Chapter 4 Role of Rhizospheric Microbes in the Management of Phytopathogens



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Abstract Medicinal plants play very crucial role in the life of people, and they are used in official and various traditional systems of medicines throughout the world, benefitting people to prevent disease, maintain health, and cure ailments. Nearly all modern pharmaceuticals are considered to be natural products or derived from plants. Fungal diseases are the major constraints in the profitable cultivation of medicinal plants. Phytopathogenic problem of medicinal plants not only reduces the vield, but it is also responsible for the deterioration of biochemical and secondary metabolites which are of immense therapeutic value. Imprudent use of insecticides, fungicides, agrochemicals, and fertilizers poses serious threat to environment. Scientists have reported various mechanisms regarding plant rhizospheric microbes, i.e., fungi and bacteria, which colonize the roots of plant and thus help the plants in maintaining its health. In the present scenario, rhizospheric microbes (biocontrol agents) have gained popularity due to their effectiveness, safety, and eco-friendliness, and hence their demand has gradually increased. Rhizospheric microbes not only manage plant diseases but at the same time also boost plant growth by different mechanisms. Many scientists have already reported the beneficial role of rhizospheric microbes on the health of various medicinal plants. Research on medicinal plants and rhizospheric microbes is inadequate as far as biotic stresses are concerned. The mechanisms of plant disease management such as mycoparasitism, antibiosis, induced systemic resistance, plant growth promotion, root colonization, siderophore production, phosphate solubilization, etc., have been studied well in reference to medicinal plants. Still due to the distinct features of medicinal plants, future research could be a major breakthrough in the significant increase in the production of medicinal plants.

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4.1 Introduction

About 80% people of the globe rely on herbal medicine for their health problems according to the World Health Organization (Goto et al. 1998). Due to severe side effects of modern medicines, drugs extracted from medicinal plants are gaining popularity in many developed countries. The basics of herbal medicines mainly depend on plant diversity and past studies of their use in maintaining human health (Table 4.1). Nearly all herbal medicines are natural products or derivatives of plants; interestingly it has also been acknowledged that the discovery of artemisinin, which is an antimalarial drug extracted from medicinal plant "sweet wormwood," has earned a 2015 Nobel Prize in medicine (George et al. 2016). Fungal diseases pose a serious threat to the profitable cultivation of crop as well as the medicinal plant. In 1845 potato late blight fungus was responsible for Irish famine which led to millions of people to migrate from Ireland. The Plasmopara viticola causal organism of downy mildew of grapes devastated the wine industry in France. In 1943 brown spot disease of rice was solely responsible for Bengal famine, and other catastrophic examples of phytopathogens also include apple scab; Panama wilt; wheat rust; wilt of Cajanus cajanus, chickpea, castor, and guava; rust; and smut of cereals. Phytopathogenic problem is not only a threat to commercial crop plants, but they are also a threat to our important medicinal plants. Alternaria leaf spot and Fusarium cause severe wilting in Ashwagandha which leads to enormous yield losses (Sharma et al. 2013; Zuhaib et al. 2016; Ansari and Mahmood 2017; Sharma and Trivedi 2010). Apart from Ashwagandha other medicinal plants were also reported to be infected by Fusarium wilt.

Various options of disease management such as chemical, botanical, and biological are available, and among them, chemicals are considered one of the best and reliable options, but they pose serious health and environmental risks, which have limited their use. About 0.1% of the agrochemicals used in crop protection reach to the target pest, rest 99.9% enter into the environment causes greater damage to the ecosystem (Ashraf and Zuhaib 2013). The indiscriminate use of chemical fungicides has consequently caused several health issues, such as toxicity in food, water, and soil, which ultimately leads to pollution of the ecosystem; hence it was recommended by the scientists to use nonchemical methods for the management of phytopathogenic problem of medicinal plants.

