Interactions of *Piriformospora indica* with Medicinal Plants

R. Prasad(✉), M. Sharma, S. Chatterjee, G. Chauhan, S. Tripathi, A. Das, S. Kamal, A.K.S. Rawat, K.K. Bhutani, M.K. Rai, P. Pushpangdan, and A. Varma

1 Introduction

The microbial world exerts a negative as well a positive impact on living plants and animals, and forms an association either pathogenic or symbiotic with the other partners of the living world. Mycorrhiza refers to an association or symbiosis between plants and fungi that colonize the roots during periods of active plant growth. The intimate symbiotic relationships developed between mycorrhizal fungi and plants, since the colonization of land by the latter, have led to interdependence between these organisms for many basic processes. The fungi require plants to accomplish their life cycle. Plants depend heavily on mycorrhizal fungi for many different functions, such as mineral nutrition and abiotic and biotic stress resistance. Substantial evidence has accumulated in the recent past about how the use of the microsymbiont could significantly contribute in decreasing use of fertilizers and pesticides in agriculture, forestry and florihorticulture, especially if combined with other beneficial soil microorganisms.

The most common and prevalent arbuscular mycorrhizal fungi play an indispensable role in upgrading plant growth, vigor and survival by a positive impact on the nutritional and hydraulic status of the plant and on soil health, by increasing the reproductive potential, improving root performance, and providing a natural defence against invaders, including pests and pathogens. The described species of arbuscular mycorrhizal fungi mainly belong to Zygomycetes placed in the order Glomerales. However, the growing of arbuscular mycorrhizae in pure culture in the absence of living host roots is a matter of global concern. Unfortunately, their biotechnological applications cannot be exploited to the level they deserve due to their axenically unculturable nature.

---

R. Prasad
Amity Institute of Microbial Technology, Amity University, Uttar Pradesh, Noida, India
e-mail: rprasad@amity.edu
2 The Medicinal Plants

In the present scenario, herbal medicines are once again gaining popularity as they are easily available, and have rare or no side effects. There is a resurgence in the demands for medicinal herbs. As a consequence, the herbs are now under pressure, also because of shrinking habitats, and are in a phase of extinction. It has, therefore, become necessary to cultivate medicinal plants on a large scale (Rai 1994). At least 20–30 medicinal herbs have been declared chronically endangered by the Government of India. The main reason is increasing biotic pressure on forests and unscientific exploitation of medicinal plants. Conservation of threatened species and promotion of high yielding varieties can be achieved by various modern techniques of biotechnology, such as tissue culture, micropropagation and protoplast culture. Applying these techniques, endangered medicinal plants have been successfully multiplied and developed.

2.1 History of Medicinal Plants

Our ancestors were well equipped with a vast knowledge regarding drugs of natural origin, but they had little knowledge of how to isolate and obtain pure chemical compound as active ingredients. “Charak Samhita” is the oldest text available having a wide resource of hundreds of herbs in the complete treatment of disorders including cholera, tuberculosis, leprosy, etc. “Indian Materia Medica” deals with the detail identification, collection and therapeutic uses of thousands of medicinal plants (Mazumder and Mazumder 2006).

The earliest evidence of humans making use of plants for healing dates back to the Neanderthal period. In the sixteenth century, botanical gardens were created to grow medicinal plants for medical schools. Herbal medicine practice flourished until the seventeenth century when more “scientific” pharmacological remedies were favored.

There are multiple reasons patients turn to herbal therapies. Often cited is a “sense of control”, a mental comfort from taking action, which helps explain why many people taking herbs have diseases that are chronic or incurable, such as diabetes, cancer, arthritis, or AIDS. In such situations, they often believe that conventional medicine has failed them. When patients use home remedies for acute, often self-limited, conditions, such as a cold, sore throat, or bee sting, it is often because professional care is not immediately available, too inconvenient, costly, or time consuming. In rural areas, there are additional cultural factors that encourage the use of botanicals.

Natural plant products are perceived to be healthier than manufactured medicines. Additionally, reports of adverse effects of conventional medications are found in the lay press at a much higher rate than reports of herbal toxicities, in part because mechanisms to track adverse effects exist for conventional medicines whereas such data for self-treatment are harder to ascertain. Even physicians often
dismiss herbs as harmless placebos, and many consumers and physicians alike mistakenly believe that the US Food and Drug Administration (FDA) have not approved anything in a pill form (Winslow and David 1998).

2.2 Herbal Remedies for Human Sufferings

Different surveys conducted in various parts of India revealed that the majority of the people are suffering from different microbial diseases, ranging in severity from mild-like cough or fever to dreadful-like tuberculosis, leprosy, etc. Researchers have found that tendency of self-medication; drug resistance, ignorance, poor health and hygiene are some of the factors responsible for the widespread occurrence of such diseases. Considerable progress has been made during the past two centuries when chemists and biologists accepted the challenge of combating these dreadful diseases by synthesizing a wide plethora of organic compounds having the capacity to combat various pathogens. However, indiscriminate use of synthetic drugs has resulted in mutation of strains making them insensitive to the chemical agent leading to global hazard of drug resistance. The scientists of the twenty-first century are generally reviving our traditional knowledge and are screening various parts of plants scientifically used in folklore medicine in search of newer lead compounds to create alternative medicines (Mazumder and Mazumder 2006).

2.3 Medicinal Importance of Plants

Since ancient times, plants have been an exemplary source of medicine. Ayurveda and other Indian literature mention the use of plants in treatment of various human ailments. India has about 45,000 plant species and, among them, several thousands have been claimed to possess medicinal properties (Grover et al. 2002). The therapeutic value of some of the medicinally important plants is given in Table 1.

