Chapter 9
Cocultivation of *Piriformospora indica* with Medicinal Plants: Case Studies

Aparajita Das, Ram Prasad, R.B. Srivastava, Shivaji Deshmukh, M.K. Rai, and Ajit Varma

9.1 Introduction

A thorough review of literature has shown that *P. indica* has enormous potential for growth promotion of diverse groups of plants by colonization of host roots (Singh et al. 2000; Rai et al. 2001; Malla et al. 2002, Rai and Varma 2005; Oelmuller et al. 2009; Achatz et al. 2010). The fungus is of utmost biotechnological importance and is multifunctional (Singh et al. 2003: Kumar et al. 2011). *P. indica* is similar to arbuscular mycorrhizal fungi in many respects (Varma and Schuepp 1994; Varma et al. 1999, 2001; Singh et al. 2000; Rai and Varma 2005). But, unlike arbuscular mycorrhizal fungi, it can be cultured on artificial medium (Varma et al. 1999, 2001; Pham et al. 2004). Thus, this revolutionary endophytic fungus functions as a plant growth promoter and biofertilizer in nutrient-depleted soils. *P. indica* promotes the growth of plants and improves their productivity, increases the drought tolerance, delays the wilting of leaves, prolongs the ageing of callus tissue, enhances the uptake of phosphate from the soil and relieves the stress conditions caused by acidity, desiccation and heavy metal toxicity (Oelmuller et al. 2009; Yadav et al. 2010; Camehl et al. 2010; Kumar et al. 2011). Plants treated with *P. indica* resulted in increase in overall growth, flowering, nutrient uptake and enhancement in secondary metabolites production in plants (Dolatabadi et al. 2011a, b; Das et al. 2012).

A. Das • R. Prasad • A. Varma (✉)
Amity Institute of Microbial Technology (AIMT), Amity University Uttar Pradesh (AUUP), Sec-125, Expressway, Noida, UP, India
e-mail: adas@amity.edu; rprasad@amity.edu; ajitvarma@amity.edu

R.B. Srivastava
Defence Institute of Higher Altitude Research, Defence Research Development Organisation, Leh-Ladakh, Jammu and Kashmir, India
e-mail: rbs1_cnb@rediffmail.com

S. Deshmukh • M.K. Rai
Department of Biotechnology, SGB Amravati University, Amravati 444 602, Maharashtra, India
e-mail: shivgajanan@rediffmail.com; mkrai123@rediffmail.com

A. Varma et al. (eds.), *Piriformospora indica*, Soil Biology 33,
DOI 10.1007/978-3-642-33802-1_9,

Pronounced growth promotional effect was recorded with terrestrial orchids also (Prasad et al. 2004). For more information readers may refer to Chap. 1.

Medicinal plants are of great importance because of their healing properties due to the presence of various active principles and different secondary metabolites (Rai 1994). A large number of remedies have been offered to mankind by the plant kingdom, of which many are provided by aromatic plants (Wyk and Wink 2004). Over three quarters of the world population rely mainly on medicinal plants and plant extracts for health care. More than 30 % of the entire plant species, at one time or other, was used for medicinal purposes. The rural folks and tribals in India as well as in other developing countries even now depend largely on the surrounding plants/forests for their day-to-day needs. Medicinal plants are being looked upon not only as a source of health care but also as a source of income. The value of medicinal plants related trade in India is of the order of 5.5 billion US dollar and is further increasing day by day. The international market of herbal products is estimated to be US $ 62 billion. India shares in the global market of medicinal plants trade is less than 0.5 % (http://www.agricultureinformation.com/forums/questions-answers/34618-cultivation-medicinal-plants-india-government-support.html, dated 17th April 2012). It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25 % of the total drugs, while in fast developing countries such as China and India, the contribution is as much as 80 %. Thus, the economic importance of medicinal plants is much more to countries such as India than to rest of the world. These countries provide two-third of the plants used in modern system of medicine, and the health care system of rural population depends on indigenous systems of medicine (Chandel and Sharma 1997; Lambert 1998; Kavitha et al. 2010). Traditional Indian Ayurveda medicines have a 70 % share of the formal medicine market in India (Lambert 1998).

India is one of the world's 12 biodiversity centres with the presence of over 45,000 different plant species. India's diversity is unmatched due to the presence of 16 different agro-climatic zones, 10 vegetation zones, 25 biotic provinces and 426 biomes (habitats of specific species). Of these, about 15,000–20,000 plants have remarkable medicinal value. However, only 7,000–7,500 species are used for their medicinal values by traditional communities (Kurian and Sankar 2007, http://megforest.gov.in dated 26th April 2012). There are about 45,000 medicinal plant species in India, with hot spots in the region of Eastern Himalayas, Western Ghats and Andaman and Nicobar Island. The officially documented plants with medicinal potential are 3,000, but traditional practitioners use more than 6,000. In rural India, 70 % of the population depends on the traditional type of medicine, the Ayurveda (http://medicinalpltsinindiamp, dated 17th April 2012). Hence, the medicinal and aromatic plants are of immense importance for welfare of the mankind. Taking into consideration, the above facts, it is the need of the hour to employ techniques which may enhance the growth and yield of economically important plants. In this context, arbuscular mycorrhizal fungi and other endophytes like *P. indica* play a crucial role (Oelmuller et al. 2009; Harman 2011).

It has been extensively studied that symbiotic fungus *P. indica* promoted growth and enhanced active ingredients of the medicinal as well as economically important

plants by forming association with their roots. This novel fungus has been also established as an agent for biological hardening of tissue culture-raised plantlets (Sahay and Varma 1999, 2000; Oelmuller et al. 2009; Gosal et al. 2010). So far, more than 148 plants have been interacted with the fungus and documented. This include plants such as *Centella asiatica*, *Coriandrum sativum*, *Artemisia annua*, *Spilanthes calva*, *Arabidopsis thaliana*, *Cajanus cajan*, *Arachis hypogaea*, *Mimosa pudica*, *Cicer arietinum*, *Allium cepa*, *Hordeum vulgare*, *Zea mays*, *Saccharum officinarum*, *Withania somnifera*, *Solanum lysopersicum*, etc. (Bagde et al. 2010). This chapter provides an overview of case studies of selected medicinal plants and their interaction with the wonder fungus *P. indica*.

9.2 Interaction of *P. indica* with Different Medicinal Plants

A large number of medicinal plants like *Spilanthes calva*, *Withania somnifera*, *Bacopa monnieri*, *Coleus forskohlii* and others were inoculated with the *P. indica* in pots as well as in fields to study its influence on the host plants. The symbiotic fungus promoted the overall growth and development of all the medicinal plants tested so far. It also enhanced percentage flowering and fruit development in medicinal plants like *Coleus forskohlii*, *Spilanthes calva* and *Withania somnifera*. It also promoted enhancement of secondary metabolites contents in medicinal plants inoculated with *P. indica*. Details of case studies are discussed from Sect. 9.2.1 to 9.2.13.

9.2.1 Interaction with Artemisia annua

Artemisia annua is also known by many names like sweet wormwood, sweet sagewort or annual wormwood. It is a common type of wormwood which is native to temperate Asia but now found throughout the world (Kapoor et al. 2007). The plant has traditionally been grown in China for medicine and more recently in Europe for its aromatic leaves which are used in flavouring beverages. It has fern-like leaves, bright yellow flowers and with camphor-like scent (Fig. 9.1a). Its height averages about 2 m tall, and the plant has a single stem, alternating branches and alternating leaves which range 2.5–5 cm in length. It secretes many medicinal compounds including "artemisinin" which is well antimalarial drug (Kapoor et al. 2007). *P. indica* showed growth promotion in *A. annua* when inoculated in seedlings stages under field condition (Fig. 9.1c,d; Tables 9.1a and 9.1b).

P. indica promoted the growth of the tissue culture-raised plantlets when interacted in vitro. It showed higher rate of shoot and root development (Fig. 9.2a, b). Similarly, it helped in biohardening of tissue culture-raised plantlets when transferred to the natural environmental conditions. Inoculated plants showed a higher survival rate than the uninoculated control (uninoculated) plants. It was also reported that interaction with fungus not only increased the biomass but also the Artemisinin (an antimalaria drug) content.

