Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 6, Issue, 11, pp.9531-9535, November, 2014

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

USE OF LOCALLY AVAILABLE JAGGERY FOR DEVELOPMENT OF A COST EFFECTIVE MEDIA FOR MASS CULTIVATION OF *PIRIFORMOSPORA INDICA*- A STEP FORWARD TOWARDS COMMERCIALIZATION

Monika Arora, Divya Kilam, Amit C Kharkwal and *Ajit Varma

Amity Institute of Microbial Technology, Amity University, Noida, Uttar Pradesh 201303, India

ARTICLE INFO

Article History:
Received 08th August, 2014
Received in revised form
21st September, 2014
Accepted 14th October, 2014
Published online 18th November, 2014

Key words:
Biomass yield,
Economical plants,
Hill Kaefer,
Jaggery,
Piriformospora indica,
Spore Yield

ABSTRACT

Piriformospora indica is an axenically cultivable fungus that colonizes with the roots of mono and dicotyledonous plants. Colonized plants in response show enhancement in growth, yield and value addition. The fungus is normally grown on large variety of complex and synthetic media. As the readily available synthetic media are expensive, there is a need to find an alternative medium for mass production of *P. indica.* The need arises as a cheaper and cost effective medium will bring down the production cost and make it commercially competitive. In the present study, we examined the impact of cheaply available local material "Jaggery" on the growth of fungus and also checked its viability towards economically important plants. The data presented here suggests that maximum fungal biomass, radial growth and spore yield were recorded on Jaggery medium as compared with commonly used Hill-Kaefer medium that was used as control. Plants inoculated with biomass of fungus grown on Jaggery medium showed increase in shoot and root length, fresh and dry weight, photosynthetic pigments and phosphorus content. Our findings thus suggest that cheap locally available Jaggery can be used as an alternative media in place of Hill-Kaefer for the commercial production of *P. indica.*

INTRODUCTION

Plant growth promoting fungus, *Piriformospora indica* shows mutualistic interaction with many angiosperms encompassing both dicot and monocot plants (Pham *et al*., 2004; Varma *et al*., 2012). It belongs to phylum Hymenomycetes, class Basidiomycota and order Sebacinales. The fungus has been isolated from the sandy rhizospheric soil of woody shrubs *Prosopis juliflora* and *Zizypus nummularia* in Thar Desert in north western Rajasthan, India (Verma *et al*., 1998). This fungus produces pear shaped thick walled chlamydospores having long shelf life of almost 12 months at room temperature. The length and width of pear shaped chlamydospores are 16-25 µm and 10-17 µm respectively and are observed as being single or in clusters (Varma *et al*., 2001). The fungus protects the plants against acidity, desiccation and heavy metals toxicity (Kumari *et al*., 2004). It thus acts as a bio-fertilizer, bio-protector, plant regulator, fungicide and pesticide. The fungus is being commercialized under the trade name "Rootonic". Most important advantage of *P. indica* over AM fungi is that it grows axenically on wide range of synthetic solidified and broth media e.g., Aspergillus, Modified Aspergillus Medium, Potato Dextrose Agar, Woody Plant Medium, Yeast Extract Mannitol Peptone Agar, Czapek's Dox Agar, Malt Extract, M4N, Modified Melin-Norkrans, MMN 1/10, MMNC, MS, MYP (Singh *et al*., 2013). Mycelium of *P. indica* forms rhythmic rings on solid agar medium. It is propagated by chlamydospores or by mycelium. Among the tested media, most optimum was aspergillus (Hill and Kaefer, 2001). The cost of this media is high due to which mass production becomes expensive. In earlier study locally available cheap media was used as an alternative culture media for mass production of entomopathogenic fungi (Latifian *et al*., 2013). In this communication, we report the mass cultivation of the fungus on an indigenous carbon/ nutritional energy source locally known as "Jaggery" quantitatively it was comparable to that of Hill- Kaefer medium. The efficacy of fungal biomass grown on Jaggery was checked towards the plant growth and productivity. The finding has been filed for patent (Arora *et al.,* 2012).

