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Research Article

Co-cultivation of Curcuma longa with Piriformospora indica Enhances the Yield and Active Ingredients

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Abstract

The rhizome of *Curcu*ma longa is used in the traditional medicinal system. Its secondary metabolites curcumin and the volatile oil possess wound-healing properties and inhibitory activities against certain pathogenic fungi and bacteria. Piriformospora indica is a root endophytic fungus that colonizes many plant roots and promotes the growth. P. indica was cultivated in the 5 litre capacity fermentor under standard conditions. The filtered biomass was then mixed with raw talcum powder. The propagative buds were treated with this formulation containing both sterile and inoculated fungus. We demonstrated that co- cultivation of C. longa and P. indica resulted in pronounced productivity and enhanced secondary metabolites- curcumin and volatile oil in farmers' field. To the authors best of knowledge this is the first report where symbiotic fungus has added value to this medicinal plant in the agricultural field.

Keywords: *Piriformospora indica*; *Curcuma longa*; volatile oil; curcumin

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Introduction

Turmeric (*Curcuma longa* L.) is a medicinal plant belonging to the family of *Zingiberaceae* and is a medicinal plant. It's modified underground stem (rhizome) is extensively used as alternative medicines (Ayurveda, Unani and Siddha) and has a long history and is known to exhibit a variety of pharmacological effects including anti-inflammatory, anti-tumor, anti-HIV and anti-infectious activities [1-5] (Mazumder et al. 1996; Allen et al. 1998; Chan et al. 1998; Vlietinck et al. 1998; Ammon and Wahl 1991). The secondary metabolite curcumin scavenges active oxygen species including superoxide, hydroxyl radical and nitric oxide [6] (Sreejayan and Rao 1997). Furthermore, the yellow powder and raw rhizome is widely used as flavoring and coloring agent in the Asian diet. Curcumin (diferuloylmethane) comprises of Curcumin I (curcumin), Curcumin II (demethoxycurcumin) and Curcumin III (bisdemethoxycurcumin), which are found to be natural antioxidants [7] (Ruby et al. 1995). Its yellow color is imparted by curcumin (diferuloylmethane), a polyphenolic pigment [8] (Cooper et al. 1994). The formulation is used as antiseptic agent and cosmetics. Significance of the rhizome is highlighted in a recent US patent (**497586**).

Piriformospora indica, a model organism of the order Sebacinales, promotes growth as well as important active ingredients of several medicinal as well as economically important plants by forming root endophytic associations [9-15] (Monreal and Dalpe 2013; Prasad et al. 2013; Qiang et al. 2012a; Rai et al. 2012; Varma et al. 2012 a, b; Weiss et al. 2011). The fungus has been established as bioprotector [16] (Varma et al. 2013), immunoregulator and agent for biological hardening of tissue culture raised plants [17] (Deshmukh et al. 2006). It functions, in nutrient deficient soils, as a bioprotector against biotic and abiotic stresses [18] (Kumar et al. 2012) including root and leaf pathogens and insect invaders, induces early flowering [19-21] (Sherameti et al. 2005; Vadassery et al. 2008, Das et al. 2012) and promotes growth [22] (Pedersen et al. 2013). Entire genomic sequence of the fungus is available [23,24] (Zuccaro et al. 2009; 2011). Decoding of *P. indica* genome has revealed its potential for application as bioagent and for targeted improvement of crop plants in biotechnological approaches [25] (Qiang et al. 2012b).

In this communication, we report that co-cultivation of *C. longa* with *P. indica* resulted in the promotion of rhizome production, enhanced accumulation of secondary metabolites like curcumin and essential metal zinc which supports human body immunity and wound healing.

Materials and methods

Cultivation of *P. indica*

The fungus was mass multiplied in modified Hill and Kaefer Medium [26]. So as to get bulk culture, *P. indica* was cultivated in a 5 liter capacity fermenter (New Brunswick,USA). The fermenter was washed thoroughly and assembled again in place of console. After assembling and properly tightening the clamps and plugging /closing the openings with cotton and rubber bands, fermenter was made ready for sterilization by heating jacket. Various parameters were standardized and set before sterilization, such as pH, temperature and oxygen supply. Sterilization of fermenter and the medium therein was carried out at 121℃ and holding time 15 to 20 minutes.

