterise and use rhizospheric microorganisms associated with medicinal plants (Vasudha et al. 2013).

The rhizobacterial strain Jdm2 (*Bacillus subtilis*) isolated from the rhizosphere of the traditional Chinese medicinal herb *Trichosanthes kirilowii* enhances plant growth and inhibits the activity of nematode and has the potential to be a safe and effective microbial pesticide (Wei et al. 2014). The bacterial endophytes isolated from medicinal plant *Annona squamosa* L. showed antimicrobial activity (Baker and Satish 2013), and the bacterium belonged to the genus *Pseudomonas* sp., identified by using 16s rRNA and biochemical tests. Yang et al. (2012) discussed the mechanisms involved in controlling the soilborne disease of medicinal plants by different species of microorganisms as biocontrol agents from the following aspects: improving host plant nutrient uptake, the nutrient and space competition with the pathogenic bacteria, changing anatomical structure and the morphology of roots, balancing the host plants' endogenous hormones, activating the host plants' defence system and restoring the balance of host rhizosphere soil conditions. Plant growth-promoting rhizobacteria (PGPR) isolated from the medicinal weed, *Cassia occidentalis*, are an attractive ecofriendly alternative to chemicals in agriculture and open up possibilities for the utilisation of these in plant growth increase and subsequent boost of yield for agricultural crops (Arun et al. 2012).

The mycorrhizal medicinal plants have higher nutrient uptake capacity and growth than non-mycorrhizal plants (e.g. Karagiannidis et al. 2011; Nisha and Rajeshkumar 2010). The mycorrhizal inoculation increased the dry matter of five medicinal plants (*Abelmoschus moschatus*, *Clitoria ternatea*, *Plumbago zeylanica*, *Psoralea corylifolia* and *Withania somnifera*) grown in five different types of soil (Chandra et al. 2010). The shoot height and root biomass of *Poncirus trifoliata*, *Piper longum*, *Salvia officinalis* and *Plectranthus amboinicus* medicinal plants were promoted by mycorrhizal colonisation (Wang et al. 2006; Rajeshkumar et al. 2008; Geneva et al. 2010; Gogoi and Singh 2011).

2.4 Effect of Rhizosphere Microbes on P Solubility and Availability to Medicinal Plants

The *Aspergillus niger*, *A. fumigatus* and *Penicillium pinophilum* fungal isolates, identified in the rhizosphere of different plants, can effectively solubilise rock phosphate or tricalcium phosphate (Wahid and Mehana 2000) and increase the uptake of phosphorus (P) by the growth of plants. *Pseudomonas aeruginosa* is a plant growth-promoting rhizobacterium. The application of *P. aeruginosa* with a medicinal plant *Launaea nudicaulis* as soil amendment resulted in maximum reduction in *Macrophomina phaseolina* infection on mung bean roots (Mansoor et al. 2007). The endophytic strain of *Bacillus pumilus* isolated from tissues of the medicinal plant *Ocimum sanctum* can be used as a bioinoculant to enhance plant growth and also as a probiotic (Murugappan et al. 2013). Gupta et al. (2011) evaluated the potential of phosphate-solubilising bacteria, *Burkholderia gladioli*, *Enterobacter aerogenes* and *Serratia marcescens*, for utilising Mussoorie rock

phosphate to enhance the medicinal plant growth as biofertiliser because some medicinal plants are less dependent on chemical fertilisers. The strains differed in the extent of rhizosphere colonisation, carbon source utilisation pattern and whole cell fatty acid methyl esters composition.