Plant rhizospheric microbes including soil fungi and bacteria colonize plant roots and, in turn, help in maintaining plant health. In the present scenario, rhizospheric microbes (biocontrol agents) have gained popularity due to their effectiveness, safety, and eco-friendliness, and hence, their demand has gradually increased. Rhizospheric microbes not only manage the plant diseases but also enhance plant growth by adhering and multiplying at the root hair surface; increase in seedling emergence, functioning of premature nodules, and nodulation; and increase in area

Traditional Scientific			Part in		
name	name	Family	use	Medicinal use	
Amla	Emblica officinalis	Euphorbiaceae	Fruit	Rich source of ascorbic acid, cough, cold, hyperacidity, laxative, prevention of cancer	
Ashok	Saraca asoca	Caesalpiniaceae	Bark, flower	Diabetes disorder, menstrual pain, uterine problems	
Ashwagandha	Withania somnifera	Solanaceae	Root	Curative tonic and helps in nerves disorder	
Guggul	Commiphora wightii	Burseraceae	Gum resin	Rheumatism, laxative, hyperlipidemia	
Aloe	Aloe vera	Liliaceae	Leaves	Used in cosmetic industry	
Bael/bilva	Aegle	Rutaceae	Fruit, bark	Diarrhea, dysentery, constipation	
Tulsi (perennial)	Ocimum sanctum	Lamiaceae	Leaves/ seed	Helps in bronchitis, acts as expectorant, anticancerous	
Sarpagandha (H)	Rauvolfia serpentina	Apocynaceae	Root	Hypertension, insomnia	
Bhumiamla (H)	Phyllanthus amarus	Euphorbiaceae	Whole plant	Provide strength, lower the bilirubin	
Shatavari	Asparagus racemosus	Liliaceae	Tuber, root	Pregnant women, anti-fatigue, lowers blood sugar	
Brahmi	Bacopa monnieri	Scrophulariaceae	Whole plant	Anxiety, improve the memory enhancer,	
Makoi/ Kakamachi	Solanum	Solanaceae	Fruit/ whole	Dropsy, general weakness, anticancerous	
Isabgol	Plantago ovata	Plantaginaceae	Seed coat	Constipation and gastrointestinal irritations. Also used in food industry	
Coleus	Coleus forskohlii	Lamiaceae	Tuberous root	Used in glaucoma, heart functioning, and various types of carcinoma	
Henna/mehndi	Lawsonia inermis	Lythraceae	Leaf, seed	Burning, steam, anti-inflammatory	
Pashanbheda	Coleus barbatus	Lamiaceae	Root	Stone problems, diabetes	
Peppermint	Mentha	Lamiaceae	Leaves,	Digestive, painkiller	
Sadabahar	Vinca rosea	Apocynaceae	Whole plant	Blood cancer, blood pressure muscle spasm	
Vringraj	Eclipta alba	Compositae	Seed/ whole	Anti-allergic, digestive, hair	
Neem	Azadirachta	Meliaceae	Whole plant	Sedative, analgesic, epilepsy	

 Table 4.1
 Medicinal plants and their uses in human health

Modified from of Shahzad et al. (2015)

of leaf surface, vigor, biomass, phytohormone, and nutrient, water, and air uptake, hence stimulating the accumulation of important nutrients in plants (Shrivastava et al. 2015). This review will focus on major fungal diseases of medicinal plants and also the recent developments in the field of biological control of medicinal plant diseases by rhizospheric microbes, which will emphasize on the mechanism. In this chapter we will limit our discussion on important rhizospheric microbes, viz., species of *Trichoderma, Pseudomonas*, and *Bacillus*.