Inoculum of *P. indica* was developed in the Hill and Kaefer broth [26] to subsequent higher

volume to get enough inoculum for addition in the fermenter. Aseptic transfer of inoculum of *P. indica* was carried out to avoid contamination using flame near the port and using peristaltic pump, it was transferred from the flask containing inoculum. After inoculation was over, inoculation port was again aseptically closed. After inoculation with *P. indica* necessary growth conditions were set to carry mass cultivation of fungus in the controlled environment.

The parameters were set as per the requirement. The pH was set to 6.7. Oxygen in the form of air was forced through air meter at the rate of 0.2 vvm. The temperature was initially set at 30 ℃ and maintained in the narrow range of 28-30 ℃. Air was filtered through fibrous filter and then delivered through sparger in the form of fine air bubbles. Reservoirs containing acid and alkali were connected to the fermenter and using the pumps acid or alkali was delivered to fermenter manually as per requirement or put on automatic mode for that purpose. Impeller agitator was set at initially 100-200 rpm. Samples were removed periodically to check for the growth in the form of O.D. at 600 nm with blue filter on spectrophotometer. Microscopic observations were made to check the contamination if any [27].

Formulation

Raw talcum powder [bulk density (gm/ml) 0.40 - 0.55; pH (8 – 9); moisture (0.58); SiO_2 (60 - 62); MgO (30 - 32); Al_2O_3 (Traces); Fe_2O_3 Traces); CaO (0.5)] obtained from western rock deserts of Rajasthan, India. It was autoclaved thrice at the intervals of 24 hours each. The biomass was thoroughly mixed with talcum powder to maintain live propagules, cfu values 10^9 with average moisture about 12%. Talcum powder with equal amount of sterile fungal biomass served as control.

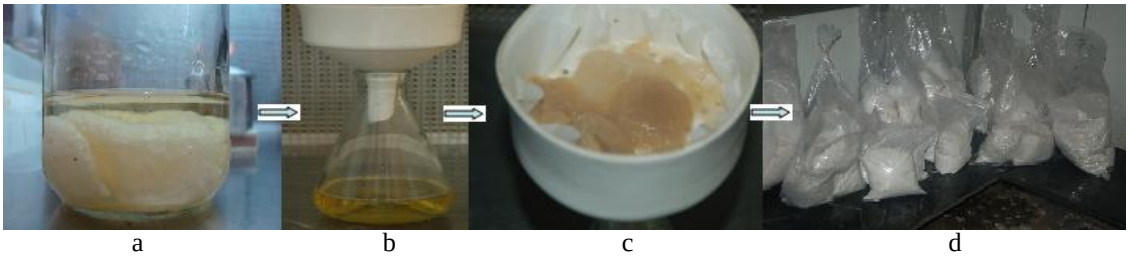
Propagative bud treatment:

50 kg Salem variety *Curcuma longa* propagative buds were thoroughly mixed with 100 g of respective formulation. 100 g Jaggery obtained from juice of *Saccharum officinarum* (Chemical compostion: 60-85% Sucrose, 5-15% Glucose, variable – Fructose, 0.4% of protein, 0.1g of fat, 0.6 to 1.0g of minerals (8mg -calcium, 4mg - phosphorus and 11.4mg - iron) and traces of vitamins and amino acids) was suspended in water and was sprinkled over the treated propagative buds which served as adhesive substance. It was kept for 5 hours in shade. In June 2011, they were transferred to the field in Balachaur, Shahid Bhagat Singh Nagar District, Punjab, North India. The prescribed dose of irrigation and chemical fertilizers were given at regular interval.

Field design and preparation

The experiment was set on 1.6 hectare land. The control and treated plots were further divided into three blocks each of the area (83 x 32 square meters) in a randomized manner shown in Fig.1. There was a space of 1 meter between the two treatments which acted as the buffer zone. Rhizomes were plotted in 80 rows in each plot with a distance of 0.3 m between them. Each row had 10 seedlings.

The soil was sandy loam with pH 7.9. The fertilizer treatment to the field was 50 kg of urea, 10 tons of farmyard manure, 50 kg diammonium phosphate and 25 kg of potassium chloride (Muriate of Potash) per 0.8 hectare land.



a b c d

Figure 1 (A): Formulation of *P. indica* for field trial a) A bottle containing 10-12 days grown culture of *P. indica* b) the fungus is filtered through Büchner funnel, the culture filtrate is removed c) Filtered biomass of *P. indica* d) biomass was mechanically mixed with Talcum powder (as to maintain CFU as 10^9 and moisture content 12 %).