Despite the high concentrations of total P in soil, its P concentration in the soil solution and uptake by plants is very low (Marschner et al. 2006) due to the low availability of inorganic and organic P compounds and poorly available inorganic P forms (Ca phosphates, Fe/Al phosphates and P adsorbed onto Fe/Al oxides and organic matter) (Schachtman et al. 1998; Richardson and Hadobas 1997). The microbial biomass is another important P pool in soil ranging from 1 to more than 10 % of total soil P (Richardson 2001), because plants and microorganisms compete for P uptake. The microbial biomass may also represent a slow sustained source of available P through decomposition of dead microbial cells (Oberson et al. 2001). Plant P uptake causes depletion of available P in the rhizosphere due to the low solubility and slow diffusion of P in soils (Jungk and Claassen 1986). The plants with the assistance of rhizosphere microorganisms can develop various strategies to increase P uptake and overcome the low P availability in soils. Bacterial and mycorrhizal fungi symbiosis can increase the plant P uptake and P acquisition efficiency (Smith and Read 2008; Rengel 1999) by increasing root growth, mineralisation of organic P by phosphatase enzymes released by roots and microorganisms (Tarafdar and Jungk 1987) and by excretion of organic acids into the rhizosphere and/or changing the rhizosphere pH (Gerke and Meyer 1995; Imas et al. 1997). The microbe in the rhizosphere has different capacity to solubilise or mineralise poorly available P (Banik and Dey 1983) and therefore could affect P availability to medicinal plants.

2.5 Effect of Rhizosphere Microbes on Nutrient Uptake and Stress Tolerance

The AM fungal inoculation has played a significant positive role on plant growth via improved acquisition of nutrients of low mobility, especially P in low-nutrient and constrained soils. AM fungi increase plant uptake of nutrients such as P, Zn, Cu, Mn and Fe in poor soils (Chen and Zhao 2009; Hosamani et al. 2011) and increase the shoot dry weight of plants (Gupta and Janardhanan 1991; Hosamani et al. 2011). The external hyphae of AM fungi can also increase NH_4^+ and NO_3^- uptake by plants and assimilate these molecules into free amino acids (Johansen et al. 1996). However, the effectiveness of AM fungi differs with the plant species, soil fertility and plant growth environments (Smith and Smith 2011). For example, Zhao and Yan (2006) reported that leaf nitrogen contents were lower in the mycorrhizal *Camptotheca acuminata* than its non-mycorrhizal counterpart.

AM fungi-colonised plants have greater tolerance capacity over non-mycorrhizal plants to several biotic and abiotic stresses, such as toxic metals,

root pathogens, drought, high soil temperature, saline soils (Khaliel et al. 2011), adverse soil pH and transplanting shock (Evelin et al. 2009; Lu et al. 2003; Tang et al. 1999). Inoculation with AM fungi enhanced tolerance of *Rosa multiflora* to HCO_3^- as indicated by greater nutrient uptake and leaf chlorophyll and lower root iron reductase activity and alkaline phosphatase activity (Cartmill 2004). The possible drought-induced genes may enhance the tolerance of AM plants to water deficit (Fan and Liu 2011; Ruiz-Lozano et al. 2008). The AM colonisations may alleviate metal stress of plants showing capability in binding heavy metals (Joner et al. 2000; Salvaraj and Kim 2004; Prasad et al. 2011), even though the mechanisms involved in metal tolerance of AM plants are still poorly understood (Hildebrandt et al. 2007) and need to be explored.

2.6 Effect of Rhizosphere Microbes on Quantity and Quality Medicinal Compounds

Bacteria and AM fungi can improve secondary metabolite contents in medicinal plants via improving plant phosphorus status or an altered hormonal balance of the plants (Koeberl et al. 2013; Toussaint 2007). Root diseases (rot and wilt) caused by a complex involving *Fusarium chlamydosporum* (Frag. & Cif.) and *Ralstonia solanacearum* (Smith) are serious diseases affecting the cultivation of *Coleus forskohlii*, a medicinal plant producing forskolin compound (Singh et al. 2013). Coinoculation of *Pseudomonas monteilii* with *Glomus fasciculatum* significantly improved the AM root colonisation and spore numbers, and *Pseudomonas monteilii* can be a mycorrhiza helper bacterium. The forskolin content of tubers was significantly increased by the inoculation treatments of *G. fasciculatum*, *P. monteilii* and *P. monteilii* + *G. fasciculatum*.