4.2 Biotic Stresses on Medicinal Plants

Since there is a great demand for herbal medicinal in the international market, many biotic factors are responsible for the low productivity of medicinal plants. Biotic factors liable for the low productivity of medicinal plants include an attack of insects, arthropods, fungi, bacteria, and nematodes. Among them the fungi cause major yield losses after insects; in this chapter, we will focus on economic yield losses caused by fungi. Black leaf spot diseases of Aloe vera were caused by Alternaria alternata; two different isolates A and B of Alternaria alternata were isolated from the diseased leaf of Aloe vera (Alam et al. 2007). Similarly Shukla et al. (2008) also reported Pythium leaf spot of Aloe vera which was caused by Pythium aphanidermatum. An occurrence of leaf spot of Kalmegh (Andrographis paniculata) was also observed which causes severe yield loss of 30-45% (Alam et al. 2007). Leaf blight of Mentha piperita and Ocimum sanctum was also reported by (Alam et al. 2007) and (Ashraf and Zuhaib 2009), respectively. Same workers (Zuhaib et al. 2016) also reported leaf spot of Withania somnifera by Alternaria alternata and also screened the resistant cultivars of Withania somnifera for the pathogen. Twig blight of periwinkle (Catharanthus roseus) caused by Sclerotinia sclerotiorum was also studied; similarly foliar infection in the form of leaf spot of Rauvolfia serpentina was also studied by Alam et al. (2007). Medicinal plants are not only attacked by phyllospheric pathogens, but they are also attacked by rhizospheric pathogens. Fusarium causes severe wilting in Ashwagandha which leads to enormous yield losses (Bharti et al. 2014; Zuhaib et al. 2016; Sharma and Trivedi 2010). Apart from Ashwagandha other medicinal plants were also reported to be infected by Fusarium wilt. Important medicinal plants reported to be infected with Fusarium wilt include Atractylodes lancea, Dioscorea zingiberensis, Euphorbia pekinensis, Ophiopogon platyphyllum, Pinellia ternata (Dai et al. 2009), Curcuma manga (Khamna et al. 2009), Launaea nudicaulis (Mansoor et al. 2007), Jerusalem artichoke (sunchoke) (Jina et al. 2013), Panax quinquefolius (Song et al. 2014), Coleus forskohlii (Zheng et al. 2012), Papaver somniferum (Kishore et al. 1985; Sattar et al. 1995), Calotropis gigantea (Selvanathan et al. 2011), Basilicum (Elmer et al. 1994; Katan et al. 1996), and Asparagus (Lamondia and Elmer 1989). Coleus forskohlii is an important medicinal plant; wilt of Coleus forskohlii is a disease complex caused by Rhizoctonia bataticola, Fusarium chlamydosporum, Sclerotium rolfsii, and Ralstonia solanacearum (Bhattacharya and Jha 2012).

4.3 Rhizospheric Microbes as Biocontrol Agents

Plant health may be ameliorated by rhizospheric microbes (naturally present soil fungi and bacteria) by colonizing the plant roots. In the present scenario, rhizospheric microbes (biocontrol agents) have gained popularity due to their effectiveness, safety, and eco-friendliness, and hence, their demand has gradually increased. Vigor, biomass, nutrients and water absorption, yield, root hair proliferation, root hair branching, increase in seedling emergence, increase in area of leaf surface, nodulation, and promoted accumulation of carbohydrates are some of the ways in which rhizospheric microbes supplement plant growth besides providing protection to plants from diseases (Shrivastava et al. 2015). Usage of fungicides is not recommended as it is neither economical nor environmentally friendly. Moreover, its long-term use can cause the development of resistant strains of a pathogen (Ashraf and Zuhaib 2014a, b; Vinale et al. 2008). However, research on biological control gained momentum in the last quarter of the tenth century, and several books (Baker and Cook 1974; Cook and Baker 1983) and review articles (Papavizas 1985) have come up stressing the potential of microorganisms in disease management. Numerous microorganisms have been reported to cause antagonism against plant pathogenic fungi in laboratory and in vivo condition. A perfect biocontrol agent/rhizospheric microbe must have the subsequent qualities (Lucy et al. 2004; Mukerji 2000).

- 1. Prolonged survival, either in active or passive form.
- 2. Greater probability of contact with the pathogen.
- 3. Functional under variable environments.
- 4. Mass multiplication should be easy, feasible, and economical.
- 5. Proficient and cheap.
- 6. Eco-friendly.

A number of rhizospheric microbes such as *Trichoderma*, *Bacillus*, and *Pseudomonas* have been found successful against a number of important fungal diseases of medicinal plants (Scher and Baker 1982; Strashnov et al. 1985; Kaur et al. 2006; Abo-Elyousr et al. 2014; Dubey et al. 2007). The most common species of *Trichoderma* which have been successfully exploited in biological control of pathogenic fungi are *T. virens*, *T. viride*, and *T. harzianum* (Benitez et al. 2004). *T. viride* has been found to significantly reduce mycelial growth, a formation of spores, and germ tube formation of *A. solani* and *A. alternate* (Latha et al. 2009). *T. harzianum* has been found active against *F. oxysporum* inciting wilt in Ashwagandha (Sharma and Trivedi 2010). Moreover, *Trichoderma* can even stimulate plant growth; reports of which have been found in the case of *T. virens* (Kumar et al. 2011) and the stimulation of plant defense mechanisms (Chet et al. 1997).