Small volume of jaggery solution was sprinkled on rhizome. The formulated mixture was thoroughly mixed with rhizome. See material and methods

↓

Keep this mixture in shade for 5 hours

↓

Followed the normal practice of seed sowing

Figure 1 (B): Protocol for rhizome treatment

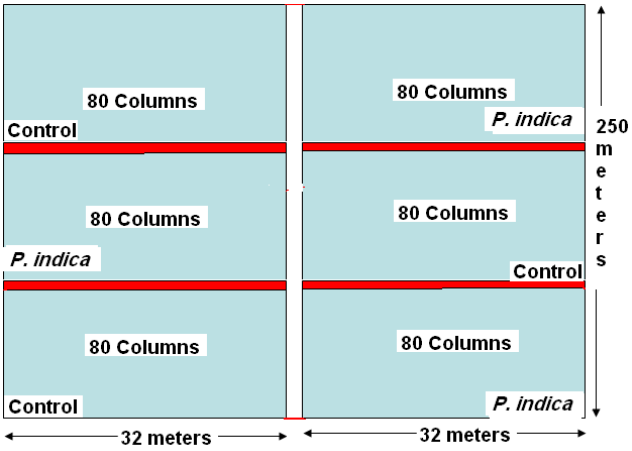


Figure 1 (C): Topography of the field. Note: 1 hectare= 10000 square Meter

Root colonization

Root samples were harvested and stained following the techniques described by Dickson et al. [28] (1998) and Phillip and Hayman [29] (1970). Thoroughly washed roots were cut into small segments (1.0 cm approximately) and heated with 10% KOH solution for 15 minutes. The root segments were neutralized with 1N HCl and washed with water. The segments were stained with 0.05% trypan blue overnight and mounted in lactophenol for observation. The root-pieces were randomly picked and examined on the hemocytometer under a light microscope at the magnification of 10x –40x (OLYMPUS CX41). Root colonization was assessed using the method proposed by Giovannetti and Mosse [30] (1980). 100 treated roots were examined and percentage colonization was calculated with the following formula:

$$\text{Percentage Colonization} = \frac{\text{Number of root segments colonized}}{\text{Number of total segments examined}} \times 100$$

The root colonization was further confirmed by molecular technique reported earlier [19] (Sherameti et al. 2005). To study colonization of plant roots with *P. indica*, RNA from turmeric roots grown in the absence or presence of *P. indica* was isolated with an RNA isolation kit (RNeasy; Qiagen, http://www.qiagen.com) and cDNA were synthesized using reverse transcription (RT-PCR). After reverse transcription, cDNA was amplified with gene-specific (Pitef1) and control primer pairs (actin). RT-PCR was performed by reverse transcription of 2µg of total RNA with gene-specific reverse primers. First-strand synthesis was performed with a kit (#K1631) from MBI Fermentas (http://www.fermentas.com). After PCR, the products were analyzed on 1% agarose gels and stained with ethidium bromide, and visualized bands were quantified with the Image Master Video System (Amersham, GE Life Sciences, http:// www.gelifesciences.com). The transcript levels of the fungal translation elongation factor 1 (cPitef1) in the roots of colonized turmeric seedlings was compared with the levels of the plant actin nucleic acids. Primer pair used for cDNA amplification are Pitef1, ACCGTCTTGGGGTTGTATCC and TCGTCGCTGTCAACAAGATG.

Yield

The rhizomes were harvested after 9 months (in

March 2012) from the date of sowing. The fresh biomass was weighed with an electronic balance.

Biochemical tests

Secondary metabolites like volatile oils, curcumin and heavy metals (Pb, Ca, Zn, As and Hg) were outsourced from FICCI Research & Analysis Centre (New Delhi, India). Standard methods and assays for the quantification of spices and condiments were described by Indian Standard Method and test for spices and condiments [31]. The whole fingers were ground to pass through sieve with 0.5 mm apertures. The ground material (0.5 g) was extracted by refluxing for 2.5 hours with ethanol, according to procedures described in ISO 10925-1984. After cooling, the extract was filtered and made up to 100ml with ethanol rinsing in a volumetric flask. The stock solution was used directly for spectrophotometric analysis of curcuminoid pigments. The absorbance of the extract was measured at 425 nm in 1 cm cells against the alcohol blank.

Statistical analysis

The data were analyzed using the paired comparison t- test [32] (Sokal and Rohlf 1981). The three plots of control were on the left side while the treated were at the right side of the field. There was a gap of 1 meter which acted as buffer between the two treatments. Each treatment had with three lines, which had 10 rhizome plants. The biochemical tests were done with replicas of three in each plot.