2.3.1 Diabetes

Researches conducted in the last few decades on plants mentioned in ancient literature or used traditionally for diabetes have shown anti-diabetic properties as observed by several experimental or clinical data showing anti-diabetic activity. The most effective and the most commonly studied plants in relation to diabetes and their complications are: Allium cepa, Allium sativum, Aloe vera, Cajanus cajan, Coccinia indica, Caesalpinia bonducella, Ficus bengalenesis, Gymnema sylvestre, Momordica charantia, Ocimum sanctum, Pterocarpus marsupium, Swertia chirayita, Syzigium cumini, Tinospora cordifolia and Trigonella foenum
| S. no. | Therapeutic component | Source | Plant part | Market demand for product | Commercial utilization | Liver diseases treatment | Remark |
|-------|-----------------------|--------|------------|---------------------------|------------------------|-------------------------|--------|
| 1.    | Andrographolid         | Andrographis paniculata | Aerial parts | 50%, 95% | Liver diseases treatment | | |
| 2.    | Artemisin             | Artemisia annua | Aerial parts | 50%, 95% | Liver diseases treatment | | |
| 3.    | Asiaticoside          | Centella asiatica | Aerial parts | 10%, 20%, 99.5% | Liver diseases treatment | | |
| 4.    | Bacopasides           | Bacopa monniera | Aerial parts | 20%, 40% | Liver diseases treatment | | |
| 5.    | Berberine             | Berberis aristata | Roots | 99.8% | Liver diseases treatment | | |
| 6.    | Boswellia aeruginosa  | Boswellia serrata | Oleo-gum-resin | 45%, 65%, 70% | Liver diseases treatment | | |
| 7.    | Colchicine            | Colchicum lanatum | Seeds | 99.5% | Liver diseases treatment | | |
| 8.    | Colchicoside          | Colchicum lanatum | Seeds | 98.5% | Liver diseases treatment | | |
| 9.    | Curcuminooids         | Curcuma longa | Rhizomes | 98.5% | Liver diseases treatment | | |
| 10.   | Curcuminoids          | Curcuma longa | Rhizomes | 98.5% | Liver diseases treatment | | |
| 11.   | Digoxin               | Digitalis lanata | Seeds | 99.5% | Liver diseases treatment | | |
| 12.   | Forskolin             | Coleus forskohlii | Oleo-gum-resin | 2.5% | Liver diseases treatment | | |
| 13.   | Guggulsterones (E+Z)  | Commiphora mukul | Oleo-gum-resin | 2.5% | Liver diseases treatment | | |
| 14.   | Gymnemic Acids        | Gymnema sylvestre | Leaves | 25%, 75% | Liver diseases treatment | | |
| 15.   | Gymnemic Acids (H)    | Garcinia cambogia | Fruit rind | 50%, 70% | Liver diseases treatment | | |
| 16.   | L-Dopa                | Macca pruriens | Seeds | 15%, 30%, 40% | Liver diseases treatment | | |
| 17.   | Lutein Esters         | Tagetes erecta | Flowers | 15%, 30% | Liver diseases treatment | | |
| 18.   | Lycopene              | Lycopersicum | Fruit | 2%, 5%, 10% | Liver diseases treatment | | |
| 19.   | Mangostin             | Mangostoa | Fruit rind | 15%, 30% | Liver diseases treatment | | |
| 20.   | Reserpine             | Rauwolfia serpentina | Root bark | 98.5% | Liver diseases treatment | | |
| 21.   | Sennoside             | Cassia angustifolia | Leaves, pods | 10%, 20%, 40% | Liver diseases treatment | | |
| 22.   | Taxol                 | Taxus brevifolia | Leaves | 99.5% | Liver diseases treatment | | |
Interactions of *Piriformospora indica* with Medicinal Plants

All plants have shown varying degrees of hypoglycemic and antihyperglycemic activity (Grover et al. 2002).

### 2.3.2 Antioxidant Property

Because of increased safety concerns about synthetic antioxidants, exploitation of cheaper and safer sources of antioxidants based on natural origins is the focus of research nowadays (Iqbal et al. 2006).

Plants as antioxidants: there are many plants as antioxidants. Screening of plants is done by measuring the antioxidant activity through various *in vitro* models mice like 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging, scavenging of superoxide anion radical-generated non-enzymatic system, ferric thiocyanate method, reducing power, hydrogen peroxide scavenging and metal-chelating activities. For example, *Ocimum basilicum* L. (Lamiaceae) assayed by different methodologies (Gülçin et al. 2007), and black pepper (*Piper nigrum*) (Gülçin 2005).

### 2.3.3 Organophosphate Exposure

Accidental (environmental or occupational) and self-inflicted (suicide) exposure to organophosphate (OP) pesticides is encountered frequently in the emergency room, especially in the developing world. These perennial public health issues are compounded by a growing concern over the potential use of OP nerve agents such as sarin as a means of terror and nonconventional warfare. OPs disrupt neurotransmission by inhibiting synaptic acetylcholinesterase (AChE-S), leading to an accumulation of acetylcholine in the synapse and neural overstimulation. The severity of the ensuing nicotinic and muscarinic symptoms is dose-dependent and can result in death due to cardiovascular and respiratory collapse. Those surviving often suffer long-term sequelae, including OP-induced delayed neuropathy, muscle weakness, permanent brain dismorphology, and social/behavioral deficits.

For the production of protein pharmaceuticals, plant systems offer low production costs (with comparable purification and regulatory costs), production scalability and flexibility (with low capital investment), and improved safety (no concern of human pathogens and prions).

Nevertheless, mammalian-based production systems seem less promising for large-scale production of AChE-R because of the low levels and relative instability of the protein and its cognate mRNA in such systems. To solve these difficulties, plant-based production is being tried, though still in its experimental infancy, for example: *Nicotiana benthamiana* and endoplasmic reticulum (ER) retention of recombinant human AChE-R. We report here the efficient production and purification of this novel therapeutic protein, a single administration of which provides prophylactic protection from otherwise lethal OP challenges and attenuates the long-term serum AChE-R excess and NMJ damages caused by OP poisoning (Evron et al. 2007).
2.3.4 Anti-carcinogenic Activity

Various components in green and black tea, the beverages made by infusing appropriately processed dried leaves of *Camellia sinensis*, notably simple catechins, have properties *in vitro* that suggest an anti-carcinogenic activity. These include: a direct bactericidal effect against *Streptococcus mutans* and *S. sobrinus*; prevention of bacterial adherence to teeth; inhibition of glucosyl transferase, thus limiting the biosynthesis of sticky glucan; and inhibition of human and bacterial amylases. Studies in animal models show that these *in-vitro* effects can translate into caries prevention. A limited number of clinical trials in man suggest that regular tea drinking may reduce the incidence and severity of caries. If substantiated, this could offer a very economical public health intervention (Hamilton-Miller 2001). Apart from this, *Withania somnifera* Dunal (Solanaceae) is also under study for anticancer activity (Mathur et al. 2006).

2.3.5 Meticillin-resistant *Staphylococcus aureus* (MRSA)

Meticillin-resistant *Staphylococcus aureus* (MRSA) is recognized as a major nosocomial pathogen that has caused problems in hospitals worldwide, with the UK having one of the highest rates of MRSA in Europe. By far the most important reservoir for MRSA, and hence the most important source for spread and subsequent infection, is patients who may be colonized without evidence of infection. The usual sites of MRSA colonization are areas of broken skin, the groin and the axillae, with MRSA infections occurring most frequently in areas of broken skin and in the bloodstream. It is common practice to attempt to clear MRSA colonization and infection in hospital patients with topical antimicrobials and antiseptics; mupirocin and chlorhexidine, for example, are currently employed as part of standard hospital MRSA decolonization protocols. However, resistance to these agents is increasing, with a marked increase in antibiotic resistance recently reported for bacterial strains isolated from superficial skin wounds and leg ulcers. Alternative agents for MRSA decolonization are therefore required.

Tea-tree oil (TTO), the essential oil of *Melaleuca alternifolia*, has been suggested as a potential agent for MRSA decolonization, as it has been shown to be an effective broad-spectrum anti-microbial with good activity *in vitro* against a variety of bacteria including MRSA. Furthermore, it has been shown that bacteria such as *S. aureus* that transiently colonize the skin were more susceptible to TTO than bacteria such as coagulase-negative staphylococci (CoNS), which are regarded as part of the normal commensal skin flora. It has been suggested, therefore, that TTO could be useful for removing transient skin flora while suppressing but still maintaining the resident flora, which acts as a natural defence against colonization by other pathogenic bacteria. Studies comparing the activity of TTO against planktonically grown clinical skin isolates of MRSA, meticillin-sensitive *S. aureus* (MSSA) and CoNS using both a modified broth microdilution method and a quantitative *in vitro* time–kill test method have been carried out (Pinto et al. 2006).
2.3.6 Candiasis

Fungal infections have been increasing in recent years due to a growing number of high-risk patients, particularly immunocompromised hosts. *Candida* is the third- or fourth-most common isolate in nosocomial bloodstream infections in the USA. In addition, candidosis is the most common invasive fungal infection in critically ill nonneutropenic patients. The mortality rate due to invasive aspergillosis increased by 357% between 1980 and 1997 in the USA. Dermatomycoses are common infections caused by members of the genus *Candida* and by filamentous fungi, particularly the dermatophytes. Superficial candidosis and dermatophytosis can be severe in immunocompromised patients.