Corresponding author: Ajit Varma
Amity Institute of Microbial Technology, Amity University, Noida, Uttar Pradesh 201303, India

MATERIALS AND METHODS

Experiment I: Growth of *P. indica* on different concentration of Jaggery and Hill-Kaefer under *in-vitro* conditions

Microbial culture and their maintenance

The pure culture of *P. indica* strain (DSM11827) was retrieved from the culture collection of Amity Institute of Microbial

Technology, Amity University, Uttar Pradesh (AUUP) and culture was maintained on Hill-Kaefer medium (Prasad *et al*., 2005). One agar disc (having 8 mm diameter) punched with a sterilized cork-borer was placed in the center of the Petri plate containing solidified Hill-Kaefer medium. After inoculation plates were incubated at 30°C for 7 days and then stored at 4°C.

Medium for the growth of *P. indica*

Jaggery was obtained from local shop and ground into fine powder using electric grinder. Powdered jaggery was suspended in double distilled water at concentration of 2, 4, and 6 percent (w/v), respectively. Hill-Kaefer medium served as a control. Before autoclaving, pH of media was set to 6.5. With the help of sterilized cork borer, 8 mm agar disc having chlamydospores and live propagules were punched and transferred in 500 ml conical flasks containing 100 ml media. Control was maintained by transferring fungal discs on Hill-Kaefer media. Incubation was carried out at 28^{o}C under constant shaking at 100 rpm on a Rotary Shaker. For solid medium one disc was placed in the centre of the Petri plate and incubated at 28^{o}C.

Dry cell weight and radial growth

A known volume of culture broth was filtered through Whatmann filter paper No. 1 and dried to a constant weight in hot air oven at 60 ^{o}C for 24 h. After complete drying, weight was determined. Three flasks were removed each day for analysis of the dry cell weight (DCW). Radial growth of mycelia from disc on the agar plate was measured every alternate day.

Measurement of spore yield

P. indica culture grown on Hill-Kaefer and different concentration of Jaggery were used to count the pear shaped spores that were attached to mycelium. For collection of spores, sterilized water containing 0.05% Tween- 20 was dispensed on the agar plates and gently swirled. With the help of spatula the surface of plates was scratched to detach the spores. This suspension was collected in centrifugation tube and centrifuged at 3500 rpm for 10 min followed by washing with sterilized Tween-20 water. Spore density was determined by hemocytometer in combination with a Microscope (Olympus CX41RF, model no. SN = 6K09628).

Experiment II: Co-cultivation of *P. indica* grown on jaggery with plants under *in-vivo* conditions

Plant material and growth conditions

Viability of fungal spores grown on jaggery was checked towards monocot and dicot plants. Seeds of *Triticum aestivum, Pennisetum glaucum*, *Cicer arietinum* and *Lathyrus sativus* were collected from seed testing division, Indian Agricultural Research Institute, New Delhi. Seeds were surface sterilized with sodium hypochlorite solution (5%) for 2-3 min followed by washing with sterile distilled water (Gamborg and Phillips 1996). After surface sterilization seeds were placed onto water agar plates (0.8%) for germination in a growth chamber with a photoperiod of 16 h light/8 h dark for 2-3 days. The green house conditions were maintained at temperature range from 27 ± 2 ^{o}C with 16 h light/8 h dark regime and the relative humidity was 70%. Soil used in the experiment was a sandy loam with pH 7.5.

Plant inoculation with *P. indica*

For preparation of fungal formulation, active propagules containing chlamydospores and hyphae grown on Jaggery medium (From experiment 1; 4% Jaggery showed maximum growth so this medium named as Jaggery medium) were harvested after 8 days from the liquid culture through filtration. Mycorrhizal inoculation was carried out by mixing 2gm of fungal mycelium with 100 gm of soil (2% fungus w/w) and thin layer of mycorrhizal inoculum was placed 2 cm below the surface of soil. Three pre germinated seedlings were transplanted to pots, with 12 cm diameter, containing sterilized peat and soil in the ratio 1:1. Pots were placed in environmentally controlled green house. Plants were fertilized with 1/10 Hoagland solution on every alternative week and allowed to grow for 60 days.

Assessment of plant growth parameters

Plants were harvested after 60 days and growth parameters such as shoot and root length, fresh and dry weights were recorded. The length of shoot was recorded (cm/plantlet) from roots at 0.5 cm above the soil surface and shoots were separated for fresh and dry weight. Roots were separated and length was measured (cm/plantlet) from the soil by washing with running tap water and fresh weight of roots was measured (gm/plantlet) immediately. Dry weight of shoot and root was measured by drying in an oven at 40 ^{o}C for 4 days and expressed in gm/plantlet.