Results and discussion

The field trial of *Curcuma longa* showed a positive interaction with *P. indica*. Although there was no change in the pattern of leaflet formation in treated and control plants, the treated plants were greener. The analysis of chlorophyll content also validated the observation (data not given). The number of leaves in treated plants was significantly higher compared to the controls, although the heights of the stem were almost equal for both treatments (Fig 2 upper panel and Table 1).

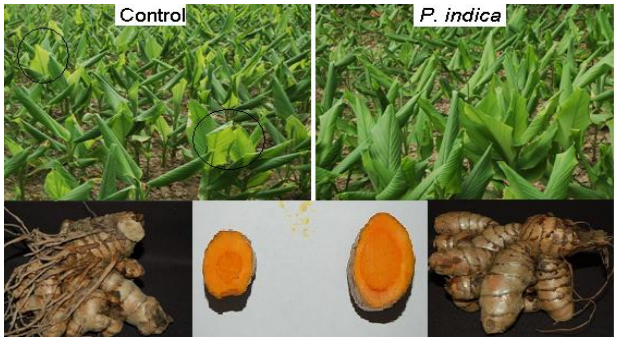


Figure 2 Field trial of *P. indica* with *C. longa*

Table 1 Leaves, roots and rhizome yield as a result of interaction with *Piriformospora indica*

Parameters	Control	*P. indica*-treated	P value	% increase over control
Number of leaves/plant	4 ±0.8	8 ±0.5	P <0.05	100
Number of adventitious roots/plant	26 ±1.2	18 ±2.1	P <0.05	(-)30.76
Rhizome yield per block	8566 ±42	9658 ±36	P <0.05	12.67

Microscopic studies of turmeric roots treated with *P. indica* along with an untreated control using trypan blue showed extensive inter- and intra-cellular root colonization with intra cellular chlamydospores. Hyphal colonization could be seen on the surface of roots as well as in the inter- and intra- cellular spaces of root cortex. Chlamydospores were found as single, double, tetrad, long chains and sometimes clusters. The shape of the spores varied from round and ovoid while only a few of the spores showed typical pear shaped structure. The root colonization was ranged from 60- 70% (Fig 3). Colonized roots showed larger nuclear size than the control, which may be due to excessive gene expression or polyploidy [10] (Prasad et al. 2013).

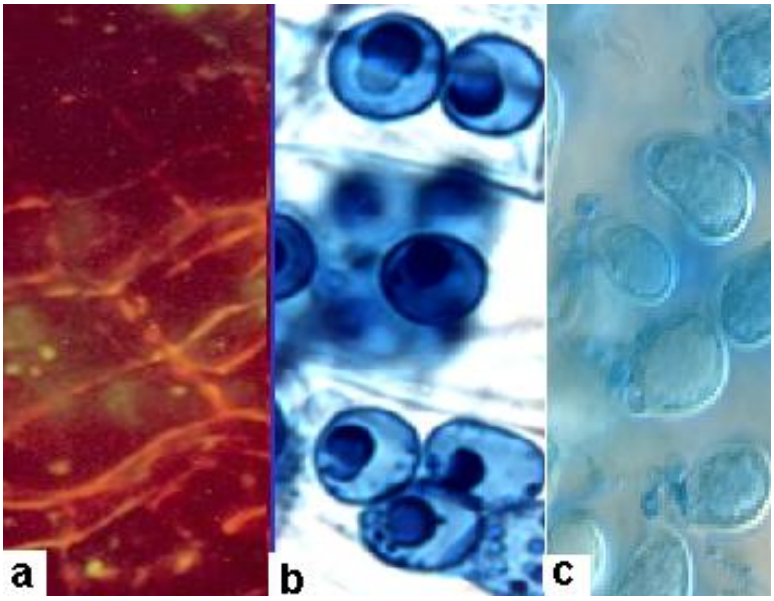


Figure 3 (a) indicates early auto-fluorescent chlamydospores as observed under phase contrast microscope, b) Spores observed under conventional microscope (OLYMPUS CX41), c) extracellular enlarged spores forming thick cell wall.

The morphology and shape of rhizomes are shown in Fig. 1 (lower panel). *P. indica*-treated rhizomes were bigger, shinier and contained only a few adventitious hairy roots. In contrast, untreated rhizomes were visibly smaller, dull and contained a large number of adventitious roots. The treated plants produced at-least 12.7 % higher yield than the control.