In spite of the introduction of new antifungal drugs, they are limited in number. The increase of fungal resistance to classical drugs, the treatment costs, and the fact that most available anti-fungal drugs have only fungistatic activity, justify the search for new strategies.

Aromatic plants have been widely used in folk medicine. It is known that most of their properties are due to their volatile oils. Essential oils from many plants are known to possess antifungal activity, but only limited information exists about activity towards human fungal pathogens. They have been empirically used as antimicrobial agents, but the mechanisms of action are still unknown.

According to our preliminary results some essential oils show an important antifungal activity against yeasts, dermatophyte fungi and *Aspergillus* strains, which could predict therapeutic benefits, mainly for diseases with mucosal, cutaneous and respiratory tract involvement.

Several studies have shown that thyme oils, particularly those of *Thymus vulgaris* and *T. zygis* possess antimicrobial activity, those of the phenol type being the most active. The limited occurrence of these phenols in nature is one of the reasons why *Thymus* oils containing thymol and carvacrol have been of great interest for some time.

*Thymus pulegioides* is widely distributed on the European continent south of the Mediterranean islands. In Portugal, it grows in the northeast, and it is locally used as an antiseptic. Previous results have demonstrated that this species is polymorphic, and that the thymol/carvacrol chemotype is one of the most abundant in Portugal (Pinto et al. 2006).

2.3.7 Plants Showing Anti-fungal Properties

The essential oils of *Origanum vulgare* ssp. *hirtum*, *Mentha spicata*, *Lavandula angustifolia*, and *Salvia fruticosa* exhibited antifungal properties against the human pathogens *Malassezia furfur*, *Trichophyton rubrum*, and *Trichosporon beigelii*. Of the four oils, *O. vulgare* ssp. *hirtum* oil showed the highest fungicidal activity and at a dilution of 1/50,000 caused a 95% reduction in the number of metabolically active cells within 6 h of exposure. Among the main components of the four oils, carvacrol and thymol exhibited the highest levels of antifungal activity. The therapeutic
efficacy of the *O. vulgare*ssp. *hirtum* essential oil was tested in rats experimentally infected with *T. rubrum* and yielded promising results. Furthermore, the above essential oils were tested with the Ames test and did not exhibit any mutagenic activity (Adam et al. 1998).

### 2.3.8 Plants Showing Contraceptive Effects

Since early times, plants were used to control fertility, but now this knowledge is restored only to the tribal population. The prosecution of witches in early modern Europe led to the decline of “wise women”, who had for centuries transmitted the lore of contraception. By the seventeenth and eighteenth centuries, that knowledge was everywhere disappearing from Europe, and it remained for researchers in the twentieth century to rediscover it. There is an absence of evidence of the widespread use of effective contraceptives before the modern era. It is assumed that, because of social constraints, knowledge of contraception remained a secret lore, which was transmitted orally or alluded to in written sources in coded form (Riddle 1997).

But this knowledge is under study again and the following are a few of the plants which are being studied for their contraceptive property: *Ancistrophyllum secundiflorum* (Odesanmi et al. 2002), *Tripterygium wilfordii*, a Chinese herbal plant (Kutney et al. 1992), Neem oil from *Azadirachta indica* (Juneja et al. 1994), *Emblia ribes* (Williamson 2002), *Montanoa tomentosa* (Browner and Bernard 1986), *Carica papaya* (Lohiya et al. 1994), *Trigonella foenum graecum* (Fenugreek) (Kassem et al. 2006), *Vicoa indica* (Banjauri) (Dhall and Dogra 1988), and *Gloriosa superba* (Dixit et al. 1983).

### 2.4 Regulation: Dietary Supplement and Health Education Act

In 1993, the FDA began scrutinizing the herbal and supplement industry, which triggered a massive letter-writing campaign organized by health food stores. Under pressure, the FDA created the supplement category, which includes vitamins, minerals, and herbs, and created the Dietary Supplement and Health Education Act (DSHEA), signed October 1994. The DSHEA requires no proof of efficacy, no proof of safety, and sets no standards for quality control for products labeled as supplements. Although the DSHEA requires that supplements do not promise a specific cure on the label, they may claim an effect. Now, if questions arise, the burden lies with the FDA to prove a product unsafe, rather than a company proving its product safe. Manufacturers must put a message on the label stating that the FDA has not reviewed claims, but this statement can be subtle. In contrast, regulating agencies in Germany, France, the United Kingdom, and Canada enforce standards of herb quality and safety assessment on manufacturers.

Because of the lack of requirements for quality control, safety, and efficacy, consumers cannot determine if a herb’s active ingredients are actually in the product, if the ingredient is bioavailable, if the dosage is appropriate, if the next bottle
they buy will have the same components, or what else is in the pill besides the claimed ingredients (Winslow and David 1998).

## 2.5 Present Status of Herbal Medicines

The age-old system of medicine has been neglected mainly because of the rapid expansion of allopathic medical treatment. Presently, the Indian system of medicine uses over 1,100 medicinal plants and most of them are collected regularly from the wild, of which over five dozen species are said to be in great demand (Mazumder and Mazumder 2006).

## 2.6 Future Prospects and Constraints of Herbal Drug Industry

An upsurge in the use of products based on plants is booming. Medicinal plants and their derivatives will continue to play a major role in medical therapy in spite of advances in chemical technology and the appearance of cheap, synthesized, complex molecules from simple ones through highly specific reaction mechanisms. The reactions involved are either difficult or expensive to duplicate by classical chemical methods.

Since production of drugs from medical plants is less expensive than chemical synthesis (Mazumder and Mazumder 2006) and other associated benefits, the use of herbal products is going to increase not just nationally but internationally, requiring a need for the conservation of natural flora. Along with the flourishing herbal industry there is an urgent need to develop ethics for the use of herbs. The world is already facing issues like global warming and deterioration of the natural environment, so if the herbal industry is to be promoted to the desired level there will be a requirement to set up stringent rules and regulations for the conservation of flora and fauna. We are facing problems in the conservation of endangered species and, with the advent of massive herbal production, the current scenario is getting worse. There is a need to develop techniques to enhance the active ingredient from its usual quantity present in the plant, and to enhance the biomass and better growth of medicinally important herbs.

## 3 Piriformospora indica – Model Symbiotic Fungus

Varma and his collaborators, from the School of Life Sciences, Jawaharlal Nehru University, New Delhi, have screened a novel endophytic root-colonizing fungus which mimics the capabilities of a typical AM fungus. However, the unique feature is that this fungus is axenically culturable, and this is a golden lining for AM
fungi for the scientist dealing with the mycorrhizal research. The fungus has been named *Piriformospora indica* based on its characteristic pear-shaped chlamydospores (Fig. 1), and is related to the Hymenomycetes of the Basidiomycota (Verma et al. 1998).