Mechanism of disease suppression by rhizospheric microbes *Trichoderma* spp. is reported to suppress plant pathogenic fungi through a combination of different mechanisms (Table 4.2) such as mycoparasitism, synthesis of antibiotics (Harman 2006; Harman et al. 2004), enzymes degrading cell wall (Jayalakshmi et al. 2009),

Trichoderma species	Target organism	Factor responsible for biocontrol	Disease control
T. harzianum 1051, T. harzianum 39.1	Crinipellis perniciosa	Chitinase, <i>N</i> -acetylglucosaminidase, β -1,3-glucanase,total cellulase, endoglucanase, aryl- β -glucosidase, β -glucosidase, protease, and amylase	Witches' broom disease (<i>Crinipellis</i> <i>perniciosa</i>) of cocoa
T. lignorum, T. virens, T. hamatum, T. harzianum, T. pseudokoningii (Rifai)	Rhizoctonia solani	Extracellular, metabolites or antibiotics, or lytic enzyme action	Damping-off disease of bean plants
T. viride, T. harzianum	Aspergillus flavus and Fusarium moniliforme	Lipolytic, proteolytic, pectinolytic, and cellulolytic enzymes. Unknown (mycotoxins) antibiotic compounds (e.g., peptides, cyclic polypeptides)	Fungal seed-associated
<i>T. harzianum</i> , BAFC 742	Sclerotinia sclerotiorum, BAFC 2232	β -1,3-Glucanase and chitinase	Fungal-soybean plant
T. harzianum 25, T. Serpula viride lacrymans		Antibiotic; anthraquinones	Fungal wood decay
T. virens "Q" strain Rhizopus oryzae/ Pythium sp.		Plant phytoalexin induction by antibiotic compound, gliovirin	Seedling disease of cotton
T. virens isolates GL3 and GL21; T. harzianum T-203	Rhizoctonia solani, Pythium ultimum, Meloidogyne incognita	Antibiotics gliovirin and gliotoxin and other inhibitory metabolites	Damping- off disease of cucumber

 Table 4.2
 Trichoderma species, their target organism, and mechanisms involved in suppression of plant pathogens

Source: Leng et al. (2011)

contesting for the availability of important nutrients and increase in plant health (Zimand et al. 1996), parasitism of host fungus (Komatsu 1968; Gao et al. 2001), inducing plant defense (Jayalakshmi et al. 2009), and/or induced systemic resistance (Harman et al. 2004; Sriram et al. 2009).

4.3.1 Mycoparasitism

The most common mechanism used by *Trichoderma* for the suppression of phytopathogens is mycoparasitism (Howell 2003; Vinale et al. 2008). Mycoparasitism is a diverse process involving recognition of the host by the mycoparasite; hyphal attachment and coiling of pathogen hyphae (Whipps 2001; Woo and Lorito 2007). The biocontrol of *R. solani* by *T. lignorum* through mycoparasitism was very well described by Weindling (1932). Enzymes such as chitinases, proteases, and β -1, 3-glucanases lyse hyphal cell walls of pathogens during mycoparasitic activity (De La Cruz et al. 1993; Schirmbock et al. 1994). β -1, 3-Glucanases have properties for degrading the cell wall and inhibit the mycelial growth and spore germination of phytopathogenic fungi (Benítez et al. 2004; Lin et al. 2007). Degradation of pathogen hyphal membranes and cell walls was achieved by proteases produced by *T. harzianum*. Application of T. harzianum may inhibit the synthesis of hydrolytic enzymes such as endo-polygalacturonase and exo-polygalacturonase, produced by *Botrytis cinerea*, a causal agent of gray mold, resulting in reduced disease severity (Elad and Kapat 1999). Mustafa et al. (2009) and Kotze et al. (2011) also observed the mycoparasitic activity of *Trichoderma* species against wide range of plant pathogenic fungi.