The results of the biochemical test are summarized in Table 2. The concentration of volatile oils and curcumin increased by 21% and 19% respectively in the treated rhizomes. *P. indica* is also reported to promote antifungal spilanthol in *Spilanthus calva* [33] (Rai et al. 2004), podophyllotoxins from *Linum album* [34] (Baldi et al. 2010), saponin from *Chlorophytum* sp. [35] (Gosal et al. 2010), asiaticoside from *Centellla asiatica* [36] (Santheesan et. 2012) and essential oils in *Foeniculum vulgare* and *Thymus vulagis* [37,38] (Dolatabadi et al. 2011 a, b)

Table 2 Biochemical test of *C. longa*

Parameters	Control	*P.indica*	P	% increase over control	Test Method
volatile oil, %, w/w	1.28	1.55	P<0.05	21.09	IS:1797
curcumin, %, w/w	3.63	4.32	P<0.05	19.00	IS:10925
lead, mg/kg	3.6	3.47	P>0.05	-3.61	APHA 3030D3030J,1992
cadmium, mg/kg	<0.02	<0.02		-	APHA 3030D3030J,1992
zinc, mg/kg	12.8	18.8	P<0.05	46.87	APHA 3030D3030J,1992
arsenic, mg/kg	<0.002	<0.002		-	APHA 3030D3030G,1992
mercury, mg/kg	<0.02	<0.02		-	APHA 3030D3030G,1992

In the treated rhizome the lead decreased by 3.61%, and zinc concentration enhanced by 46.87%. Other heavy metals such as cadmium, arsenic and mercury showed no change.

P. indica is a well established plant growth-promoting mycorrhiza- like fungus isolated from deserts of Rajasthan [39, 40] (Verma et al. 1998, Rai et al. 2001). The increased growth of *P. indica*-colonized plants could be associated with an enhanced nutrients uptake (especially of phosphorus and nitrogen) from the soil, observed for AM fungi. In *Arabidopsis*, upon cultivation with the fungus, the early protein was modified in endoplasmic reticulum and plasma membrane along with stimulated nitrate reduction in roots [41] (Peskan-Berghofer et al. 2004).

In particular, *P. indica* promotes phosphorus uptake and modulates nitrogen uptake from the soil [42,19] (Oelmüller et al, 2009; Sherameti et al. 2005). Sherameti et al. [19] (2005) also suggested that *P. indica* stimulates NADH dependent nitrate reductase activity in roots of *Arabidopsis* and tobacco. Enhanced enzyme activity is related with increased transcription of corresponding plant gene and helps in nitrate uptake from soil. *P. indica* is involved in the transportation of the phosphate to the host plant which takes place by a phosphate transporter (PiPT) [18,22] (Kumar et al. 2012; Pedersen et al 2013). Like in mycorrhizal associations, the fungus promotes the nutrient uptake from the soil due to a better hyphal penetration into the soil as compared to the penetration of the thicker root hairs. The colonized plants acquire phosphate from the extensive network of fine extra radical hyphae which extend beyond root depletion zones to mine new regions of the soil [43] (Yadav et al. 2010). In retreat, the plant delivers photo- assimilates to the fungus. This is a clear advantage for agriculture applications because the fungus can be applied even in anthropogenic ecosystems with high concentrations of phosphate where it also exerts plant growth promoting effects [44] (Achatz et al. 2010). *P. indica* also influences sulphate reduction leading to higher levels of sulphur-containing proteins and glutathione contents. This in turn influences resistance against biotic and abiotic stress [45] (Nongbri and Oelmüller 2013). Therefore we have to understand the interaction of root with its environment and in addition they could be used as biological agents to improve plant production system [46] (Franken 2012). Taken together, better access to soil nutrients could be the major reason for the growth-promoting effect of *P. indica* on turmeric. This is

accompanied by an increased synthesis and accumulation of secondary metabolites.

An increase in uptake of soil components is inevitably associated with the uptake of soluble ions. While some of them are essential, others such as heavy metals could be toxic. The fungus regulates the uptake and transportation of important macro-nutrients like Fe, Zn, Mg, or Cu. Several studies have demonstrated that the interaction has also a strong effect on the plant's nitrogen metabolism, because the symbiotic interaction is accompanied by an increase uptake of nitrate [42, 47] (Oelmüller et al. 2009; Unnikumar et al. 2013). We determined heavy metal concentrations in the plants and found that their uptake is quite differently affected by *P. indica*. While uptake of zinc appears to be strongly promoted under our field conditions, that of other heavy metals is either not affected by *P. indica* or the fungus has only a minor effect on the uptake of these ions. This clearly demonstrates specificity for individual ions. It also excludes that colonization by *P. indica* results in an unspecific and uncontrolled increase in heavy metal uptake.