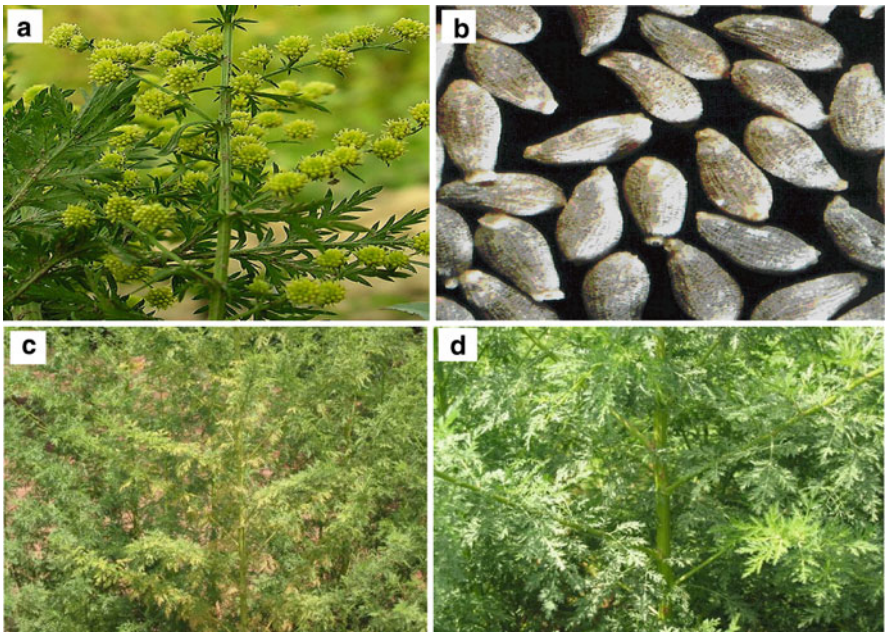

Fig. 9.1 Field grown plants of *Artemisia annua* (**a**) at flowering stage (**b**) Seeds; Five months old plants (**c**) Control plants without *P. indica* showing yellow-brown leaves, (**d**) Plants inoculated with *P. indica* showing dark green leaves

Table 9.1a Interaction of *Artemisia annua* seedlings with *P. indica* (field trial expt.)

Treatments	Height (in cm)	Mean
Control (Without *P. indica*)	152–165	158.5
Piriformospora indica	210–282	246.0

Data mean of 20 independent plants. Measured after 150 days. No chemical added. Irrigation by normal water

Table 9.1b Interaction of *Artemisia annua* seedling with *P. indica* (field trial expt.)

Treatment	Dry weight (in gm)/plant	Mean
Control (Without *P. indica*)	19–22	20.5
Piriformospora indica	64–82	73.0

Data mean of 20 independent plants. Measured after 150 days

9.2.2 Interaction with Tridax procumbens

Tridax procumbens Linn. belongs to family Asteraceae, found throughout India and is employed as indigenous medicine. It is reported for treatment of variety of ailments including jaundice (Kumari 2005; Bhagwat et al. 2008). It is commonly known as "Ghamra" and in English popularly called "coat buttons" because of the

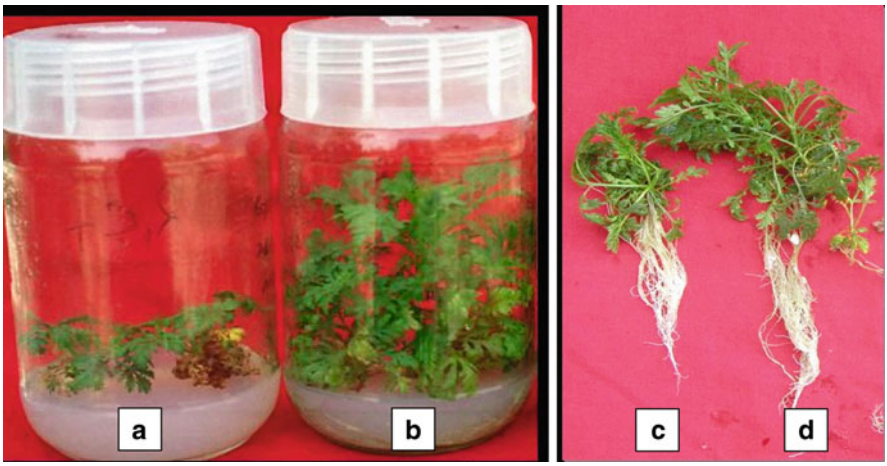

Fig. 9.2 Effect of *P. indica* on tissue culture-raised plantlets with high rate of shoots and root development. (**a**) Shoots without *P. indica*, (**b**) shoots with *P. indica*, (**c**) roots without *P. indica*, (**d**) roots with *P. indica*

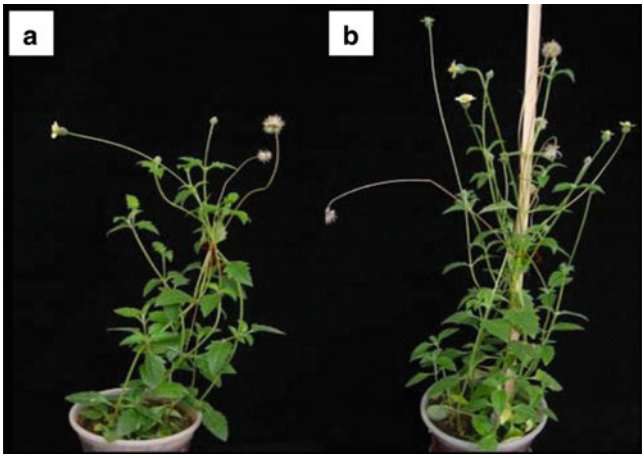

Fig. 9.3 Effect of *P. indica* on plant growth and biomass of *T. procumbens*. (**a**) Plants without fungus, (**b**) plants with fungus

appearance of its flowers. It has been extensively used in Indian traditional medicine as anticoagulant, antifungal and insect repellent, in bronchial catarrh, diarrhoea and dysentery. Moreover, it possesses wound healing activity and promotes hair growth. Antioxidant properties have also been demonstrated (Bhagwat et al. 2008). Interaction of *T. procumbens* with *P. indica* promoted the growth and biomass as depicted in Fig. 9.3 and Table 9.2 (Kumari 2005).

Table 9.2 Comparison of different growth parameters in control and *T. procumbens* plants inoculated with *P. indica*

Parameters	Control (without *P. indica*)	*P. indica* treated	Percent increase
Height	87.80 ± 1.58	108.44 ± 1.12	23
Shoot length	57.30 ± 0.99	73.04 ± 0.89	27
Root length	30.48 ± 0.89	35.44 ± 0.82	16
Fresh shoot weight	18.99 ± 0.35	23.55 ± 0.50	24
Dry shoot weight	06.80 ± 0.28	09.17 ± 0.29	35
Fresh root weight	06.87 ± 0.05	11.14 ± 0.46	62
Dry root weight	02.39 ± 0.27	04.65 ± 0.31	51
Percent colonization	nil	41	

Dead/sterile fungal biomass served as control. Height is given in centimetre, and weight is in gram. Each figure is an average of five independent replicates

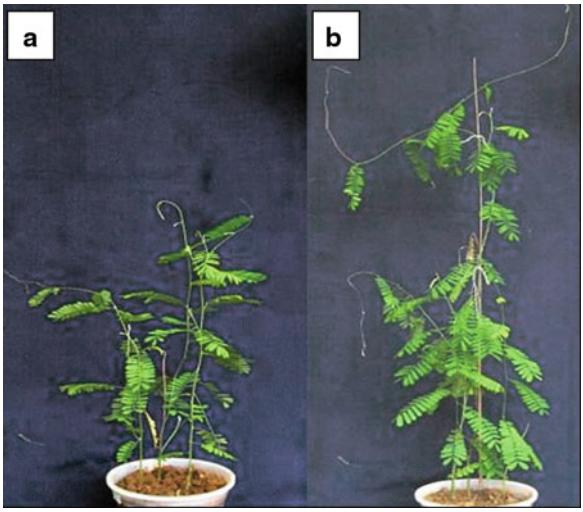

Fig. 9.4 Effect of fungi on plant growth and biomass of *A. precatorius* (**a**) without *P. indica*, (**b**) with *P. indica*

9.2.3 Interaction with Abrus precatorius

Abrus precatorius is a plant of the Fabaceae family. It is also known as Indian liquorice, jequirity, crab eye, *Glycyrrhiza glabra*, among others. The plant grows widely in fairly dry climates of tropical and subtropical regions, such as India, Sri Lanka, Nigeria and the West Indies. Plant pacifies vitiated "pitta", "vata", inflammation, vitiligo, skin disease, wounds, ulcer, alopecia, asthma, stomatitis and fever (Kumari 2005). The principal active ingredients present are abrin, abraline, choline, precatorine, abricin and abridin. Studies showed positive influence of the fungus *P. indica* on *Abrus precatorius* (Fig. 9.4 and Table 9.3).