Estimation of chlorophyll concentration in leaves

Chlorophyll a and b were measured by the method of Hiscox and Israelstam (1979) from the leaves of monocot and dicot plants and concentration was estimated with the help of Arnon's formulae (Arnon, 1949). The absorbance was measured at 645 and 663 nm in a UV-Vis spectrophotometer (U-2910, HITACHI). Chlorophyll content was expressed as mg/gm fresh weight.

Estimation of phosphorus content in leaves

Phosphorus content in leaves was measured following the method described earlier (Gericke and Kurmies, 1952). Dry leaves were taken and dissolved in 5 ml of 21% HNO_3 and ashed for 4 h at 550^{o} C. 3M HCl was added to the ash followed by vanadomolybdate reagent. The mixture was stirred and left for 20 min for colour development. Phosphorus concentration was analysed calorimetrically with a spectrophotometer at wavelength of 436nm.

Microscopy and lacto phenol staining

Fine roots from treated and untreated samples were collected, rinsed well in distilled water, and cut into 1 cm pieces. Root

segments were boiled with 10% KOH to soften the tissue and then neutralized with 2% HCl for 3-4 min. Roots were then stained with 0.5% lactophenol blue solution and examined under bright field microscope at 40 X magnification.

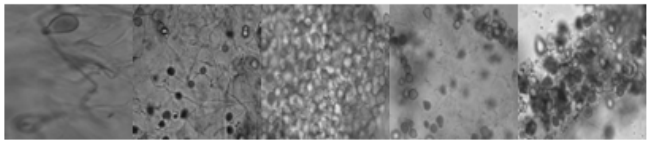
Statistical analysis

The results were confirmed in 3 independent experiments. Data expressed as mean ± standard deviation of five replicates of each group. Statistical analysis was performed using student's t-test and differences were considered to be significant at $p<0.05$.

RESULTS

Media selection and cultivation of *P. indica*

Jaggery at different concentrations and Hill-Kaefer media were used for the cultivation of *P. indica*. The study for the possibility of fungal growth on Jaggery showed that *P. indica* was able to grow on jaggery. The optimum growth was recorded at 4% jaggery. The growth was comparatively less at 2, and 6% Jaggery, respectively (Table 1). No significant difference was recorded on 3 and 5% Jaggery (Data not given here).



A B C D E

Fig. 1. Photomicrograph of *P. indica* grown on varying concentrations of different media showing pear shaped chlamydospores viewed under 40X of phase contrast microscope where A) Control (only distilled water); B-D) Media containing different concentration of Jaggery 2, 4 and 6%; E) Hill-Kaefer

Comparison of plant growth parameters

The biomass of *P. indica* grown on medium containing 4% Jaggery was used for checking its viability towards economically important plants. A visual examination of the plants indicated healthy and greener leaves in the treated plants in comparison to control. The impact of fungus biomass was tested with 2 mono and 2 di-cot plants. Results obtained from present study indicated that the overall growth of plants (*Triticum aestivum*, *Pennisetum glaucum, Cicer arietinum* and *Lathyrus sativus*) treated with the *P. indica* grown on 4% jaggery supplemented media was significantly higher as compared with plants that were not treated with *P. indica* and served as control. (Table 2 a&b)

Table 1. Growth of *P. indica* on variable concentrations of 'Jaggery'

Different Media	Radial Growth (RG) (cm)	Maximum DCW (g/L)	Day of sporulation starts	*Spore Yield (spores/ml)	Day of achieve maximum		
					RG	DCW	Sporulation
2% Jaggery	3.82	8.65	12	$(3.36\pm0.48) \times 10^4$	10	10	15
4% Jaggery	7.36	16.34	7	$(6.43\pm0.67) \times 10^7$	7	6	11
6% Jaggery	4.88	5.45	12	$(1.25\pm0.55) \times 10^2$	12	11	14
Hill-Kaefer	5.94	12.12	10	$(7.35\pm0.18) \times 10^6$	8	9	12

DCW= Dry Cell Weight; RW= Radial growth; different concentration of Jaggery (2%, 4% and 6% respectively) and Hill-Kaefer, in 500 ml Erlenmeyer flask;
*Values are mean ± SD of three replicates