To summarize, *P. indica* promotes growth and development of turmeric, similar to reports for other plant species. This has agricultural implications, because the increase growth rate and better performance of the plant is associated with higher levels of curcumin, an important spice in Asia.

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References

1. Mazumder A, Wang S, Neamati N, Nicklaus M, Sunder S, Chen J, Milne GW, Rice WG, Burke JR TR, Pommier Y. Antiretroviral agents as inhibitors of both human immunodeficiency virus type 1 integrase and protease. ***J Med Chem***. 1996, 39:2472-2481
2. Allen PC, Danforth HD Augustine PC. Dietary modulation of avian coccidiosis. ***Int J Parasitol***. 1998, 28:1131-1140
3. Chan MMY, Huang HI, Fenton MR, Fong D. *In vivo* inhibition of nitric oxide synthase gene expression by curcumin, a cancer preventive natural product with anti-inflammatory properties. ***Biochem. Pharmacol.*** 1998, 55:1955-1962
4. Vlietinck AJ, Debruyne T, Apers S, Pieters LA. Plant-derived leading compounds for chemotherapy of human immunodeficiency virus (HIV) infection. ***Planta Med***. 1998, 64:97-109
5. Ammon HPT, Wahl MA. Pharmacology of *Curcuma longa*. ***Planta Med***. 1991, 57:1-6
6. Sreejayan A, Rao MNA. Nitric oxide scavenging by curcuminoids. ***J Pharm Pharmacol***. 1997, 49:105-107
7. Ruby, AJ, Kuttan G, Dinesh Babu K, Rajasekharan KN, Kuttan R. Antitumor and antioxidant activity of natural curcuminoids. ***Cancer Letters***. 1995, 94:79-83
8. Cooper TH, Clark G, Guzinski J. Teas, spices and herbs. In: *Food Phytochemicals*, ed. Ho, C.T. Vol. I, pp. 231-236. Washington, DC: ***American Chemical Society***. 1994