Table 9.3 Comparison of different growth parameters in control and *A. precatorius* plants inoculated with *P. indica*

Parameters	Control (without *P. indica*)	*P. indica* treated
Height	102.23 ± 1.26	131.46 ± 0.80
Shoot length	66.96 ± 1.46	88.03 ± 1.58
Root length	35.26 ± 2.65	43.43 ± 0.75
Fresh weight	37.93 ± 1.02	43.95 ± 1.06
Fresh shoot weight	31.63 ± 1.40	35.66 ± 0.66
Dry shoot weight	14.00 ± 1.25	16.62 ± 0.50
Fresh root weight	6.30 ± 0.48	8.28 ± 0.56
Dry root weight	2.14 ± 0.12	3.06 ± 0.21

Dead/sterile fungal biomass served as control. Height is given in centimetre, and weight is in gram. Each figure is an average of five independent replicates

9.2.4 Interaction with Bacopa monnieri

Bacopa monnieri commonly known in India as Brahmi is an important Ayurvedic medicinal plant belonging to Scrophulariaceae family. In the traditional system of medicine, Brahmi is a reputed nervine tonic; it is also used to treat asthma, insanity, epilepsy, hoarseness, enlargement of the spleen, snake bite, rheumatism, leprosy, eczema and ringworm and as a diuretic, appetitive and cardiotonic. The main active ingredient of *B. monnieri* is believed to be the bacosides (Prasad et al. 2004).

Tissue culture technology could play an important role in the clonal propagation, germplasm conservation and improvement of *B. monnieri*. Shoot regeneration has been reported from the distal ends of 1–12 mm long internode segments of *B. monnieri* cultured on growth regulator-free medium, longer internode being more conducive to regeneration (Fig. 9.5). *P. indica* is documented to promote plant growth and protects the host against root pathogens and insects. *P. indica* promoted the plant growth and enhanced antioxidant activity as well as the active ingredient bacoside by several folds (Prasad et al. 2004). Plant treated with *P. indica* also showed significant increase in number of leaves, number of branches, root and shoot length as depicted in Fig. 9.5 and Table 9.4.

It was found that there was an inherent problem with the micropropagated plants during the time of transplantation from the laboratory to field. It was noticed that the rate of survival was very low, up to 40 % in the field conditions. The salient reason could be the "transient transplant shock" that resulted in the stunted growth. For employing the technique, inoculation of micropropagated plantlets with active cultures of AMF or mycorrhiza-like fungi appears to be critical for their survival and growth. These pre-acclimatized plantlets when transferred to field, overcome the transient transplant shock, and were able to cope up with changed environment of transplantation which also helps in their successful establishment. Pre-establishment of mycorrhiza in the host roots also helps in the development of the synergistic effect with other rhizosphere microflora that competes in the ecosystem for successful survival (Prasad et al. 2004).

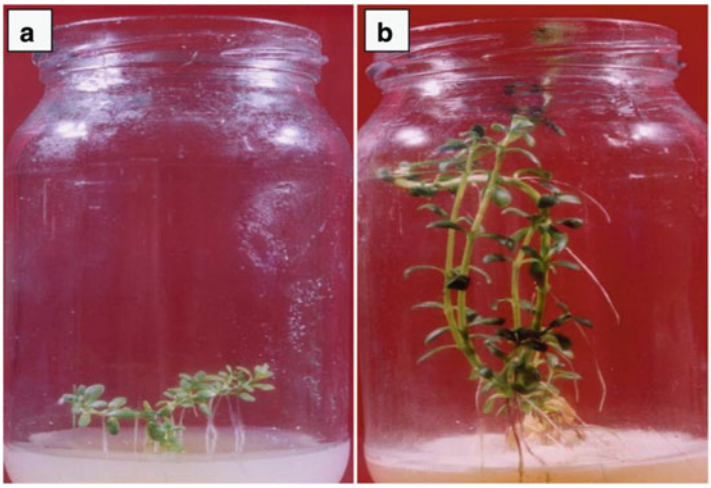

Fig. 9.5 In vitro grown plants of *B. monnieri*. (**a**) Plants without *P indica*, (**b**) plants with *P. indica*

Table 9.4 Effect of *P. indica* on growth of *B. monnieri* in 90 days time on Kaefer semisolid medium. Control: Without *P. indica*

Bio-inoculant	Number of leaves	Number of branches	Number of aerial roots	Root length (cm)	Shoot length (cm)	Total weight (f. w. in g)	
						Shoot	Root
Control	46.75	2	22.5	2.12	11.06	0.21	0.38
P. indica	76.5	6.6	38	6.5	23.12	1.26	0.96

9.2.5 *Interaction with* Coleus forskohlii

Coleus forskohlii (willd.) (Briq. syn. *C. Barbatus* (Andr.) Benth) is an aromatic herbaceous species belonging to the family Lamiaceae. It is native to India and is recorded in Ayurvedic *Materia Medica* under the Sanskrit name "Makandi" and "Mayani" (Shah 1996; Patil et al. 2001). It grows wild in the subtropical warm temperate climates of India, Nepal, Burma, Sri Lanka and Thailand. It is also found in Egypt, Arabia, Ethiopia, tropical East Africa and Brazil (Willemse 1985; Chandel and Sharma 1997; Kurian and Sankar 2007). *C. forskohlii* is a perennial plant that grows to about 45–60 cm tall. It has four angled stems that are branched, and nodes are often hairy. Leaves are 7.5–12.5 cm in length and 3–5 cm in width, usually pubescent, narrowed into petioles. Inflorescence is raceme, 15–30 cm in length; flowers are stout, 2–2.5 cm in size, usually perfect and calyx hairy inside. Upper lip of calyx is broadly ovate. The blue or lilac corolla is bilabiate. The root is typically golden brown, thick, fibrous and radially spreading. Roots are tuberous, fasciculated, 20 cm long and 0.5–2.5 cm in diameter, conical fusiform, straight, orangish within and strongly aromatic. *C. forskohlii* is the only species of the genus

to have fasciculated tuberous roots (Kavitha et al. 2010). The entire plant is aromatic, and whole plant *C. forskohlii* (roots, flowering shoots and leaves) have commercial importance. The plant contains 0.05–0.1 % forskolin/g fresh weight which is a diterpenoid and used as drug. Roots are the major source of forskolin (coleonol), although diterpenoids are found in almost all parts (Chandel and Sharma 1997). Forskolin from *C. forskohli*i is an activator of adenylate cyclase and thus leads to the production of second messenger cyclic AMP (cAMP) (Kavitha et al. 2010). Leaves also contain diterpenoid methylene quinine, coleonol, barbatusin and cyclobutatusin. Barbatusin is used against lung carcinoma and lymphatic leukaemia (Kurian and Sankar 2007). Other secondary compounds found in *C. forskohlii* are monoterpenes, monoterpene glycosides, sesquiterpenes and phenolic glycosides (Ahmed and Viswakarma 1988; Ahmed and Merotra 1991; Petersen 1994). In the traditional Ayurvedic medicine, *C. forskohlii* has been used for treating heart diseases, abdominal colic, respiratory disorder, insomnia, convulsions, asthma, bronchitis, intestinal disorders, burning sensation, constipation, epilepsy and angina (Ammon and Muller 1985). The plant is also used for veterinary purposes (De Souza and Shah 1988). Forskolin is also used in the preparation of medicines that suppresses hair greying and restoring grey hair to its normal colour (Keikichi et al. 1988). Furthermore, forskolin is valued for anti-allergic activity (Gupta et al. 1991). Roots are hypotensive and spasmolytic and are given to children in constipation. It is effective against thrombosis and is employed in glaucoma therapy, owning to its adenylated cyclase stimulant activity (Kurian and Sankar 2007; Chandel and Sharma 1997; Kavitha et al. 2010). This indigenous species, besides being used as a medicinal plant, is used as a potent source of essential oil (Patil et al. 2001). The essential oil present in tubers has an attractive and delicate odour with a spicy note (Misra et al. 1994). The essential oil has potential use in the food flavouring industry and can be used as an antimicrobial agent (Chowdhary and Sharma 1998). There is a wide variation in morphology, essential oil content and yield parameters among the genotypes of *C. forskohlii* (Patil et al. 2001). Vishwakarma et al. (1988) screened 38 genotypes collected from various locations to identify the potential genotypes for forskolin. The content of forskolin varied substantially with different genotypes, from 0.01 to 0.44 % on the fresh weight basis.