*Piriformospora indica* tremendously improves the growth and overall biomass production of diverse hosts, including legumes (Varma et al. 1999, 2001), medicinal and economically important plants (Rai et al. 2001; Peškan-Berghöfer et al. 2004; Rai and Varma 2005; Shahollari et al. 2005; Prasad et al. 2007). A pronounced growth-promoting effect was seen with terrestrial orchids (Blechert et al. 1999; Bhatnagar and Varma 2006). A study suggested that *P. indica* is able to colonize the rhizoids of liverworts and that the thalli failed to grow under in situ conditions in the absence of this fungus (Varma et al. 2000). The fungus also provides protection when inoculated into the tissue culture-raised plants by overcoming the ‘transient transplant shock’ on transfer to the field, and provides almost 100% survival on transplant (Sahay and Varma 1999, 2000). Based on anatomical and genomic studies, *P. indica* has been attributed to the highly evolved Hymenomycetes (Basidiomycetes) (Fig. 2).

However, neither clamp connections nor sexual structures could be observed. The morphological features and 18S gene sequences certainly placed the fungus in the group. The septal pores consisted of dolipores with continuous parenthosomes. The dolipores were very prominent, with a multilayered cross wall. The parenthosomes were in contact with the ER membranes, which were mostly found near the dolipore (Verma et al. 1998).

The fungus colonizes the roots and improves the health, vigor and survival of a wide range of mono-and dicotyledonous plants. This fungus grows on a large varieties of inorganic, organic and polyphosphates, and thus serves as a good model organism to study phosphorus metabolism (Malla et al. 2004). The molecular mass

![Fig. 1](Image) Electron micrograph of *Piriformospora indica* showing typical coiling and pear shaped spores
of denatured acid phosphatase (ACPase) of *P. indica* was found to be 66 kDa on SDS PAGE. This fungus mediates uptake of phosphorus from the substratum and its translocation to the host by an energy-dependent active process, serves as a strong agent for biological hardening of tissue culture-raised plants, protecting them from “transplantation shock”, and renders almost 100% survival rate on the hosts tested. This fungus also functions as a potential “bio-control agent” against potent root pathogens. Thus, it displays immense potential to be utilized as a biological tool for plant promotion, protection from pests, and for relieving stress conditions such as those due to acidity, desiccation and heavy metal toxicity. Thus, it may be concluded that this novel fungus has immense potential for biotechnological applications.

### 3.1 Phylogenetic Position of *P. indica*

Recently, molecular techniques like polymerase chain reaction (PCR), molecular cloning, and sequencing showed that members of *Sebacinaceae* have been involved in various mycorrhizal associations. Proteomics and genomics data about this fungus has recently been described (Peškan-Berghöfer et al. 2004; Kaldorf et al. 2005; Shahollari et al. 2005). However, sebacinoids were demonstrated recently to be ectomycorrhizal (Selosse et al. 2002). Observations on ectomycorrhizae and basidiomes suggest that species of *Sebacinaceae* are fairly common mycobionts in various ectomycorrhizal plant communities (Urban et al. 2003). The phylogenetic position of the *Sebacinaceae* within the Basidiomycota gives an overview of phylogenetic relationships inside this subgroup of Hymenomycetes for which the new order *Sebacinales* is proposed. The ultrastructural data also indicate that *P. indica* is a member of the Hymenomycetes (Basidiomycota), and studies on the molecular phylogeny will help to reveal the closest relatives of this species (Fig. 3).
Immunological characterization showed its strong cross-reactivity with the members of Zygomycota (*Glomerales*) instead of Basidiomycota (Varma et al. 2001; Singh et al. 2003b), which needs further critical appraisal.

A neighbor-joining analysis on comparisons of partial 18s rDNA sequences (525 nucleotide position) placed *P. indica* close to the *Rhizoctonia solani* group (*Ceratobasidiales*) within the Basidiomycota. A maximum-likelihood analysis on complete 18s rDNA sequences (1,550 nucleotide positions) confirmed this finding. A comprehensive phylogenetic analysis of *Rhizoctonia* using sequences from mitochondrial and nuclear rDNA on more representatives may provide an insight into the evolution of this important group and its evolutionary relationship with *P. indica* within Hymenomycetes. Analysis of 28s rDNA exhibited no change with respect to the taxonomic status of *P. indica* (Varma et al. 2001). Thus, based on the 18s and 28s rDNA analysis and the ultrastructure of the septal pore, it is placed within the Hymenomycetes (Basidiomycota).

### 3.2 Applications and Diverse Functions

The fungus *P. indica* associates with the roots of various plant species in a manner similar to mycorrhiza and promotes their growth (Varma et al. 1999, 2001; Singh et al. 2002, 2003a; Pešken-Berghöfer et al. 2004; Pham et al. 2004a; Oelmüller et al. 2004, 2005; Shahollari et al. 2005; Deshmukh et al. 2006). The fungus possesses unique properties to act as biofertilizer, bioprotector and immunoregulator.
It also plays a key role in protecting roots from insects by increasing the tolerance of the host roots (Varma et al. 1999; Waller et al. 2005; Serfling et al. 2007). It also promotes the antifungal potential of the medicinal plant Spilanthes calva due to an increase in spilanthol content after interaction (Rai et al. 2004).

Among the compounds released in root exudates infected with P. indica, flavonoids are found to be present. Flavonoids have been suggested to be involved in stimulation of precontact hyphal growth and branching (Gianinazzi-Pearson et al. 1989; Siqueira et al. 1991), which is consistent with their role as signaling molecules in other plant–microbe interactions (Giovannetti and Sbrana, 1998). Cell wall degrading enzymes like cellulase, polygalacturonase and xylanase were found in significant quantities both in the culture filtrate and in the root exudates colonized by P. indica.

P. indica showed profound effects on disease control when challenged with a virulent root and seed pathogen, Gaeumannomyces graminis, by completely inhibiting the growth of this pathogen. It indicates that P. indica acted as a potential agent for biological control of root diseases, although the chemical nature of the inhibitory factor is still unknown (Varma et al. 2001).

### 3.3 Eco-Functional Identity

P. indica colonizes the root cortex and forms inter- and intracellular hyphae. Within the cortical cells, the fungus often forms dense hyphal coils or branched structures intracellularly. The fungus also forms spore- or vesicle-like structures within or between the cortical cells. Like AM, hyphae multiply within the host cortical tissues and never traverse through the endodermis. Likewise, they also do not invade the aerial portion of the plant (stem and leaves). However, under certain modified cultural conditions, fungus may also invade the stem and leaves without damaging the plant. The characteristic features of P. indica are axenically culturable: no clamp connections, anastomosis present, hypha–hypha aggregation, no hyphal knots, simple septum with dolipores and continuous, straight parenthesomes, chlamydospores 16–25 μm in length and 10–17 μm in width and 8–25 nuclei per spore.

### 3.4 Host Spectrum

The host spectrum of P. indica is very much like AM fungi: it has been calculated that AM fungi interact with almost 90% of the terrestrial plants (Bagyaraj and Varma, 1995; Giovannetti and Sbrana 1998; Smith and Read, 1997; Varma et al. 1999). However, only limited members of the plant community have failed to interact and these belong to the family of Amaranthaceae, Chenopodiaceae, Cyperaceae, Junaceae, Proteaceae, or lupines and Cruciferae, etc. (Denison et al. 2003). A careful
perusal of the literature indicates that this statement may not be true (Leake 1994; Tester et al. 1987). Denison et al. (2003) have emphasized that model systems are also important as a new research tool to understand the co-operation between microbes and the plants. Cruciferae includes the model plant, Arabidopsis thaliana, that lacks symbiotic interactions such as mycorrhizae and rhizobia. However, most species of plants are normally infected by mycorrhizae, but some plant taxa do not usually form recognizable mycorrhization.