4.3.2 Competition and Rhizosphere Competence

Biocontrol agents multiplication and their multiplication depends upon various factors like rhizosphere competence, successful root colonization, proliferation along the growing plant roots (Chet 1990; Irtwange 2006). Rhizospheric competence is very crucial which provides appreciable understanding pertaining to mode of action of rhizospheric microbes against wide range of plant pathogens (Whipps 2001; Bais et al. 2004; Howell 2003). *Trichoderma, Pseudomonas*, and *Bacillus* are considered as potent biocontrol agents and offer excellent competition in terms of food and space to the pathogens (Wells 1988). Among these three rhizospheric microbes, *Pseudomonas* was reported to be more effective comparatively *Trichoderma* followed by *Bacillus* (Weller 1988).

The mass culture of *Trichoderma* can be prepared by using different media which can be thereafter used directly either by mixing with the soil or indirectly by biopriming methods (Zhang et al. 1996; Howell et al. 2000). *T. viride* have been reported to reduce the disease severity of *Chondrostereum purpureum*, the silver leaf pathogen of plum trees (Corke and Hunter 1979).

A race for obtaining carbon in the rhizosphere was also observed in the evaluation of antagonistic activity of *Trichoderma* spp. against different plant pathogens, especially *F. oxysporum* (Sivan and Chet 1989). Competition for carbon is involved in the suppression of *F. oxysporum* f. sp. *vasinfectum* and *F. oxysporum* f. sp. *melonis* by *T. harzianum* T-35 in the rhizosphere of cotton and melon, respectively (Sivan and Chet 1989). The case of root colonization by bacteria consists of two phases, attachment to roots followed by colonization of roots (Howie et al. 1987). It was also reported that motile isolates were far more better colonizers than non-motile isolates (Toyota and Ikeda 1997). The capability of bioagents to synthesize certain antibiotics has a direct relation to being a good colonizer. Mazzola et al. (1992) suggested that phenazine antibiotic production contributes to the ecological competence of *P. fluorescens* in the rhizosphere of wheat. A decrease in disease severity for take - all disease of wheat and radish wilt caused by *Fusarium* has a direct relation with the establishment *of Pseudomonas* strains (Bull et al. 1991; Raaijmakers et al. 1995). Berger et al. (1996) after thorough studies have drawn a conclusion that decrease in disease severity has a direct relation with the rhizospheric establishment by *B. subtilis*.

4.3.3 Antibiosis

Suppression or destruction of diseases producing propagules (spores, conidia, conidiophore) by the synthesis of antibiotics or other chemicals synthesizing the bioagents (fungi or bacteria) is known as antibiosis (Irtwange 2006; Viterbo et al. 2007; Haggag and Mohamed 2007). Most of the biocontrol agents including Trichoderma, Pseudomonas spp., and Bacillus species produce several types of antibiotics (Kumar et al. 2011; Handelsman and Stabb 1996). The antibiotics produced by Trichoderma species include gliotoxin (Anitha and Murugesan 2005), harzianic acid (Vinale et al. 2014), trichoviridin (McAlees and Taylor 1995), viridian (Zafari et al. 2008), viridiol (Phuwapraisirisan et al. 2006), alamethicins (Aidemark et al. 2010), and others (Goulard et al. 1995). Gliovirin an antibiotic isolated from Trichoderma (Gliocladium) virens shows a strong inhibitory effect against *Pythium ultimum* and *Phytophthora* species (Howell and Stipanovie 1983). Thielaviopsis basicola, Phymatotrichum omnivorum, Rhizopus arrhizus, or Verticillium dahliae. B. thuringiensis was not inhibited by gliovirin. Secretion of T. harzianum strain against Gaeumannomyces graminis var. tritici exhibited inhibitory effects supporting the fact that bioagent synthesizes antibiotics plays a vital role in the inhibition of the pathogen.

Bacillus and *Pseudomonas* species are also effective microbes in managing plant diseases by the production of antibiotics (Weller 1988; Kumar et al. 2011). Plant disease suppression due to *P. fluorescens* may be due to synthesis of pyoluteorin, phenazine, oomycin A, IAA, siderophores, phenazine, siderophore (Whistler et al. 2000; Schoonbeek et al. 2002; Suzuki et al. 2003; Johri et al. 2003; Rachid and Ahmed 2005), extracellular hydrolytic enzymes (Siddiqui 2006), alginate, HCN (Bagnasco et al. 1998), and pseudomonic acid. The antimicrobial compounds discussed above are responsible to cause fungistasis, inhibition of spore germination, and degradation of a mycelial wall and also induce other fungicidal effect (Thomashow and Weller 1990). Production of iturin and surfactin by *B. subtilis* RB 14 played important role in the protection of tomato plant against *R. solani* (Asaka and Shoda 1996). *B. subtilis* synthesize about five antibiotics, namely, subtillin, bacitracin, bacillin, subtenolin, and bacillomycin (Young et al. 1974). Pukall et al. (2005) isolated four toxin-producing strains of *Bacillus* spp., such as *B. pumilus*, *B. fusiformis*, *B. subtilis*, and *B. mojavensis* apart from *B. cereus*.