C. forskohlii was interacted with root endophytic fungus *P. indica* that mimics AMF under field condition. Growth of *C. forskohlii* in the presence of *P. indica* resulted in an enhancement in aerial growth and biomass production of medicinal plants. The height, number of branches, average length of the branches and number of leaves and leaf area of *P. indica*-treated 6-month-old plants was significantly increased compared to the untreated control plants (Table 9.5a and Fig. 9.6). Although the fungus also promoted the number (22 %) and lengths (32 %) of the roots, they looked fibrous and tough in texture (Table 9.5a). This demonstrates that the initial promotion of rooting shifted towards the development of fibrous root structures in the presence of the fungus. Consequently, after 6 month on the field, the overall weights of colonized roots were lower than the weight of the uncolonized controls (Table 9.5b). Also the thickness of colonized roots was dramatically reduced. As a result, shoot growth was promoted, and root growth

Table 9.5a Influence of *P. indica* on plant length (cm), number of branches, average length of branches (cm), number of leaves, leaf area (cm^2), number of roots and root thickness (cm) of field grown *C. forskohlii* plants (growth period: 6 months)

Parameters	Without *P. indica*	*P. indica*	Percent increase/ decrease over the control
Shoot length(cm)	43.94 ± 2.43a	55.07 ± 1.68b	+20.19
Number of branches	15.00 ± 1.24a	20.00 ± 1.32b	+24.21
Average length of branches (cm)	19.04 ± 2.35a	32.88 ± 2.05b	+42.14
Number of leaves	89.44 ± 6.46a	169.22 ± 13.91b	+46.05
Leaf area (cm^2)	8.79 ± 1.97a	12.50 ± 2.92b	+29.71
Number of roots	44.00 ± 3.08a	56.00 ± 2.87b	+22.81
Root length (cm)	16.44 ± 0.77a	24.06 ± 0.89b	+31.67
Root thickness (cm)	6.04 ± 4.61a	1.18 ± 2.29b	−80.46

Each data represents the mean of two independent replicates (Year 2008 and 2009), and each replicate represents 18 plants; control and *P. indica* represent non-treated and treated with symbiotic fungus *P. indica*, respectively; per cent increase represents per cent increase of treated over non-treated data; mean values within a same row followed by different letters differ significantly at $p \leq 0.05$ according to student's *t* test

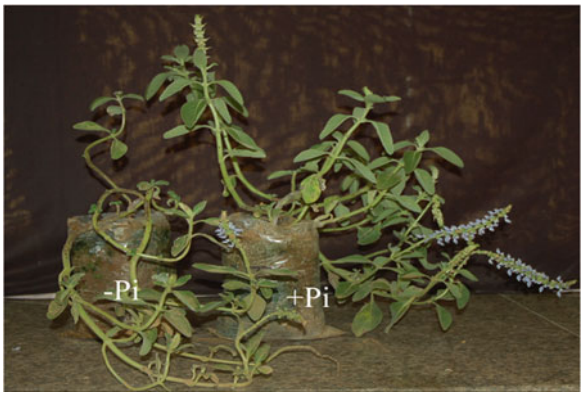

Fig 9.6 Influence of *P. indica* on 6-month-old *C. forskohlii* in field condition. Photographed after 6 months. Figure represents plant morphology as a result of interaction between *P. indica* on *C. forskohlii* under field condition. Each polythene bag contained 2.5 kg of unsterile sand, field soil and compost (1:1:0.25 w/w). The fungal inoculum was 2 % (w/v). Each bag contained 30-day-old rooted plant cuttings. Irrigation was done on alternate days using underground water. -Pi: Plants without inoculum of *P. indica*; +Pi: Plants treated with fungus

was retarded by *P. indica* when medicinal plants *C. forskohlii* when treated with *P. indica*. Flowering occurred earlier and more vigorously in *P. indica*-colonized plants. Colonized plants flowered at least 7 days earlier than the untreated controls. After 180 days, 31 % of non-treated but 81 % of *P. indica*-treated plants flowered. Moreover, the number and length of the inflorescence were significantly higher in colonized plants compared to their untreated counterparts (Das et al. 2012).

Table 9.5b Influence of *P. indica* on plant biomass (growth period 6 months)

Parameters	Without *P. indica*	*P. indica*	Percent increase/decrease over control
Dry shoot weight, g	16.63 ± 02.92a	30.82 ± 03.27b	+46.04
Dry root weight, g	08.54 ± 03.57a	04.34 ± 03.01b	−49.18
Percent root colonization	nil	25.55	

Each data represents the mean of two independent replicates (Year 2008 and 2009), and each replicate represents 18 plants; control and *P. indica* represent non-treated and treated with symbiotic fungus *P. indica*, respectively; per cent increase represents per cent increase of treated over non-treated data; mean values within a same row followed by different letters differ significantly at $p \leq 0.05$ according to student's *t* test; each figure in percent root colonization (Spores of *P. indica*) represents the mean of three independent replicates, and each replicate represents 90 root segments

9.2.6 *Interaction with* Adhatoda vasica

Adhatoda vasica Nees, commonly known as Malabar nut belongs to family Acanthaceae. It is an evergreen shrub. The plant is used for the preparation of medicine for asthma, bronchitis and other pulmonary disorders. It is also used as antiarthritis, antiseptic, antimicrobial, expectorant, sedative and antituberculosis (Dey 1980; Singh and Jain 1987). Glycodin®, a well-known product used for the cure of bronchitis, is extracted from the leaves of the plant. In Ayurveda, a number of medicines are manufactured by this plant. There is a pressing need of rapid multiplication of this plant due to increasing demand by pharma industries.

Rai and Varma (2005) studied the role of *P. indica* in growth promotion of *A. vasica*. The cuttings of *A. vasica* were inoculated with *P. indica* to assess the growth promoting property of *P. indica*. They reported that *P. indica* enhanced the growth of *A. vasica* (Fig. 9.7). The authors further observed profuse proliferation of roots of *A. vasica* after inoculation of *P. indica*. Root colonization of *A. vasica* by *P. indica* augmented with time from 53 % after 2 months to 95 % after 6 months. There was a significant enhancement in the growth rate of the plants inoculated with *P. indica*. The growth was very fast up to 2 months (Fig. 9.8) and slowed down thereafter. At each observation, growth was significantly higher for the plants inoculated with *P. indica* as compared to the control plants.

The fresh and dry weight of shoots and roots of *A. vasica*-inoculated plants was higher than that of the corresponding controls (Fig. 9.9). This suggests that *P. indica* is an appropriate endophyte for fast growth of the plants. After additional trials and evaluation of active principles or secondary metabolites production, *P. indica* may be recommended for growth enhancement of *A. vasica*.

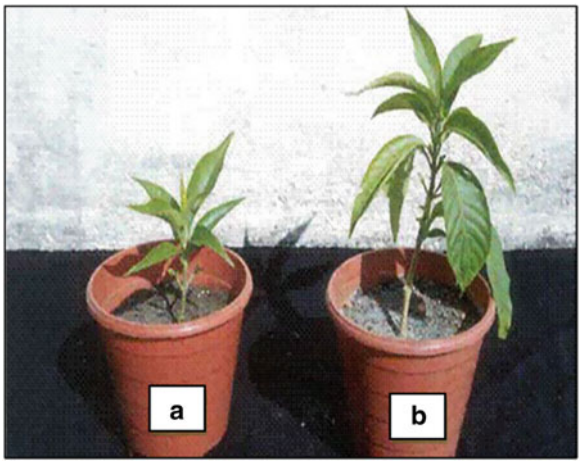

Fig. 9.7 Effect of *P. indica* on *A. vasica* (**a**) Plants without *P indica*, (**b**) Plants with *P. indica*