P. indica colonizes the roots of host plants of diverse groups of economically important crops: medicinal (Rai et al. 2001, 2004), horticultural, forest and ornamental plants (Varma et al. 1999, 2001). The similar host range of P. indica and AM fungi suggests that this phenomenon may be correlated with some identical functional aspects as indicated by the serological data (ELISA, Western blotting, immunofluorescens and immunogold labeling) showing close similarities between AMF and P. indica (Singh et al. 2003b; Varma et al. 1999).

One of the striking differences is that, unlike AM, the host range of P. indica also includes terrestrial orchids Dactylorhiza purpurella (T. & T.A. Stephenson), Soo, D. incarnata (L.) Soo, D. majalis (Rchb.) P.F. Hunt & Summerh. and D. fuchsii (Druce) Soo (Blechert et al. 1999; Singh and Varma 2000; Singh et al. 2001; Varma et al. 2001) (Table 2).

However, exceptions are those belonging to members of the Cruciferae and some members of Chenopodiaceae and Amaranthaceae (Read 1999; Varma et al. 2001). Literature reports that the members of these group normally do not accept

| Family            | Genera                                  |
|-------------------|-----------------------------------------|
| Bryophyte (liverwort) | Aneura pinguis L. Dumort. (liverwort)   |
| Acanthaceae       | Adhatoda vasica L. syn. (malabar nut)   |
| Apiaceae (Umbelliferae) | Daucus carota L. Queen Anne’s-lace (carrot) |
| Asteraceae        | Artemisia annua L. (chinese wormwood)   |
| Asteraceae        | Spilanthes calva DC (clove)             |
| Brassicaceae      | Arabidopsis thaliana L. Heynh. (mouse ear cress) |
| Combretaceae      | Terminalia arjuna L. (Arjun tree/stembark) |
| Fabaceae (Mimosoideae) | Acacia catechu L.f. Willd (black catechu) |
| Fabaceae          | Glycine max L. Merr. (soybean)          |
| Fabaceae (oak family) | Quercus robur L. (clone DF 159) (oak)   |
| Liliaceae         | Chlorophyrum borivilianum Baker (musli) |
| Meliaceae         | Azadirachta indica A. Juss (neem)       |
| Orchidaceae       | Dactylorhiza fuchsi Druce (Soo’) (spotted orchid) |
| Poaceae           | Oryza sativa L. (rice)                  |
| Rhamanaceae       | Zizyphus nummularia Burm. fil. (jujube)  |
| Rubiaceae         | Coffea arabica L. (English coffee)      |
| Salicaceae        | Populus tremula L. (aspen)              |
| Scrophularaceae   | Bacopa monniera L. Wett. (brahmi)       |
| Solanaceae        | Nicotiana tabacum L. (tobacco)          |
| Solanaceae        | Withania somnifera L. Dunal (winter cherry) |
| Verbenaceae       | Tectona grandis Linn. f. (teak)         |
AM fungi. In vitro studies, on *P. indica* and *S. vermifera* sensu recorded that these two symbiotic fungi profusely interacted with the root system of the crucifer plants viz., mustard (*Brassica junacea*), spinach (*Spinaceae oleracea*), cabbage (*Brassica oleracea var capitata*) (Kumari et al. 2003) and *Arabidopsis thaliana* (Pham et al. 2004a; Peškan-Berghöfer et al. 2004; Shahollari et al. 2005). It would be useful to assess the non-hosts of AM fungi with respect to their interaction with *P. indica* for its further functional characterization. In order to enlighten the molecular events that promote the root growth, the difference in protein expression was analyzed and modification arises due to the interaction with the fungus. Membrane-associated proteins from roots were separated by two-dimensional gel-electrophoresis (2D-PAGE) and identified by electrospray ionization mass spectrometry (ESI-MS) and tandem mass spectrometry (MS-MS). *P. indica* consists of secondary metabolites like hydroxamic acids (DIBOA, DIMBOA) which act as natural pesticides (Varma et al. 2001).

### 4 Interaction Between Novel Symbiotic Fungus *P. indica* and Medicinal Plants

*P. indica* tremendously improves the growth and overall biomass production of diverse hosts, including legumes (Varma et al. 1999, 2001), medicinal and other economically important plants (Pham et al. 2004b; Rai et al. 2001; Peškan-Berghöfer et al. 2004; Shahollari et al. 2005). *P. indica* colonizes the roots of host plants of a diverse group of plants belonging to monocots, dicots including orchids (Blechert et al. 1999; Pham et al. 2004b, Prasad et al. 2005), herbs, shrubs and woody trees. The effect of *P. indica* interaction with various plants such as *Bacopa monniera*, *Azadirachta indica*, *Tridex procumbans*, *Abrus precatorius*, *Withania somnifera*, *Chlorophytum borivilianum* and *Spilanthes calva* (Rai et al. 2001, 2004) have been tested in laboratory conditions as well as in the extensive field trial.

#### 4.1 *Spilanthes calva*

*Spilanthes calva* DC (Family Asteraceae), commonly known as toothache plant or virus blocker, is well known for enhancing immunity. Because of its high medicinal value, it is costly and there is much demand of this plant in the market. It is cultivated in tribal pockets for herbal treatment in various diseases. This plant has anti-ageing properties and cures various diseases of tooth and gums including pyorrhoea. It is antimicrobial in nature and economically very useful as tooth powder, which is prepared from this plant (Dey 1980) Its leaves stimulate salivation, which is due to the presence of an active chemical spilanathol. Manifold enhancement of the antifungal activity and quantity of spilanthol was recorded on cocultivation with *P. indica* (Fig. 4). The chemical analysis of the roots of the plant revealed a slight increase in spilanathol content.
4.2 Adhatoda vasica

*Adhatoda vasica* Nees (common name, Malabar nut; family, Acanthaceae) is an evergreen shrub. It is well known for preparation of medicine for bronchitis, asthma and other pulmonary infections. *Glycodin®*, a famous product used for the cure of bronchitis, is extracted from the leaves of this plant. It is also known for its antiarthritis, antiseptic, antimicrobial, expectorant, sedative and antituberculosis properties (Dey 1980; Singh and Jain 1987). In Ayurveda, several medicines are manufactured from this plant. Due to increasing demand for *A. vasica* by pharmacies, there is a need for its rapid multiplication. In the observations, cuttings of *A. vasica* were inoculated with *P. indica* to assess the growth-promoting property of *P. indica* on this important medicinal plant. Profuse proliferation of roots of *A. vasica* after inoculation of *P. indica* was repeatedly recorded (Fig. 5). Root-colonization of *A. vasica* by *P. indica* increased with time from 53% after 2 months to 95% after 6 months (Rai and Varma 2005).
4.3 Withania somnifera

*Withania somnifera* is also known as Indian Ginseng and belongs to the family Solanaceae. More than 91 pharmaceutical products are produced from the roots of this plant. Multiple shoot cultures of *Withania somnifera* were established from single shoot tip explants and their potential for the production of two principal withanolides, withaferin A and withanolide D, was investigated (Ray and Jay 2001; Ganzera et al. 2003). The plantlets were then transferred to pots and maintained in a greenhouse for 4 months. 90% of these *in vitro*-propagated plantlets survived and showed normal growth. Leaves from these plants were used for isolation of the withanolides. Methanolic extract of leaves from plantlets growing in tissue culture and those transferred to the greenhouse were evaluated for immunomodulatory activity. While the extract from greenhouse samples showed potent immunosuppressive activity, those from tissue culture samples did not show any activity (Furmanowa et al. 2001). Withaferin A acts as radiosensitizer from *Withania somnifera*. SER (sensitizer enhancement ratio) increased with drug dose, but at higher doses the increased lethality appears to be due to two effects, drug toxicity and radiosensitization. The applicability of this drug as a radiosensitizer in cancer therapy needs to be explored (Devi et al. 1996; Devi 1996).