Terpenoids, phenolics and alkaloids are the three major groups of secondary plant metabolites and natural medicinal products used for pharmacological and therapeutical purposes. Essential oils mostly consisting of monoterpenes, sesquiterpenes and phenylpropanoids are often used as flavours and fragrances, antimicrobials and antioxidants and medicines (Deans and Waterman 1993). AM fungi increased the content of essential oil and alterations of its composition, such as in the medicinal plant basil (*O. basilicum*) (Copetta et al. 2006). *Andrographis paniculata* that has been used to treat gastrointestinal tract, upper respiratory infections, fever, herpes, sore throat and other chronic and infectious diseases in Asian countries from ancient time contains the primary medicinal compound of andrographolide, a colourless diterpene lactone with a bitter taste. The AM symbiosis after inoculation with *Gigaspora albida* produced the high concentration of andrographolide in the leaf extracts of *A. paniculata* (Radhika and Rodrigues 2011), mostly at flowering growth stage.

The inoculation of *Glomus intraradices*, either alone or in a mixture with *G. mosseae*, significantly increased total phenolic content in leaves and flower heads of *Cynara cardunculus* (Ceccarelli et al. 2010). The AM fungi colonisation

increased the concentrations of isoflavone in roots of legume plants (Catford et al. 2006); flavonoid in white clover (*Trifolium repens*) (Ponce et al. 2004), *Bupleurum chinense*, *Ginkgo biloba* and *Astragalus membranaceus* (Meng and He 2011); rosmarinic acid, a highly antioxidant phenolic compound, in basil (Toussaint et al. 2008); and total coumarin and imperatorin in *Angelica dahurica* (Zhao and He 2011). The AM fungal colonisation could induce two different signalling pathways in the accumulation of phenylpropanoid metabolism: one is through the induction of phenylalanine ammonia-lyase and chalcone synthase, and the other is through the suppression of isoflavone reductase (Zhao and Yan 2006).

The camptothecin in *Camptotheca acuminata* and vinca alkaloids in vinca (*Catharanthus roseus*) are two important anticancer compounds (Rosa-Mera et al. 2011). The castanospermine is effectively used in the treatments against AIDS and cancers (Spearman et al. 1991). Sweet basil has been traditionally used for the treatment of headaches, coughs and diarrhoea (Jayasinghe et al. 2003). AM fungal inoculation significantly enhanced plant growth and the total content of vinblastine in Vinca leaves (Rosa-Mera et al. 2011), castanospermine content in seeds and leaves of *Castanospermum australe* (Abu-zeyad et al. 1999), rosmarinic acid (antioxidant activity) in sweet basil shoots (Toussaint et al. 2007), camptothecin in *Camptotheca acuminata*, vinca alkaloids in vinca (Rosa-Mera et al. 2011) and total phenols, ortho-dihydroxyphenols, flavonoids, alkaloids and tannins in the root and leaf of *O. basilicum* and *Coleus amboinicus* (Hemalatha 2002).

However, a few other studies reported some controversial results for mycorrhizal effects on phenolic contents in medicinal plants. Zeng et al. (2013) showed neutral effects of AM colonisation on the composition of phenolic ingredients. AM symbiosis did not alter the total concentrations of phenolic and rosmarinic acid in roots of *Salvia officinalis* (Nell et al. 2009) and the polyphenolic profile in leaves and stems of basil (Lee and Scigel 2009) after AM fungal inoculation.

2.7 Conclusions

The quality of medicinal plants (active compound content) is largely influenced by abiotic and biotic factors of the rhizosphere. The rhizosphere microbes play an important role in improving medicinal values of medicinal plants. The role of microbes in plant growth, nutrient availability, disease resistance, yield and quality of medicinal compounds is demonstrated in medicinal plants. There are increasing interests in the research of the interaction between medicinal plant and their rhizosphere microbes for the improvement of medicinal plants. A wide variety of bacteria and fungi diversity including AM fungi is recognised in the rhizosphere of medicinal plants that have high significance in plant nutrient acquisition and secondary metabolite alteration. The inoculation of PGPR and/or AM fungi is a sustainable technology to enhance the quantity and quality of the medicinal plant compounds. However, selecting and inoculating specific and efficient bacteria and/or fungi for a particular plant are essential for the cultivation of medicinal

plants in order to obtain the high-quality secondary plant metabolites. Therefore, further research is recommended to better understand the diversity and function of rhizosphere bacteria and/or fungi and their uses in the increased production of medicinal plants by identifying relationship between genetic and functional diversity of bacteria and/or fungal species.

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