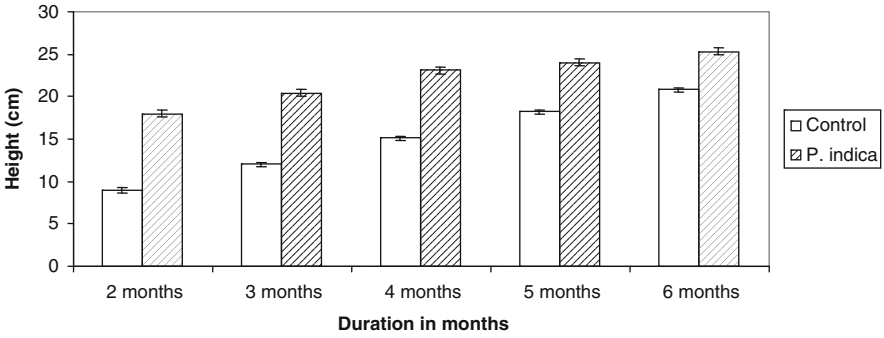

Fig. 9.8 Growth response of *A. vasica* (in cm) after inoculation with *P. indica*, control plants are without *P. indica*

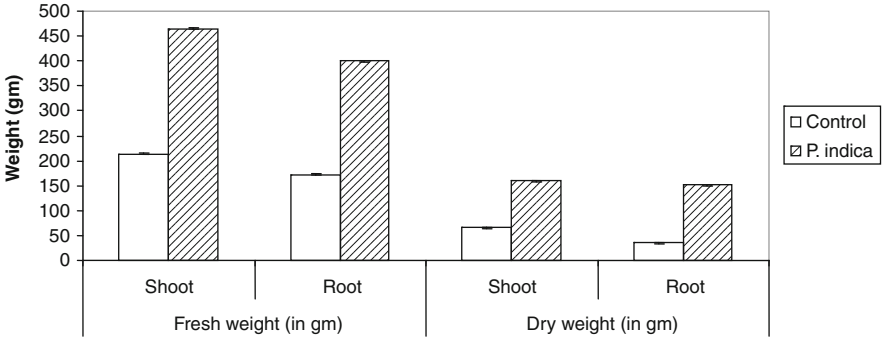

Fig. 9.9 Fresh and dry weight of *A. vasica* after 6 months after interaction with *P. indica*, control plants are without *P. indica*

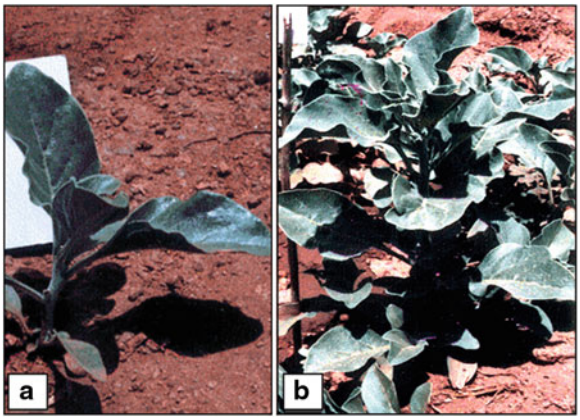

Fig. 9.10 Pronounced growth response in *W. somnifera* after inoculation with *P. indica*: (**a**) control (without *P. indica*), (**b**) inoculated with *P. indica*

9.2.7 *Interaction with* Withania somnifera *and* Spilanthes calva *in Field Trials*

W. somnifera is also known as Indian ginseng and belongs to the family Solanaceae. There are more than 91 pharmaceutical products manufactured from the roots of this plant and are potential sources of a promising drug for cancer (Devi 1996). *S. calva* is a member of Asteraceae and is called as "toothache plant" and is well known for enhancing immunity (Rai et al. 2001). The plant has anti-ageing property and cures diseases of gums like pyorrhoea and is also useful in toothache. The leaves of this plant stimulate salivation which is due to an active chemical known as "spilanthol". Because of high medicinal value, there is an increasing demand for these plants in national and international market (Rai et al. 2001). Therefore, there is a greater need to augment the growth and secondary metabolites of these plants. This inoculation of beneficial microorganisms like arbuscular mycorrhizal fungi or other growth promoting endophytes serves the purpose.

Pronounced growth response in *W. somnifera* and *S. calva* after inoculation with *P. indica* was noted (Figs. 9.10a, b and 9.11a, b). The data revealed that the plants treated with *P. indica* were superior in development compared to control plants (uninoculated with *P. indica*). A significant increase in the shoot length was observed in the inoculated plants. The microscopic examination of stained root samples revealed a high colonization of *S. calva* and *W. somnifera* by *P. indica* in 62 and 73 % root length, respectively (Fig. 9.12).

The basal and leaf area of treated plants was also increased (Table 9.6). Interestingly, in inoculated *S. calva* plants, some large, kidney-shaped heads were observed among the normal round heads. These kidney-shaped heads were never observed in control plants. The length of the inflorescence and the number of flowers in inoculated *S. calva* were also increased (Table 9.6; Fig 9.11a, b). Similarly, in the inoculated plants of *W. somnifera*, the number of flowers was higher (Table 9.6) as compared to controls. In both medicinal plants, seed count was higher as compared to controls.

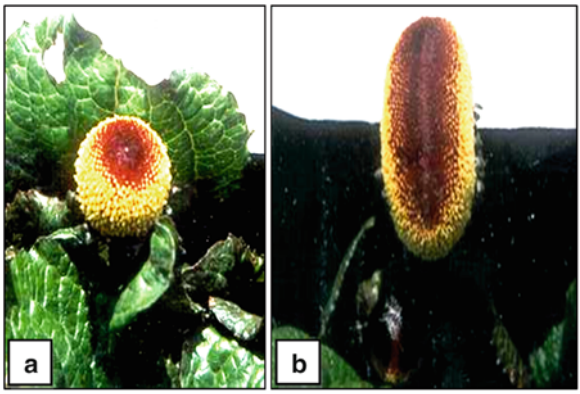

Fig. 9.11 Pronounced growth response and flowering in *S. calva* after inoculation with *P. indica*. (**a**) Control (without *P. indica*), (**b**) inoculated with *P. indica*

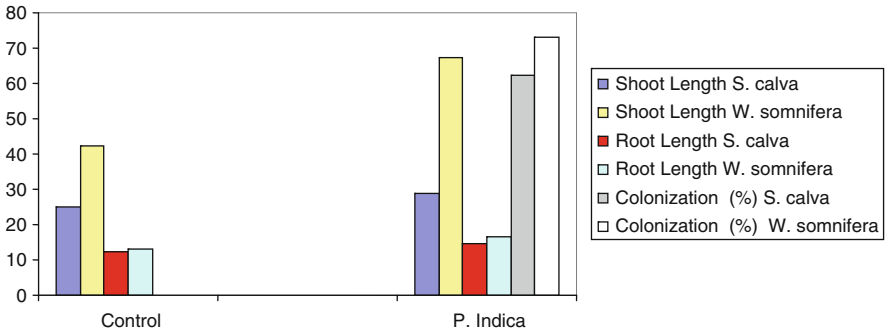

Fig. 9.12 Influence of *P. indica* on shoot and root length and on per cent root colonization of S. *calva* and *W. somnifera* in a field trial. The control plants were treated with an equal amount of autoclaved mycelium

*9.2.8 Interaction with Turmeric (*Curcuma longa *L) in a Field Trial*

Turmeric (*Curcuma longa* L.) is a perennial crop of Zingiberaceae family, widely cultivated in India and other parts of the world. In medieval Europe, turmeric became known as Indian saffron, since it was widely used as an alternative to the far more expensive saffron spice (Ruby et al. 1995). The plant has wide spectrum of medicinal properties and commercial applications due to presence of the well-known bioactive component curcumin. The microbial bioinoculants such as, phosphate solubilizing bacteria (PSB) and arbuscular mycorrhizal fungi (AM fungi) were used for the improvement of the crop, which are shown by the enhancement in the productivity (Eigner et al. 1999). It is a very important medicinal plant and extensively used in Ayurveda, Unani and Siddha medicine as the treatment for

Table 9.6 Influence of *P. indica* on morphology and growth of host plants (*S. calva* and *W. somnifera*) 90 days after inoculation in a field trial. The control plants were treated with equal amount of autoclaved mycelium; control: without *P. indica*