4.3.4 Plant Growth Promotion

PGPR helps in improving the plant health by the producing of different metabolites such as siderophore and hydrocyanic acid (HCN) (Bhatia et al. 2008); other metabolites also include phytohormones like indole acetic acid, gibberellins, cytokinins, and ethylene (Patten and Glick 2002). Another mechanism is the breaking of ethylene molecules which inhibits the growth of roots by certain rhizobacteria and also improves the plant health (Glick et al. 1999). Great number of rhizospheric microbes produces the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which breaks down the ACC molecule, the direct originator of the plant hormone ethylene (Belimov et al. 2001; Glick 1995). They kindle the root propagation of different crop plants (Belimov et al. 2001). The abovementioned mode of action (breakdown of ACC) is most efficient in plants which undergo stresses like flooding, drought, and phytopathogens (Grichko and Glick 2001; Wang et al. 2000). The plant health improvement by rhizospheric microbes lies in the fact of initiation of phosphorous plant nutrition (Bertrand et al. 2001). (The increase in yield of groundnut by Pseudomonas strains is the best example of initiation of phosphorous plant nutrition which leads to easy uptake of soil phosphorus by plants) (Dey et al. 2004). Effect of rhizospheric microbes on plant growth is because of synthesis of siderophores; synthesis of phytohormones which leads to increase in plant growth (Garcia de Salamone et al. 2001); and initiation of phosphorous plant nutrition leading to readily available phosphorous (Richardson 2001). Sen et al. (2012) reported that Stevia rebaudiana Bertoni, a natural sweetener, is composed of two main sweetest compounds which make it 300 and 450 times sweeter than sucrose. Pseudomonas BRL-1 isolated from the rhizosphere showed both in vitro and in vivo antagonistic activity against the pathogen Alternaria alternata inciting leaf blight disease in Stevia rebaudiana. Siderophore produced by fluorescent Pseudomonas has very high affinity for ferric ion and is secreted during growth under low iron conditions (Johri et al. 2003) which is then converted to ferrous ions and thus reduces iron availability to pathogens. However, the producing strain can utilize this via a very specific receptor in its outer cell membrane (Buyer and Leong 1986). In this way the bacteria may restrict the growth of deleterious bacteria and fungi at the plant root surface (Loper and Buyer 1991). Consequent iron starvation condition prevents the germination of fungal spores. Elad and Baker (1985) have demonstrated the direct relationship between synthesis of siderophores and their tendency to control the germination of chlamydospores of Fusarium oxysporum. Johri et al. (2003) have also reported that fluorescent pseudomonas during low iron concentration secrete siderophores which reduces ferric into ferrous ions, and thus no more iron is available to pathogens. However, the synthesizing strain has a tendency to use this with the help of specific receptors in its outer cell membrane (Buyer and Leong 1986). In this is how the bacteria can check the growth of harmful bacteria and fungi at the surface of roots (Loper and Buyer 1991) and hence promote the plant growth.

4.4 Conclusions

Now it is very much clear that rhizospheric microbes have a positive trend in increasing the growth and yield of medicinal plants under biotic stresses. Although understanding the mechanism of rhizospheric microbes as a plant growth promoter is still an interesting field of qualitative research, therefore, it is the right time to think about the potential candidate of microbes which can improve the plant health even under biotic stresses. Application of suitable strain of microorganisms in the field infested with the soil borne pathogens may exert some reliable results. Consortium of microorganism of different origin can enhance the potentiality of the bioagents which may be very useful in the disease management. However, mechanisms behind the control of diseases are still the matter of research as this will unravel various important facts related to disease management. Due to the distinct features of medicinal plants, future research could also pave a new platform in understanding the subject. Adequate research in this thrust area could be a major breakthrough in the improvement of health of various economically important medicinal plants.

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