Radial growth and dry cell weight

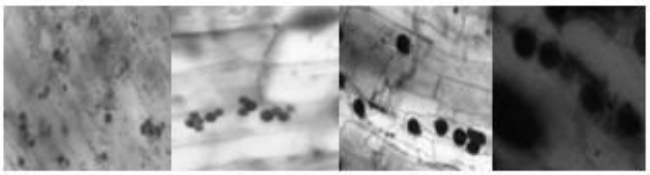
The morphology of leading hyphae in the peripheral colonies of *P. indica* growing on medium containing jaggery at different concentrations and Hill-Kaefer was investigated (Table 1). Measurements of diameters of leading hyphae of colonies grown on different agar media revealed no difference in the morphology. Growth on 4% Jaggery led to a significant increase in diameters of the leading hyphae to 7.36 cm. The analysis of mean radial growth of mutualistic fungus was noted every alternative day till a time the fungus reached the edges of the Petri dish (Table 1). Maximum dry cell weight of the fungus was obtained on 4% Jaggery medium i.e. 16.34 gm/L. Thereafter this medium was selected as basal medium or called Jaggery medium for further studies. Fungal growth on other medium was comparatively less as compared to media containing 4% Jaggery (Table 1).

Spore yield

Among the media, 4% jaggery produced significantly higher spore production of 6.43×10^7 spores/ml and sporulation of fungus started at 7th day (Table 1& Fig 1). The lowest spore production occurred on media containing 6% Jaggery (1.25×10^2 spores/ ml) and sporulation started at 12th day.

Chlorophyll and phosphorus content in leaves

More than 50 % enhancement in concentration of Chlorophyll a and b (mg/g fresh weight) was found in the leaves of colonized plants as compared with the non-colonized plants (Table 2c). The content of phosphorus in leaves of treated plants was significantly enhanced in plants inoculated with *P. indica* grown on medium containing 4% Jaggery as compared with control (Table 2 d).



A B C D

Fig. 2. Root colonization; where inter and intra chalamydospores were seen in the roots of *Triticum aestivum, Pennisetum glaucum, Cicer arietinum*, *Lathyrus sativus* (A-D)

Root colonization

Roots of plants treated with *P. indica* obtained from 4% Jaggery were selected and stained with lactophenol cotton blue.

Table 2. A comparative growth of monocot and dicot plants when co cultured with *P. indica* culture grown on Jaggery Medium

a)Shoot and Root length

Treatment with	*Triticum aestivum*		*Pennisetum glaucum*		*Cicer aretinum*		*Lathyrus sativus*	
	Shoot Length	Root Length	Shoot Length	Root Length	Shoot Length	Root Length	Shoot Length	Root Length
Control	9.18±0.38	3.98±0.34	11.1±0.32	5.32±0.61	12.86±0.46	5.98±0.43	15.9±0.70	2.74±0.29
P. indica	15.9±0.72	6.78±0.71	18.98±0.30	9.7±0.69	20.68±0.71	9.22±0.42	26.9±1.16	6.76±0.43

b)Fresh and Dry Weight

Treatment with	*Triticum aestivum*		*Pennisetum glaucum*		*Cicer aretinum*		*Lathyrus sativus*	
	Fresh Weight	Dry Weight	Fresh Weight	Dry Weight	Fresh Weight	Dry Weight	Fresh Weight	Dry Weight
Control	3.53±0.18	1.41±0.11	4.84±0.14	1.97±0.11	5.30±0.29	2.82±0.12	5.89±0.33	2.76±0.16
P. indica	5.34±0.14	2.73±0.18	6.39±0.36	2.91±0.19	8.71±0.35	3.85±0.43	9.23±0.21	4.61±0.19

c)Chlorophyll Content

Treatment with	*Triticum aestivum*		*Pennisetum glaucum*		*Cicer aretinum*		*Lathyrus sativus*	
	Chl a	Chl b	Chl a	Chl b	Chl a	Chl b	Chl a	Chl b
Control	2.51±0.25	0.90±0.21	2.50±0.16	1.33±0.26	3.39±0.27	0.98±0.15	3.28±0.23	0.91±0.39
P. indica	3.49±0.22	1.28±0.11	4.32±0.20	2.37±0.36	4.29±0.49	1.93±0.17	4.31±0.26	0.23±0.15

d)Phosphorus Content (%)

Treatment with	*Triticum aestivum*	*Pennisetum glaucum*	*Cicer aretinum*	*Lathyrus sativus*
Control	0.17	0.33	0.24	0.11
P. indica	0.28	0.40	0.45	0.19

Each data represents the mean of two independent replicates and each replicate represents five samples. In case of *P. indica* colonized plants; Shoot and root length (a), fresh and dry weight (b), chlorophyll a and b contents (c), and Phosphorus content (d) were found significantly increased at $p<0.005$ according to Student's t-test as compared with control plants.