The basal stem and leaf areas of treated plants were also enhanced. The lengths of the inflorescence and the number of flowers on inoculated *S. calva* plants were also increased relative to controls. Similarly, the number of flowers on the flowers on the inoculated plants of *W. somnifera* was higher than on controls. Seed counts were higher for treated than for control plants.

The overall root biomass of the inoculated plants was higher than that of the corresponding controls. The fresh and dry weights of both underground and above-ground parts of *W. somnifera* inoculated plants were higher than controls (Fig. 6). The net primary productivity of inoculated *S. calva* and *W. somnifera* plants was 0.06 and 0.23 g/plant/day, respectively. These values were higher than those of control plants (0.02 and 0.12 g/plant/day, respectively).

4.4 Safed Musli

Safed musli, scientifically known as *Chlorophytum borivilianum*, belongs to the Liliaceae family and is endowed with Rasayana (antiageing and immunoboosting), Balya (performance-boosting) and Vrishya (aphrodisiac) properties to keep one young and healthy with a well-tuned body for better handling of stress. It lives up to the description as recent clinical trials show that musli if taken on regular basis, it maintains health and youthfulness, keeps the person energetic and active, increases working capacity, increases tolerance to stress and strain, increases working capacity, gives sound sleep, develops a firm and muscular body, and improves conjugal capability. Phytochemicals like saponins, carbohydrate and proteins are
present in the root. This plant has got immense market potential, both domestic and international. Peeled and dried roots are used for therapeutic purposes. Both tubers and seeds are used, but the tubers are a more viable option because they offer better germination rate and tuber growth than seeds. They are normally used to maintain the equilibrium of all the systems of the body and keeps the “Body-Mind-Soul Complex” in a state of harmony. On interaction with *P. indica*, significant growth promotional effect on the plant has been observed besides early flowering in the crop and 90% survival on transplantation (Fig. 7).

### 4.5 Bacopa monniera

*Bacopa monniera* commonly known in India as Brahmi is an important ancient Ayurvedic medicinal plant in the 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, aperitive and cardiotonic (Basu and Walia 1944; Basu et al. 1967; Bhakuni et al. 1969; Elangovan et al. 1995). The main active ingredient of *B. monniera* is believed to be the bacosides.

The fungus *P. indica* is documented to promote plant growth and protects the host against root pathogens and insects. Fungus root colonization by the fungus *P. indica* promoted the plant growth and enhanced antioxidant activity as well as the active ingredient bacoside by several folds (Fig. 8).
Fig. 7  >90% survival rate, establishment and full development of micropropagated plants after treatment with \textit{P. indica}

Fig. 8  Significant growth promotional effect of \textit{P. indica} on \textit{Bacopa monniera} plant in tissue culture medium. \textbf{a} Control; \textbf{b} cocultivated with \textit{P. indica}
Tissue culture technology could play an important role in the clonal propagation, germplasm conservation and improvement of *B. monniera*. Shoot regeneration has been reported from the distal ends of 1- to 12-mm-long internode segments of *B. monniera* cultured on growth regulator free medium, longer internodes being more conducive to regeneration (Thakur et al. 1976; Tiwari et al. 1998).

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. Biological hardening has proved to be fruitful. It provided better results for the overall performance of the plants. 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 preacclimatized plantlets, when transferred to the field, overcame the transient transplant shock, and were able to cope with the changed environment of transplantation which also helped 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 compete in the ecosystem for successful survival (Prasad et al. 2004).

**Conclusion**

Medicinal plants are in great demand in modern civilization to extract various herbal drugs for human welfare. These products come from a labor- and capital-intensive activity, where chemical inputs play an essential role but bring with them a set of problems linked to the degradation of the natural environment and resource base. Thus, the potential use of biological tools such as micropropagation and biological hardening with AM, which ensure adequate level of production with satisfactorily reduction of chemical fertilizer and pesticides, like technologies needed for sustainable agriculture. Among the soil inhabiting microorganisms, AM and other mycorrhizae-like fungi acquire added importance due to their role in establishment, productivity and longevity of natural and man-altered ecosystems. AM fungi share a distinct ecological niche in soil along with a variety of microorganisms including some which are pathogenic, some commensalistic and some which are symbiotic.

A newly-described species *Piriformospora indica* covers most of the characteristics of AM. It improves the growth and overall biomass production of a diverse host including medicinal and other plants of economic importance.

This fungus has another important feature that it has potency to grow axenically as an effective alternative to AM. This fungus mediates uptake of phosphorus from the substratum and it is translocated to the host by an energy-dependent active process. The fungus serves as a strong agent for biological hardening of tissue culture-raised plants, protecting them from “transplantation shock”, and rendering almost 100% survivals on the hosts tested. This fungus is also a potential “biological agent” against potent root pathogens. The growth promotion observed may have
been caused by a greater absorption of water and mineral nutrients due to extensive root colonization and the proliferation of the mycelium into the soil. Thus, it can be concluded that this root-colonizing fungus promotes growth of many plant species of medicinal and economical importance, including cereals, legumes, ornamental plants, oilseed and vegetables, is a potential candidate for the hardening of tissue culture-raised plants and exerts fungicidal and herbicidal resistance. In plant biotechnology, the emphasis is on the manpower rather than on expensive equipment in both developing and developed countries because of its vast impact on agriculture. Today, micropropagation is the most widely and successfully used technology for the mass production of horticultural, ornamentals, fruits, vegetable, cereals, plantation crops, spices and medicinal plants.