Hosts	Treatment	Basal area (cm^2)	Leaf area length of head (cm^2)	Basal area (cm^2)	Diameter of inflorescences (cm)	No. of flower inflorescences	No. seeds fruit
S. calva	Experimental	7.06 (±0.47)	37.67 (±2.28)	2.49 (±0.09)	5.06 (±0.32)	48.57 (±0.4)	1006 (±7.63)
	Control	4.11 (±0.57)	26.00 (±2.87)	1.48 (±0.03)	4.16 (±0.76)	11.50 (±3.6)	716 (±0.36)
W. somnifera	Experimental	11.40 (±0.61)	45.59 (±0.34)	Nil	Nil	307.40 (±0.53)	46.33 (±5.77)
	Control	5.57 (±0.49)	13.08 (±0.73)	Nil	Nil	81.80 (±1.57)	35.33 (±4.93)

All values are mean ± S.D.; differences between inoculated and control plants are significant at $P < 0.05$

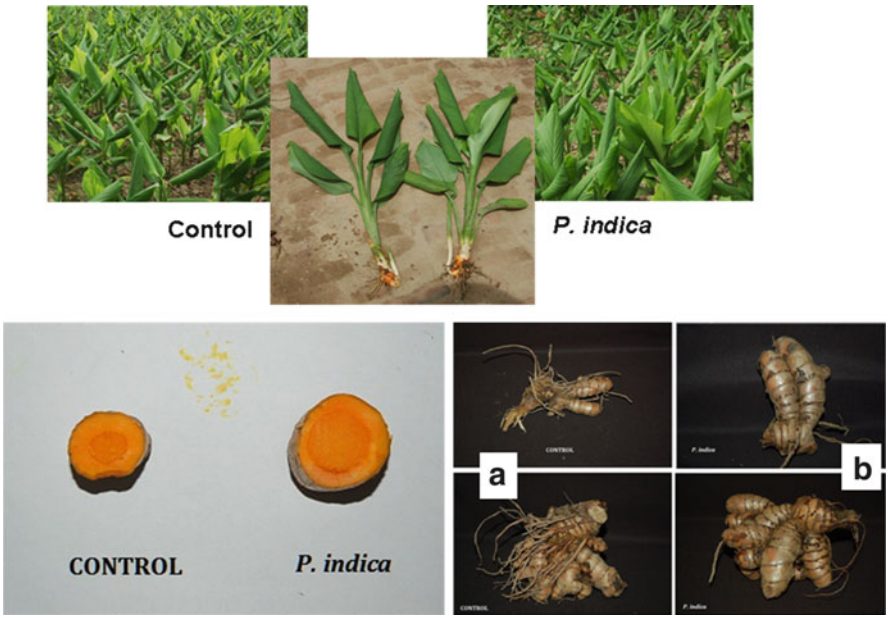

Fig. 9.13 Effect of the *P. indica* on the field grown plant and rhizomes of turmeric (*Curcuma longa* L.) (**a**) Plants without *P. indica*, (**b**) plants treated with *P. indica*. Control: plants without *P. indica*, *P. indica*: inoculated with *P. indica*

various diseases (Dasgupta et al. 1969). It is also used as a food additive (spice), preservative and colouring agent in many countries such as China and Southeast Asia. The turmeric is also used in Ayurveda for the treatment of sprains and swelling caused by injury (Ammon et al. 1992).

The present study focused to understand the interaction of the *P. indica with* turmeric plants. The impact of *P. indica* as bio-inoculant on growth and yield of turmeric was assessed by morphological parameters of the turmeric plant both in treated (with *P. indica*) and untreated (control) plants in field trials. It was observed that the plants of turmeric treated with *P. indica* demonstrated remarkable growth as compared to the control (Fig. 9.13). There was enhancement of 12 % in yield of treated turmeric plants. The rhizomes of the plant treated with *P. indica* were found to be healthier and thicker than the control (Fig. 9.13).

9.2.9 Estimation of Total Alkaloids and Withanolides in Inoculated and Control Plants

Quantitative determination of total alkaloids, withanolides and withaferin A was made by TLC densitometry. Ashwagandha roots were extracted with methanol (20 ml × 3), filtered and evaporated. The extract thus obtained was defatted with

Table 9.7 Percentage of alkaloid content in inoculated *Withania somnifera* (with *P. indica*) and uninoculated plants

Alkaloids	Inoculated with *Piriformospora indica (%)*	Uninoculated plants (without *P. indica*) (%)
Withanolides	0.52	0.60
Total alkaloids	0.34	0.38

n-hexane (10 ml × 3) and then extracted with 1 % sulphuric acid (5 ml × 3), basified with ammonia, extracted with chloroform (10 ml × 3), dried over anhydrous sodium sulphate, filtered, evaporated and weighed for total alkaloid content. The sulphuric acid insoluble was extracted with diethyl ether (10 ml × 3), dried over anhydrous sodium sulphate, filtered, evaporated and estimated as crude withanolide. The total withanolides and alkaloid contents increased after inoculation of *P. indica*. This suggests that *P. indica* can be used for enhancement of active principles (Table 9.7).

9.2.10 *Enhancement of Antimycotic Activity in* Spilanthes calva *due to Increase in Active Principles after Inoculation of* P. indica

Plants of *Spilanthes calva* inoculated with *P. indica* and uninoculated (control) exhibited antifungal activity against *Fusarium oxysporum* (opportunistic human pathogen) and *Trichophyton mentagrophytes* (potential human pathogen). A significant antimycotic activity in aqueous and petroleum ether extracts of *S. calva* was recorded. Petroleum ether extract of *S. calva was* more effective than aqueous extract in inoculated as well as uninoculated plants. The minimum inhibitory concentration of *F. oxysporum* was recorded as 125 µl/ml when treated with aqueous extract of inoculated plants, whereas 250 µl/ml aqueous extract of inoculated plant was needed to inhibit the growth of *T. mentagrophytes*. *F. oxysporum was* found to be more sensitive than *T. mentagrophytes*. The value of MIC for *F. oxysporum* was very high (1,000 µl/ml) when aqueous extract of uninoculated plant was tested. In addition, aqueous extract of uninoculated plant of *S. calva* did not inhibit the growth of *T. mentagrophytes* which is a potential fungal pathogen causing skin and nail infections in human beings (Rai et al. 2004).

Petroleum ether extract of inoculated plants of *S. calva* showed a remarkable antifungal activity. Extract (62.5 µl/ml) was sufficient to inhibit the growth of 1 ml spore suspension of *F. oxysporum*, whereas in extract of uninoculated plants the MIC value reached to 500 µl/ml of spores. Similarly, for *T. mentagrophytes*, 125 µl extract of inoculated plants was enough to arrest the growth, while MIC value was quite high (1,000 µl/ml) in case of petroleum ether extract of uninoculated plants.

The extract of inoculated plants of *S. calva* showed significantly higher activity compared to uninoculated plants. Additionally, extract of petroleum ether was

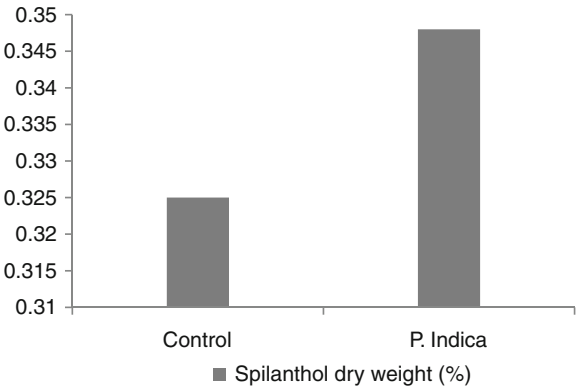

Fig. 9.14 Estimation of spilanthol in roots of *Spilanthes calva*. Control: plants without *P. indica*, *P. indica*: plants treated with *P. indica*

found to be more active than aqueous extract which shows synthesis of higher quantity of spilanthol in inoculated plants. The enhancement of spilanthol was revealed by the chemical analysis of the roots of the plant (Fig. 9.14).

*9.2.11 Interaction with Safed Musli (*Chlorophytum sp.*)*

Chlorophytum sp., which is commonly called as "Safed Musli", is an important medicinal herb belonging to family Liliaceae, having fleshy roots of medicinal importance for the cure of physical weakness, diabetes and arthritis. The dried fasciculed storage roots of this herb, popularly known as "Musli", have strong aphrodisiac, antistress and immuno-modulatory properties due to the presence of steroidal saponins and polysaccharides. The continued collection of these medicinal plants has resulted in the fast depletion of its population, may be due to low rate of multiplication through vegetative means and shy flowering behaviours (Mathur et al. 2008; Gosal et al. 2010).