Root colonization was seen under microscope. Treated plants showed inter and intracellular chlamydospore single and in chain (Fig 2).

DISCUSSION

An optimal nutrient medium should provide good or adequate growth of microorganisms according to clinical laboratory standards (McGinnis and Rinaldi, 1991). The nutrient medium is a major factor that influences the results of susceptibility tests (Espinel-Ingroff *et al*., 1998; Rex *et al*., 1993). For cultivation of various groups of fungi there are a large number of media compositions available in the literature, but no literature is available for axenic cultivation of obligate symbiotic fungi since they cannot be grown without the plant host, on synthetic media (Hart and Trevors, 2005). *P. indica* has potency to grow axenically on number of complex and synthetic media, but these are not cost effective for commercialization of the fungus. Because of various advantageous properties of fungus with plants, optimization of media is an important aspect to be considered for examining the growth of fungus (Kumar *et al*., 2011). In order to commercialize the fungus obviously we have to consider reducing the cost of mass production of fungus. An attempt was therefore made to cultivate the fungus on cheaply available and rich energy source, Jaggery. In present study, different concentrations of Jaggery were used to grow *P. indica* and its growth was compared with fungus growing on Hill-Kaefer medium (Table 1). Low cost technology means an advanced generation technology, in which cost reduction is achieved by improving process efficiency and better utilization of resources (Savangikar, 2002). Low cost options should reduce the cost of production without compromising the spore viability, number and its efficacy towards plant productivity. Therefore, low cost alternatives are needed to reduce cost of cultivation of mycorrhizal fungi. Fungus growing on different nutrient composition has shown significant quantitative and morphological changes. In Kumar *et al*., 2011 demonstrated that nutrition can significantly influence spore yield, stability, and biocontrol efficacy, once the media for mass cultivation is optimized. These findings match with our results. In present study, the spore yield, radial growth and Dry cell weight varied on different media (Tables 1). In the present investigations the hyphal growth of *P. indica* was found to be the best on 4% Jaggery followed by Hill and Kaefer medium. It is well documented that the fungus grows best on Hill and Kaefer medium with the rhythmic pattern of growth (Pham *et al*., 2004) but no finding have shown the growth of fungus on Jaggery.

Significant increase in shoot and root length, fresh and dry weight in all the tested plants (*Cicer arietinum* L., *Lathyrus sativus* L., *Triticum aestivum* L. and *Pennisetum glaucum*) inoculated with *P. indica* grown on medium containing 4% Jaggery was recorded relative to the control plants. The efficacy of the fungus was therefore not affected when grown on 4% Jaggery. Increase in chlorophyll and phosphorus content was also seen in *P. indica* colonized plants. According to our calculation one litre of Hill-Kaefer medium costs a sum of Rs. 482.00 for production of 48 gm fresh weight of fungus whereas a similar quantity was produced in Jaggery medium at

a cost of only Rs. 2.00 or One US Cent. Jaggery is highly cost effective medium which could be used for reducing the cost of mass production of fungus at commercial level. Significant increase in biomass productivity and reduction in time to achieve better yield were obtained for *P. indica* with an indigenous carbon/nutrition energy source 'Jaggery'. Also, the positive effect of fungal biomass on plants was unaffected. This promises a tool for commercializing the fungus for application in Agricultural, Floriculture & Horticulture at very cheap price comparing the cost with Hill-Kaefer media.

Conclusion

The present invention relates to an improved, inexpensive carbohydrate enriched nutrient media-Jaggery, a by-product of sugarcane industry was used to reduce the production costs for cultivation of *P. indica* on commercial scale. It is effectively used as low-cost substrate without any pre-treatment. This medium does not pose any threat to environment and also it is a boon for bio-fertilizer industry.

Acknowledgement

This study was supported by Department of Biotechnology (DBT), Government of India and MA is grateful to DBT for providing junior research fellowship.

REFERENCES

Arnon, D.I. 1949. Copper enzyme in isolated chloroplast polyphenoloxidase in *Beta vulgaris* L. *Plant physiol.,* 24: 1-15.

Arora, M., Kharkwal, A.C., Bains, K.S. and Varma, A. 2012. Novel and new nutrient for cultivation of *Piriformospora indica*, Indian Pat. *Application number* 944/DEL/2012.