9. Monreal M, Dalpe Y Arbuscular mycorrhizal fungi strain potential on flax, In: 7th International Conference on Mycorrhiza "Mycorrhiza for All: An Under- Earth Revolution" 6-11 Jaunuary 2013, New Delhi, India, 2013
10. Prasad R, Kamal S, Sharma PK, Oelmüller R, Varma A. Root endophyte *Piriformospora indica* DSM 11827 alters plants morphology, enhances biomass and antioxidant activity of medicinal plant *Bacopa monniera*. ***Journal of Basic Microbiology***. 2013, 53:1016–1024
11. Qiang X, Weiss M, Kogel KH, Schafer P. *Piriformospora indica* a mutualistic basidiomycete with an exceptionally large plant host range. ***Mol Plant Pathol***. 2012a, 13:508-518
12. Rai M, Gade A, Rathod D, Dar M, Varma A. Review: Mycoendophytes in medicinal plants: diversity and bioactivities. ***Nusantara Bioscience***. 2012, 4:86-96
13. Varma A, Bakshi M, Lou B, Hartmann A, Oelmüller R. *Piriformospora indica*: A novel plant growth-promoting mycorrhizal fungus, ***Agric Res***. 2012a , 1:117-131
14. Varma A, Kharkwal AC, Bains KS, Agarwal A, Bajaj R, Prasad R. *Piriformospora indica*: The model microbes for organic green revolution, ***Organic Farming Newsletter***. 2012b, 20:3-8
15. Weiss M, Sy 'korova ´Z, Garnica S, Riess K, Martos F, Krause C, Oberwinkler F, Bauer R, Redecker D. Sebacinales everywhere: previously overlooked ubiquitous fungal endophytes. ***PLOS ONE***. 2011, 6:e16793
16. Varma A, Tripathi S, Prasad R, Das A, Sharma M, Bakshi M, Arora M, Rastogi K, Agrawal A, Kharkwal AC, Tsimilli-Michael M, Strasser RJ, Bagde US, Bisaria VS, Upadhyaya CP, Malla R, Kost G, Joy K, Sherameti I, Chen Y, Ma J, Lou B, Oelmüller R. The symbiotic fungus *Piriformospora indica*: update. In: Hock B (ed) The Mycota IX, ***Springer Verlag***, Berlin, 2013, pp: 231-254
17. Deshmukh S, Hückelhoven R, Schäfer P, Imani J, Sharma M, Weiss M, Waller F, Kogel KH. The root endophytic fungus *Piriformospora indica* requires host cell death for proliferation during mutualistic symbiosis with barley. ***Proc Natl Acad Sci USA***. 2006, 103:18450-18457
18. Kumar M, Sharma R, Jogawat A, Singh P, Dua M, Gill SS, Trivedi DK, Tuteja N, Varma AK, Oelmüller R, Johri AK *Piriformospora indica*, a root endophytic fungus, enhances abiotic stress tolerance of the host plant. In: Tuteja N, Gill SS, Tiburcio AF, Tuteja R (eds) Improving crop resistance to abiotic stress. ***Wiley-Blackwell, Weinheim***, 2012:543-548
19. Sherameti I, Shahollari B, Venus Y, Altschmied L, Varma A, Oelmüller, R. The endophytic fungus *Piriformospora indica* stimulates the expression of nitrate reductase and the starch degrading enzyme glucan-water dikinase in tobacco and Arabidopsis roots through a homeodomain transcription factor that binds to a conserved motif in their promoters. ***J Biol Chem***. 2005, 280:26241-26247
20. Vadassery J, Ritter C, Venus Y, Camehl I, Varma A, Shahollari B, Novk O, Strnad M, Ludwig-Müller J, Oelmüller R. *Piriformospora indica* mediated growth promotion in Arabidopsis is sensitive to high auxin levels, requires trans-cytokinin biosynthesis and the cytokinin receptor combination CRE1/AHK2. ***Mol Plant Microbe Interact***. 2008, 21:1271-1282
21. Das A, Kamal S, Shakil NA, Sherameti I, Oelmüller R, Dua M, Tuteja N, Johri AK, Varma A. The root endophyte fungus

Piriformospora indica leads to early flowering, higher biomass and altered secondary metabolites of the medicinal plant, *Coleus forskohlii.*, ***Plant Signaling & Behavior*****.** 2012, 7:1-10

22. Pedersen BP, Kumar H, Waight AB, Risenmay AJ, Roe-Zurz Z, Chau BH, Schlessinger A, Bonomi M, Harries W, Sali A, Johri AK, Stroud RM. Crystal structure of a eukaryotic phosphate transporter. ***Nature*****.** 2013, 496:1-6
23. Zuccaro A, Basiewicz M, Zurawska M, Biedenkopf D, Kogel KH. Karyotype analysis, genome organization, and stable genetic transformation of the root colonizing fungus *Piriformospora indica*. ***Fungal Genet Biol***. 2009, 46:542-550
24. Zuccaro A, Lahrmann U, Ldener UG, Langen G, Pfiffi S, Biedenkopf D, Wong P, Samans B, Grimm C, Basiewicz M, Murat C, Martin F, Kogel KH. Endophytic life strategies decoded by genome and transcriptome analyses of the mutualistic root symbiont *Piriformospora indica*. ***PLoS Pathog***. 2011, 7:e1002290
25. Qiang XY, Zechmann B, Reitz MU, Kogel KH, Schafer P. The mutualistic fungus *Piriformospora indica* colonizes *Arabidopsis* roots by inducing an endoplasmic reticulum stress-triggered caspase-dependent cell death. ***Plant Cell***. 2012b , 24:794-809
26. Hill TW, Kaefer E. Improved protocols for Aspergillus medium: trace elements and minimum medium salt stock solution. ***Fungal Genet Newsl*****.** 2001, 48:20-21
27. Bagde US, Prasad R, Varma A (2010) Interaction of *Piriformospora indica* with medicinal plants and of economic importance. Afr J Biotechnol 9(54):9214-9226
28. Dickson S, Mandeep SM, Smith SM. Evaluation of vesicular arbuscular mycorrhizal colonization by staining. In: Varma A (ed) Mycorrhiza manual. ***Springer, Berlin***.1998, pp 77-84
29. Phillip JM, Hayman DS. Improved procedures for clearing roots and staining parasitic and VAM fungi for rapid assessment of infection. ***Trans Br Mycol Soc.*** 1970, 55:158-161
30. Giovannetti M, Mosse B. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. ***New Phytologist*****.** 1980, 84:489-500
31. International Standard Organization. Specification for Turmeric Oleoresin**, ISO 10925** , 1984
32. Sokal RR, Rohlf FJ. **Biometry**, 2nd ed, W. H. Freeman, San Francisco, 1981
33. Rai MK, Varma A, Pandey AK. Antifungal potential of *Spilanthes calva* after inoculation of *Piriformospora indica*. ***Mycoses*****.** 2004, 47:479
34. Baldi A, Farkya S, Jain A, Gupta N, Mehra R, Datta V, Srivastava AK, Bisaria VS. Enhanced production of podophyllotoxins by co-culture of transformed *Linum album* cells with plant growth-promoting fungi. ***Pur Appl Chem*****.** 2010, 82:227-241
35. Gosal SK, Karlupia A, Gosal SS, Chhibba IM, Varma A. Biotization with *Piriformospora indica* and *Pseudomonas fluorescens* improves survival rate, nutrient acquisition, field performance and saponin content of micropropagated *Chlorophytum sp*. ***Indian J Biotechnol***. 2010, 9:289-297
36. Satheesan J, Narayanan AK, Sakunthala M. Induction of root colonization by *Piriformospora indica* leads to enhanced asiaticoside production in *Centella asiatica*. ***Mycorrhiza*****.** 2012, 22:195-202
37. Dolatabadi HK, Goltapeh EM, Jaimand K, Rohani N, Varma A. Effects of *Piriformospora indica* and *Sebacina*