Chlorophytum plants when inoculated with the fungus *P. indica*, after 30 days of growth in green house, significant increase in root length and the number of lateral roots were observed over uninoculated control (Gosal et al. 2010). Survival rate of musli plants as recorded after transplantation in soil in the greenhouse was improved with microbial *P. indica* biotization (Mathur et al. 2008; Gosal et al. 2010). In vitro plantlets with roots when interacted with P. indica, there was 86 % of plantlets survival in green house, at biohardening stage. An important observation was noted that even the micropropagated un-rooted shoots could form roots under in vivo conditions when treated with *P. indica* with more than 43 % plantlet establishment (Mathur et al. 2008). Biotization with *P. indica* alone or in combination with *P. fluorescens* has led to increase in saponin content (Gosal et al. 2010).

9.2.12 *Interaction of* P. indica *with Fennel (*Foeniculum vulgare*)*

Fennel (*Foeniculum vulgare*) a member from the family Apiaceae is one of the most important aromatic plants widely applied in culinary and medicinal preparations. It is generally considered indigenous to the Mediterranean area, but it is also cultivated elsewhere (Russia, India, China and Japan). Fennel is used against digestive disorders such as spasmodic gastrointestinal complaints and bloating. It may be an effective diuretic and a potential drug for the treatment of hypertension, nervous disturbances, paediatric colic and some respiratory disorders due to its antispasmodic effects. Essential oils are mainly concentrated in the fruits and provide their unique aroma and taste. Anethole and fenchone are the most important volatile components of *F. vulgare* essential oil (Dolatabadi et al. 2011a).

P. indica significantly increased growth of the inoculated of fennel plants in comparison to uninoculated control plants. As well as it significantly increased dry weight of 1,000 fruits in comparison to controls. Not only biomass but the concentration of essential oil increased in pot cultures inoculated with *P. indica* in comparison to controls (Dolatabadi et al. 2011a). Their work revealed through GC and GC-MS studies that the level of anethole was also enhanced with *P. indica* inoculation.

9.2.13 *Interaction with* Linum album

Lignans constitute a large group of secondary metabolites synthesized by many plants. These compounds are usually formed from two phenylpropanoid units and manifest considerable biological activity. Podophyllotoxin, a lignan with antiviral and antineoplastic activities, is used today primarily as a precursor for the semi-synthesis of established cancer therapeutics such as etoposide, teniposide and Etopophos. *Linum album* is an herbaceous and medicinal plant that has important lignan such as podophyllotoxin. Podophyllotoxin and 6-methoxy podophyllotoxin have antiviral and anticancer properties (Chashmi et al. 2011). Podophyllotoxin has been obtained by solvent extraction from the rhizomes of plants *Podophyllum peltatum* and *P. hexandrum* that belong to the family Berberidaceae. Due to endangered status of its natural source and economically unfeasible chemical synthesis, there is an imperative need to search for alternate ways to produce these lignans by cell cultures. Cell cultures of *Linum album* are known to produce these lignans with highest productivity (Baldi et al. 2008).

Baldi et al. (2008) developed cell suspension cultures of *Linum album* from internode portions of in vitro-germinated plant in Gamborg's B5 medium supplemented with 0.4 mg naphthalene acetic acid/l. The highest biomass was 8.5 g/l with podophyllotoxin and 6-methoxypodophyllotoxin at 29 and 1.9 mg/l, respectively, after 12-day cultivation. They were able to successfully coculture *L. album* cells with axenically cultivable arbuscular mycorrhiza-like fungi,

P. indica and *Sebacina vermifera*, for the first time. These enhanced podophyllotoxin and 6-methoxypodophyllotoxin production by about four- and eight-fold, respectively, along with a 20 % increase in biomass compared to the control cultures.

9.3 Conclusions

From the studies of the interaction of *P. indica* with selected medicinal plants, it can be concluded that *P. indica* acts as a potential colonizer and the plant growth promoter fungus which induced a faster development of the aerial part by promoting early maturation with respect to flowering and biomass. Besides this, it also enhances the absorption of the nutrients by underground roots. The increased growth of *P. indica*-colonized plants is due to enhanced nutrient uptake such as phosphorus and nitrogen from the soil. *P. indica* is also involved in the transportation of the phosphate to the host plant. The fungus colonizes both monocots and dicots. The wonder fungus also provided protection when inoculated into the tissue culture-raised plants by overcoming the "transient transplant shock" on transfer to the field or green house and rendered very high survival percentage on transplanted in vitro plants. Finally, the fungus is multifunctional and thus should be used for the growth promotion of medicinally important plants.

Acknowledgements Authors are thankful to Dr. Amit C. Kharkwal for providing turmeric (*Curcuma longa* L) data and figures. Ajit Varma is thankful to ICAR, DBT and DRDO for partial financial support.

References

Achatz B, Kogel KH, Franken P, Waller F (2010) Piriformospora indica mycorrhization increases grain yield by accelerating early development of barley plants. Plant Signal Behav 5:1685–87

Ahmed B, Merotra R (1991) Coleoside-B: a new phenolic glycoside from *Coleus forskohlii*. Pharmazie 46:157–158

Ahmed B, Viswakarma RA (1988) Coleoside, a monoterpene glycoside from *Coleus forskohlii*. Phytochemistry 27:3309–3310

Ammon HPT, Anazodo MI, Safayhi H, Dhawan BN, Srimal RC (1992) Curcumin: a potent inhibitor of leukotriene B4 formation in rat peritoneal polymorphonuclear neutrophils (PMNL). Planta Med 58:26–28

Ammon HPT, Muller AB (1985) Forskolin: from an ayurvedic remedy to a modern agent. Planta Med 46:473–477

Bagde US, Prasad R, Varma A (2010) Interaction of mycobiont: *Piriformospora indica* with medicinal plants and plants of economic importance. Afr J Biotechnol 9:9214–9226

Baldi A, Jain A, Gupta N, Srivastava AK, Bisaria VS (2008) Co-culture of arbuscular mycorrhiza-like fungi (Piriformospora indica and Sebacina vermifera) with plant cells of Linumalbum for enhanced production of podophyllotoxins: a first report. Biotechnol Lett 30:1671–1677

Bhagwat DA, Killedar SG, Adnaik RS (2008) Antidiabetic activity of leaf extract of *Tridax procumbens*. Int J Green Pharm 2:126–128

Camehl I, Sherameti I, Venus Y, Bethke G, Varma A, Lee J, Ralf O (2010) Ethylene signalling and ethylene-targeted transcription factors are required to balance beneficial and non beneficial traits in the symbiosis between the endophytic fungus *Piriformospora indica* and *Arabidopsis thaliana*. New Phytol 185:1062–1073

Chandel KPS, Sharma N (1997) Micropropagation of *Coleus forskohlii* (Willd.) Briq. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry. Hi Tech and micropropagation. Springer, Berlin, pp 74–84

Chashmi NA, Sharifi M, Yousefzadi M, Behmanesh M, Palazon J (2011) The production of cytotoxic lignans by hairy root cultures of *Linum Album*. World Acad Sci, Engineer Technol 80:401–402

Chowdhary AR, Sharma ML (1998) GC-MS investigations on the essential oil from *Coleus forskohlii* Briq. Indian Perfumer 42:15–16

Das A, Kamal S, Shakil NA, Sherameti I, Oelmüller R, Dua M, Tuteja N, Johri AK, Varma A (2012) The root endophyte fungus *Piriformospora indica* leads to early flowering, higher biomass and altered secondary metabolites of the medicinal plant. Coleus forskohlii. Plant Signal Behav 7:103–112

Dasgupta SR, Sinha M, Sahana CC, Mukherjee BP (1969) A study of the effect of an extract of Curcuma longa Linn. on experimental gastric ulcers in animals. Ind J Pharmacol 1:49–54

De Souza NJ, Shah V (1988) Forskolin—an adenylate cyclase activating drug from an Indian herb. Econ Med Plant Res 2:1–16

Devi PU (1996) *Withania somnifera* Dunal (Ashwgandha): potential plant source of a promising drug for cancer chemotherapy and radiosensitization. Ind J Exp Biol 34:927–932

Dey AC (1980) Indian medicinal plants used in ayurvedic preparations. In: Bishen Singh, Mahendra Pal Singh (eds), Dehra Dun, pp 202

Dolatabadi HK, Goltapeh EM, Jaimand K, Rohani N, Varma A (2011a) Effects of *Piriformospora indica* and *Sebacina vermifera* on growth and yield of essential oil in fennel (*Foeniculum vulgare*) under green house conditions. J Basic Microbiol 51:33–39