References

Adam K, Sivropoulou A, Kokkini S, Lanaras T, Arsenakis M (1998) Antifungal activities of Origanum vulgare sub sp. hirtum, Mentha spicata, Lavandula angustifolia, and Salvia fruticosa essential oils against human pathogenic fungi. J Agric Food Chem 46: 1739 –1745
Bagyaraj DJ, Varma A (1995) Interaction between arbuscular mycorrhizal fungi and plants, and their importance in sustainable agriculture in arid and semi-arid tropics. In: Jones JG (ed) Advances in microbial ecology, vol 14. Plenum, NewYork, pp 119–142
Basu N, Rastogi RP, Dhar ML (1967) Chemical examination of Bacopa monniera Wettst. Part III: bacoside B. Indian J Chem 5: 84–86
Basu NK, Walia JS (1944) The Chemical investigation of the leaves of Herpestis monniera. Indian J Pharm 4: 84–91
Bhakuni Ds, Dhar ML, Dhar MM, Dhawan BN, Mehrotra BN (1969) Screening of Indian plants for biological activity. Part II. Indian J Exp Biol 7: 398–402
Bhatnagar K, Varma A (2006) The healthy marriage between terrestrial orchids and fungi. J Hill Res India 19: 1–12
Blechert O, Kost G, Hassel A, Rexer RH, Varma A (1999) First remarks on the symbiotic interactions between Piriformospora indica and terrestrial orchids. In: Varma A, Hock B (eds) Mycorrhiza, 2nd edn. Springer, Heidelberg, pp 683–688
Browner CH, Bernard ROM (1986) Herbal emmenagogues used by women in Colombia and Mexico. In: Etkin NL (ed) Plants in indigenous medicine & diet: bio-behavioral approaches. Routledge, London, pp 32–34
Denison RD, Bledsoe C, Kahn M, Gara FO, Simms EL, Thomashow LS (2003) Cooperation in the rhizosphere and the “free rider” problem. Ecology 84: 838–845
Deshmukh S, Huckelhoven R, Schafer P, Imani J, Sharma M, Weiss M, Waller F, Kogel KH (2006) The root endophytic fungus Piriformospora indica requires host cell death for proliferation during mutualistic symbiosis with barley. Proc Natl Acad Sci USA 103: 18450–18457
Devi PU (1996) Withania somnifera Dunal (Ashwagandha): potential plant source of a promising drug for cancer chemotherapy and radiosensitization. Indian J Exp Biol 34: 927–32
Devi PU, Akagi K, Ostapenko V, Tanaka Y, Sugahara T (1996) Withaferin A, a new radiosensitizer from the Indian medicinal plant Withania somnifera. Int J Radiat Biol 69: 193–197
Dey AC (1980) Indian medicinal plants used in Ayurvedic preparations. Bishen Singh Mahendra Pal Singh, Dehradun
Dhall K, Dogra M (1988) Clinical trials with Vicoa indica (Banjauri), an herbal medicine, as an antifertility agent. Contraception 37: 75–84
Dixit VP, Joshi S, Kumar K (1983) Possible antispermatic effect of G. superba in male ger-berils. Comp Physiol 8:17–22
Elangovan V, Govindasamy S, Ramamoorthy N, Balasubramanian K (1995) *In vitro* studies on the anticancer activity of *Bacopa monnieri*. Fitoterapia 66: 211–215

Evron, T, Geyer BC, Cherni I, Muralidharan M, Kilbourne J, Fletcher SP, Soreq H, Mor TS (2007) Plant-derived human acetylcholinesterase-R provides protection from lethal organophosphate poisoning and its chronic aftermath. FASEB J 21: 2961–2969

Furmanowa M, Gajdzis-Kuls D, Ruszkowska J, Czarnocki Z, Obidoska G, Sadowska A, Rani R, Upadhyay SN (2001) *In vitro* propagation of *Withania somnifera* and isolation of withanolides with immunosuppressive activity. Planta 67: 146–149

Ganzer M, Choudhary MI, Khan IA (2003) Quantitative HPLC analysis of withanolides in *Withania somnifera*. Fitoterapia 74: 68–76

Gianinazzi-Pearson V, Branzanti B, Gianinazzi S (1989) *In vitro* enhancement of spore germination and early hyphal growth of a vesicular-arbuscular mycorrhizal fungus by host root exudates and plant flavonoids. Symbiosis 7: 243–255

Giovannetti M, Sbrana C (1998) Meeting a non-host: the behaviour of AM fungi. Mycorrhiza 8: 123–130

Grover JK, Yadav S, Vats VJ (2002) Medicinal plants of India with anti-diabetic potential. J Ethnopharmacol 81: 81–100

Gülçin I (2005) The antioxidant and radical scavenging activities of black pepper (*Piper nigrum*) seeds. Int J Food Sci Nutr 56: 491–9

Gülçin I, Elmasstas M, Aboul-Enein HY (2007) Determination of antioxidant and radical scavenging activity of Basil (*Ocimum basilicum* L. Family Lamiaceae) assayed by different methodologies. Phytother Res 21: 354–61

Hamilton-Miller JMT (2001) Anti-cariogenic properties of tea (*Camellia sinensis*). J Med Microbiol 50: 299–302

Iqbal S, Bhanger MI, Akhtar M, Anwar F, Ahmed KR Anwer T (2006) Antioxidant properties of methanic extracts from leaves of *Rhzaya stricta*. J Med Food. 9: 270–5

Juneja SC, Pfeifer T, Williams SR, Chegini N (1994) Neem oil inhibits two-cell embryo development and trophectoderm attachment and proliferation *in vitro*. J Assist Reprod Genet 11: 419–427 http://www.isetlink.com/content/7hhx8n434427229u/

Kaldorf M, Koch B, Rexer K-H, Kost G, Varma A (2005) Patterns of interaction between Populus Esch5 and *Piriformospora indica*: a transition from mutualism to antagonism. Plant Biol 7: 210–218

Kassem A, Aghbari AA, Habori AM, Mamary AM (2006) Evaluation of the potential antifertility effect of fenugreek seeds in male and female rabbits. Contraception 73: 301–306

Kumari R, Yadav H K, Bhoon YK, Varma A (2003) Colonization of Cruciferous plants by *Piriformospora indica*. Curr Sci 85: 1672–1674

Kutney PJ, Hewitt GM, Gin L, Piotrowska K, Roberts M, Rettig SJ (1992) Studies with tissue cultures of the Chinese herbal plant, *Tripterygium wilfordii*. Isolation of metabolites of interest in rheumatoid arthritis, immunosuppression, and male contraceptive activity. Can J of Chem 70: 1455–148

Leake JR (1994) The biology of myco-heterotrophic (saprophytic) plants, New Phytol. 127: 171–216

Lohiya NK, Goyal RB, Jayaprakash D, Ansari AS, Sharma S (1994) Anti-fertility effects of aqueous extracts of *Carica papaya* seeds in male rats. Planta Med 60: 400–404

Malla R, Prasad R, Kumari R, Giang PH, Pokharel U, Oelmüller R, Varma A (2004) Phosphorus solubilizing symbiotic fungus: *Piriformospora indica*. Endocytobiosis Cell Res 15: 579–600

Mathur R, Gupta SK, Singh N, Mathur S, Kuchupillai V, Thirumurthy Velpandian (2006) Evaluation of the effect of *Withania somnifera* root extracts on cell cycle and angiogenesis. J Ethnopharmacol 105: 336–346

Mazurek A, Mazumder R (2006) Traditional knowledge, herbal remedies to anti bacterial therapy: its current status in the 21st century. In: Trivedi PC (ed) Medicinal plants: ethnobotanical approach. Agrobios, Jodhpur, pp 50–51

Odesanmi OS, Dawodu AO, Magbagbeola OA (2002) Comparison of Metabolic effects of ethanolic extracts of *Ancistrophyllum secundiflorum* and menstrogen (orthodox contraceptive) on metabolic parameters in pregnant rabbits. Nigerian J Health Biomed Sci 1: 117–120.
Oelmüller R, Peškan-Berghöfer T, Shahollari B, Trebicka A, Sherameti Irena, Varma A (2005) MATH-domain proteins represent a novel protein family in Arabidopsis thaliana and at least one member is modified in roots in the course of a plant/microbe interaction. Physiol Plant 124:152–166

Peškan-Berghöfer T, Shahollari B, Giang P.H., Hehl S, Markent C, Blank V, Kost G, Varma A, Oelmüller R (2004) Association of Piriformospora indica with Arabidopsis thaliana roots represent a novel system to study beneficial plant-microbe interactions and involve in early plant protein modifications in the endocytosomal reticulum and in the plasma membrane. Physiol Plant 122: 465–471