vermifera on growth and yield of essential oil in fennel (*Foeniculum vulgare*) under greenhouse conditions. ***J Basic Microbiol***. 2011a, 51:33-39

38. Dolatabadi HK, Goltapeh EM, Moieni A, Jaimand K, Sardrood BP, Varma A. Effect of *Piriformospora indica* and *Sebacina vermifera* on plant growth and essential oil yield in *Thymus vulgaris* in vitro and in vivo experiments. ***Symbiosis***. 2011b , 53:29-35
39. Verma SA, Varma A, Rexer KH, Hassel A, Kost G, Sarbhoy A, Bisen P, Bütehorn B, Franken P. *Piriformospora indica*, gen. et sp. nov., a new root-colonizing fungus. ***Mycologia***. 1998, 90:898-905
40. Rai M, Acharya D, Singh A, Varma A. Positive growth responses of the medicinal plants *Spilanthes calva* and *Withania somnifera* to inoculation by *Piriformospora indica* in a field trial, ***Mycorrhiza***. 2001, 11:123-128
41. Peskan-Berghofer T, Shahollari B, Giong PH, Hehl S, Markert C, Blanke V, Kost G, Varma A, Oelmuller R. Association of *Piriformospora indica* with *Arabidopsis thaliana* roots represents a novel system to study beneficial plant–microbe interactions and involves early plant protein modifications in the endoplasmic reticulum and at the plasma membrane. ***Physiol Plant***. 2004, 122:465-477
42. Oelmüller, R. Sherameti I, Tripathi S, Varma A *Piriformospora indica*, a cultivable root endophyte with multiple biotechnological applications. ***Symbiosis.*** 2009, 19:1-19
43. Yadav V, Kumar M, Deep DK, Kumar H, Sharma R, Tripathi T, Tuteja N, Saxena AK, Johri AK. A phosphate transporter from the root endophytic fungus *Piriformospora indica* plays a role in phosphate transport to the host plant. ***J Biol Chem***. 2010, 285:26532-26544
44. Achatz B, Ruden S, Andrade D, Neumann E, Pons-Kuhnemann J, Kogel KH, Franken P, Waller F. Root colonization by *Piriformospora indica* enhances grain yield in barley under diverse nutrient regimes by accelerating plant development. ***Plant Soil***. 2010, 333:59-70
45. Nongbri PL, Oelmüller R. Role of *Piriformospora indica* in sulfur metabolism in *Arabidopsis thaliana,* In: Sebacinales, eds: Varma, Kost and Oelmüller, ***Springer Verlag;*** 2013
46. Franken P. The plant strengthening root endophyte *Piriformospora indica*: potential application and the biology behind. ***Appl Microbiol Biotechnol***. 2012, 96:1455-1464
47. Unnikumar KR, Sree KS, Varma A. *Piriformospora indica*: A versatile root endophytic symbiont. ***Symbiosis***. 2013, 60: 107-113