Dolatabadi HK, Goltapeh EM, Moieni A, Jaimand K, Pakdaman B, Sardrood P (2011b) Effect of *Piriformospora indica* and *Sebacina vermifera* on plant growth and essential oil yield in *Thymus vulgaris* in vitro and in vivo experiments. Symbiosis 53:29–35

Eigner D, Scolz D (1999) *Ferula asa-foetida* and *Curcuma longa* in traditional medicinal treatment and diet in Nepal. J Ethnopharmacol 67:1–6

Gosal SK, Karlupia A, Gosal SS, Chhibba IM, Varma A (2010) Biotization with *Piriformospora indica* and *Pseudomonas fluorescens* improves survival rate, nutrient acquisition, field performance and saponin content of micropropogated Chlorophytum sp. Ind J Biotechnol 9:289–297

Gupta PP, Srimal RC, Tandon JS (1991) Antiallergic activity of coleonol, a diterpene from *Coleus forskohlii*, In: Proc 25th Indian Pharmacol Soc Conf. Dec 29–31, Ahmedabad, Gujrat, India, pp 142

Harman GE (2011) Multifunctional fungal plant symbionts: new tools to enhance plant growth and productivity. New Phytol 189:647–649

Kapoor R, Chaudhary V, Bhatnagar AK (2007) Effects of arbuscular mycorrhiza and phosphorus application on artemisinin concentration in *Artemisia annua* L. Mycorrhiza 17:581–587

Kavitha C, Rajamani K, Vadivel E (2010) *Coleus forskohlii*: a comprehensive review on morphology, phytochemistry and pharmacological aspects. J Med Plant Res 4:278–285

Keikichi S, Koji T, Akira F, Makota E (1988) Forskolin containing composing for hair graying suppression. Eur Pat Appl EP 295(903):21

Kumar K, Sarma MVRK, Saharan K, Srivastava S, Kumar L, Sahai V, Bisaria VS, Sharma AK (2011) Effect of formulated root endophytic fungus *Piriformospora indica* and plant growth promoting rhizobacteria fluorescent pseudomonads R62 and R81 on *Vigna mungo*. World J Microbiol Biotechnol. doi:10.1007/s11274-011-0852-x

Kumari R (2005) Plant–Microbe Interaction in in vitro and in vivo condition. Ph D Thesis, Ch. Charan Singh University, Meerut, India

Kurian A, Sankar A (2007) Important medicinal plants in trade. In: Peter KV (ed) Medicinal plants, vol 2. New India Publishing Agency, New Delhi, pp 294–295

Lambert JDH (1998) Medicinal plants: Their importance to National Economics. In: Medicinal plants: a global heritage. In: Proceedings of the International Conference on Medicinal Plants for survival. 16–19 Feb, Bangalore, India, pp 122–127

Malla R, Singh A, Eyaullah MD, Yadav V, Varma A, Rai M (2002) *Piriformospora indica* and plant growth promoting rhizobacteria: an appraisal. In: Rao GP, Manoharachari C, Bhat DJ, Rajak RC, Lakhanpal TN (eds) Frontiers of fungal diversity in India (Prof Kamal Festscrift). International Book Distributing Co, Lucknow, India, pp 401–419

Mathur A, Mathur AK, Verma P, Yadav S, Gupta ML, Darokar MP (2008) Biological hardening and genetic fidelity testing of micro-cloned progeny of *Chlorophytum borivilianum* Sant. Et Fernand. Afr J Biotechnol 7:1046–1053

Misra LN, Tyagi BR, Ahmad A, Bahl JR (1994) Variablity in the chemical composition of the essential oil of *Coleus forskohlii* genotypes. J Essent Oil Res 6:243–247

Oelmuller R, Sherameti I, Tripathi S, Varma A (2009) *Piriformospora indica*, a cultivable root endophyte with multiple biotechnological applications. Symbiosis 49:1–17

Patil S, Hulamani NC, Rokhade AK (2001) Performance of genotype of *Coleus forskohlii* Briq. for growth, yield and essential oil content. Indian Perfumer 45:17–21

Petersen M (1994) Coleus spp In vitro culture and the production of forskolin and Rosmarinic acid. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry, medicinal and aromatic plants VI. Springer, Berlin, pp 69–85

Pham HG, Kumari R, Singh A, Malla R, Prasad R, Sachdev M, Kaldorf M, Buscot F, Oelmüller R, Hampp R, Saxena AK, Rexer KH, Kost G, Varma A (2004) Axenic culture of symbiotic fungus *Piriformospora indica*. In: Varma A, Abbott LK, Werner D, Hampp R (eds) Plant surface microbiology. Springer, Germany, pp 593–613

Prasad R, Garg AP, Varma A (2004) Interaction of medicinal plant with plant growth promoting rhizobacteria and symbiotic fungi. In: Podila G, Varma A (eds) Basic research and applications: mycorrhizae. Microbiology series. IK International, India, pp 363–407

Rai M, Acharya D, Singh A, Varma A (2001) Positive growth responses of the medicinal plants *Spilanthes calva* and *Withania somnifera* to inoculation by *Piriformospora indica* in a field trial. Mycorrhiza 11:123–128

Rai M, Varma A (2005) Arbuscular mycorrhiza-like biotechnological potential of *Piriformospora indica*, which promotes the growth of *Adhatoda vasica* Nees. Electron J Biotechnol 8:107–112

Rai M, Varma A, Pandey AK (2004) Antifungal potential of *Spilanthes calva* after inoculation of *Piriformospora indica*. Mycosis 47:479–481

Rai MK (1994) Herbal medicines in India: retrospects' and prospectus. Fetoterapia LXV 6:483–491

Ruby AJ, Kuttan G, Dinesh Babu K, Rajasekharan KN, Kuttan R (1995) Antitumor and antioxidant activity of natural curcuminoids. Cancer Lett 94:79–83

Sahay NS, Varma A (1999) *Piriformospora indica*: a new biological hardening tool for micropropagated plants. FEMS Microbiol Lett 181:297–302

Sahay NS, Varma A (2000) Biological approach toward increasing the survival rates of micropropagated plants. Curr Sci 78:126–129

Shah V (1996) Cultivation and utilization of medicinal plants (supplement). RRL and CSIR, Jammu—Tawai, pp 385–411

Singh A, Sharma J, Rexer KH, Varma A (2000) Plant productivity determinants beyond minerals, water and light. Piriformospora indica: a revolutionary plant growth promoting fungus. Curr Sci 79:101–106

Singh AN, Singh AR, Kumari M, Rai MK, Varma A (2003) Biotechnological importance of Piriformospora indica. A novel symbiotic mycorrhiza- like fungus: an overview. Ind J Biotechnol 2:65–75

Singh V, Jain DK (1987) Taxonomy of angiosperms. Rastogi, Meerut, India
Varma A, Schuepp H (1994) Positive influence of arbuscular mycorrhizal fungus on in vitro raised hortensia plantlets. Angew Bot 68:108–113
Varma A, Singh A, Sudha Sahay NS, Sharma J, Roy A, Kumari M, Rana D, Thakran S, Deka D, Bharti K, Franken P, Hurek T, Blechert O, Rexer KH, Kost G, Hahn A, Hock B, Maier W, Walter M, Strack D, Kranner I (2001) *Piriformospora indica*: an axenically culturable mycorrhiza-like endosymbiotic fungus. In: Hock B (ed) The Mycota IX, Fungal associations. Springer, Berlin, pp 123–150
Varma A, Verma S, Sudha SN, Britta B, Franken P (1999) *Piriformospora indica*—a cultivable plant growth promoting root endophyte with similarities to arbuscular mycorrhizal fungi. Appl Appl Environ Microbiol USA 65:2741–2744
Vishwakarma RA, Tyagi BR, Ahmed B, Hussain A (1988) Variation in forskolin content in the roots of *Coleus forskohlii*. Planta Med 54:471–472
Willemse RH (1985) Notes on East African *Plectranthus* species (Labiatae). Kew Bull 40:93–96
Wyk BEV, Wink M (2004) Medicinal plants of the world. Times Edition, Singapore
Yadav V, Kumar M, Kumar DD, Kumar H, Sharma R, Tripathi T, Tuteja N, Saxena A, Johri A (2010) A phosphate transporter from the root endophytic fungus *Piriformospora indica* plays a role in the phosphate transport to the host plant. J Biol Chem 285:26532–2654