Peškan-Berghöfer T, Shahollari B, Giang P.H., Hehl S, Markent C, Blank V, Kost G, Varma A, Oelmueller R (2004) Association of Piriformospora indica with Arabidopsis thaliana roots represent a novel system to study beneficial plant-microbe interactions and involve in early plant protein modifications in the endocytosomal reticulum and in the plasma membrane. Physiol Plant 122: 465–471

Prasad R, Garg A P and Varma A (2004) Interaction of medicinal plants with plant growth promoting rhizobacteria and symbiotic fungi. In: Podila G, Varma A (eds) Basic research and applications: mycorrhiza. Microbiology series, vol 1. IK International, India, pp 363–407

Prasad R, Pham GH, Kumari R, Singh A, Yadav V, Sachdev M, Peskan T, Hehl S, Oelmluer R, Varma A (2005) Sebacinaeaceae: culturable mycorrhiza-like endosymbiotic fungi and their interaction with non-transformed and transformed roots. In: Declerck S (ed) Root organ culture of mycorrhizal fungi. Soil biology series. Springer, Heidelberg, pp 291–312

Prasad R, Malla R, Bhatnagar K, Das A, Kharkwal H, Verma N, Garg AP and Varma A (2007) Beneficial microorganisms: herbal and medicinal plants. In: Chauhan AK, Harsha K, Varma A (eds) Microbes for human life, vol 4. IK International, India, pp 49–72

Rai MK (1994) Herbal medicines in India: retrospect’s and prospects. Fitoterapia LXV 6: 483–491

Rai M, Varma A (2005) Arbucuscular mycorrhiza-like biotechnological potential of Piriformospora indica, which promotes the growth of Adhatoda vasica Nees. Electron J Biotechnol 8: 107–11

Rai MK, Singh A, Arya D, Varma A (2001) Positive growth responses of Withania somnifera and Spilanthes calva were cultivated with Piriformospora indica in field. Mycorrhiza 11: 123–128

Rai MK, Varma A, Pandey A K (2004) Antifungal potential of Spilanthes calva after inoculation of Piriformospora indica. Mycoses 47: 479–481

Ray S, Jay S (2001) Production of withaferin A in shoot cultures of Withania somnifera. Planta 67: 432–436

Read DJ (1999) Mycorrhiza: the state of art. In: Varma A, Hock B (eds) Mycorrhiza: structure, function, molecular biology and biotechnology, 2nd edn. Springer, Heidelberg, pp 3–34

Riddle JM (1997) Eve’s herb: a history of contraception and abortion in the west. Harvard University Press, Cambridge, Mass.

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

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

Schüßler A, Schwarzott D, Walker C (2001) A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycol Res 105:1413–1321

Selosse MA, Bauer R, Moyersoen B (2002) Basal hymenomycetes belonging to Sebacinaeaceae are ectomycorrhizal on temperate deciduous trees. New Phytol 155: 183–195
Serfling A, Wirsel SGR, Lind V, Deising HB (2007) Performance of the biocontrol fungus *Piriformospora indica* on wheat under greenhouse and field Conditions. Phytopathology 97: 523–531

Shahollari B, Varma A, Oelmüller R (2005) Expression of a receptor kinase in Arabidopsis roots is stimulated by the basidiomycete *Piriformospora indica* and the protein accumulates in Triton X-100 insoluble plasma membrane microdomains. J Plant Physiol 162: 945–958

Singh A, Varma A (2000) Orchidaceous Mycorrhizal fungi. In: Mukherji KG (ed) Mycorrhizal fungi. Kluwer, Amsterdam, pp 265–288

Singh An, Singh A, Rexer KH, Kost G, Varma A (2001) Root endosymbiont: *Piriformospora indica* – A Boon for Orchids. J Ore Soc India 15: 89–102

Singh An, Singh Ar, Kumari M, Rai MK, Varma A (2003a) Biotechnology importance of *Piriformospora indica*-A novel symbiotic mycorrhiza-like fungus: an overview. Plant Biotechnol (special issue) 2: 65–75

Singh An, Singh Ar, Kumari M, Kumari R, Rai MK, Sharma AP, Varma A (2003b) AMF-like-fungus: *Piriformospora indica*-a boon for plant industry. In: Prasad BN (ed) Biotechnology in sustainable biodiversity and food security. Oxford and IBH, New Delhi, pp 101–124

Singh Ar, Singh An, Varma A (2002) *Piriformospora indica*-in vitro raised leguminous plants: A new dimension in establishment and phyto-promotion. Indian J Biotech. 1: 372–376

Singh V, Jain DK (1987) Taxonomy of angiosperms, Rastogi, Meerut

Smith SE, Read DJ (1997) Mycorrhizal symbiosis, 2nd edn. Academic, London

Siqueira JO, Safir GR, Nair MG (1991) Stimulation of vesicular-arbuscular mycorrhiza formation and growth of white clover by flavonoid compounds. New Phytol. 118: 87–93

Tester M, Smith SE, Smith FA (1987) The phenomenon of “nonmycorrhizal” plants. Can J Bot 65: 419–431

Thakur S, Ganapathy PS, Johri BN (1976) Morphogenesis of organ differentiation in *Bacopa monnieri* stem cultures. Indian J Exp Biol 16: 514–516

Tiwari V, Singh BD, Tiwari KN (1998) Shoot regeneration and somatic embryogenesis from different explants of Brahmi (*Bacopa monniera*). Plant Cell Reports 17: 538–543

Urban A, Weiß M, Bauer R (2003) Ectomycorrhizae involving sebacoid mycobionts. Mycol Res 107: 3–14

Varma A, Rai MK, Sudha N, Sahay N (2000) Microbial-biotechnology: new paradigms and role in sustainable agriculture. In: Rajak RC(ed)Microbial biotechnology for sustainable development and productivity. Science Publishers, India, pp 22–37

Varma A, Singh A, Sudha, Sahay NS, Sharma J, Roy A, Kumari M, Rana D, Thakran S, Deka D, Bharti K, Hurek T, Blechert O, Rexer KH, Kost G, Hahn A, Maier W, Walter M, Strack D, Kranner I (2001) *Piriformospora indica*: an axenically culturable mycorrhiza like endosymbiotic fungus. In: Hock B (ed) The mycota, vol IX. Springer, Heidelberg, pp 125–150

Varma A, Verma S, Sudha, Sahay NS, Butehorn B, Franken P (1999) *Piriformospora indica*, a cultivable plant growth promoting root endophyte. Appl Environ Microbiol 65:2741–2744

Verma S, Varma A, Rexer KH, Hassel A, Kost G, Babshoy A, Bisen P, Buenthoen B, Franken P (1998) *Piriformospora indica*, gen. et sp. nov., a new root colonizing fungus. Mycologia 90:896–903

Wallw W, Achatz B, Baltruschat H, Fodor J, Becker K, Fischer M, Heier T, Hückelraven H, Neumann C, von Wettstein D, Franken P, Kogel KH (2005) The endophytic fungus *Piriformospora indica* reprograms barley to salt stress tolerance, disease resistance and higher yield. Proc Natl Acad Sci USA 102: 13386–13391

Williamson EM (ed) (2002) Major herbs of Ayurveda. Elsevier, London

Winslow CL, David JK (1998) Herbs as medicines. Arch Intern Med 158: 2192–2199