Espinel-Ingroff, A., Barchiesi, F., Hazen, K.C., Martinez-Suarez, J.V. and Scalise, G. 1998. Standardization of antifungal susceptibility testing and clinical relevance. *Med. Mycol.,* 36: 68–78.

Gamborg, O.L. and Phillips, G.C. 1996. Sterile Techniques. In: Gamborg OL, Phillips GC (eds) Plant Cell Tissue and Organ Culture: (Narosa New Delhi), pp. 67-79.

Gericke, S. and Kurmies, B. 1952. The colorimetric determination of phosphoric acid ammonium vanadate molybdate and its application in plant analysis. *J Plant Nutr Soil Sci.,* 159: 11–21.

Hart, M.M. and Trevors, J.T. 2005. Microbe management: Application of mycorrhyzal fungi in sustainable Agriculture. *Frontiers in Ecology and the Environment,* 3:533-539.

Hill, T.W. and Kaefer, E. 2001. Improved protocols for aspergillus medium: trace elements and minimum medium salt stock solutions. Fungal Genet News Lett., 48: 20-21.

Hiscox, J.D. and Israelstam, C. P. 1979. A method for extraction of chlorophyll from leaf tissues without maceration. *Can. J. Bot.,* 57: 1332–1334.

Kumar, V., Sahai, V. and Bisaria, V.S. 2011. High density spore production of *Piriformospora indica*, a plant growth promoting endophyte by optimization of nutritional and cultural parameters, *Bioresource Technology*, 102: 3169–3175.

Kumari, R., Pham, G.H., Prasad, R., *et al*, 2004. *Piriformospora indica*: fungus of the millennium. In: Podila G, Varma A (eds) Basic research and applications: mycorrhizae, *Microbiology series* (New York: IK International-India), pp. 259–295.

Latifian, M., Rad, B., Amani, M. and Rahkhodaei, E. 2013. Mass production of entomopathogenic fungi *Beauveria bassiano* (Balsamo) by using agricultural products based on liquid-solid diphasic method for date palm pest control. *International Journal of Agricultural and Crop Sciences,* 5: 2337-2341.

McGinnis, M.R. and Rinaldi, M. G. 1991. Antifungal Drugs: Mechanisms of action, drug resistance, susceptibility testing, and assays of activity in biological fluids, in Antibiotics in laboratory medicine. In: V. Lorian (ed) , 3rd edn, vol V, Williams and Wilkins Baltimore, Md), pp. 198–251.

Pham, H. G. 2004. Interaction of *Piriformospora indica* with diverse microorganisms and plants. In: Varma A, Abbott L K, Werner D, Hampp R (eds) Plant Surface Microbiology (Berlin: Springer-Verlag), pp. 237-265.

Prasad, R., Pham, H.G., Kumari, R., Singh, A., Yadav, V. *et al.*, 2005. Sebacinacea: culturable mycorrhiza-like endosymbiotic fungi and their interaction with non transformed and transformed roots. In: Declerck S, Strullu D G, Fortin A (eds)*In vitro* culture of mycorrhizas. Soil Biology, Vol 4 (Springer-Verlag, Berlin Heidelberg), pp. 291-312.

Rex, J.H., Pfaller, M.A., Rinaldi, M.G., Polak, A. and Galgiani, J.N. 1993. Antifungal susceptibility testing. *Clin. Microbiol. Rev.,* 6: 367–381.

Savangikar, V. A. 2002. Role of low cost options in tissue culture. In: Low cost options for tissue culture technology in developing countries. Proceedings of a technical meeting organized by the Joint FAO/IAEA Division of Nuclear techniques in food and agriculture. Vienna, IAEA, pp. 11-15.

Singh, A., Rajpal, K., Singh, M., Kharkwal, A.C., Arora, M. *et al*, 2013. Mass cultivation of *P. indica* & Sebacina Species. In: Varma A, Kost G and Oelmuller R (eds) *Piriformospora indica*, sebacinales & their biotechnological applications, (Springer-Verlag, Berlin Heidelberg), pp. 377-392.

Varma, A., Singh, A., Sahay, S., Sharma, J., Roy, A. *et al*, 2001. *Piriformospora indica*: an axenically culturable mycorrhiza-like endosymbiotic fungus. In: Hock B (ed) Fungal Associations, The Mycota, vol IX (Springer, Berlin Heidelberg, New York), pp. 123-150.

Varma, A., Sherameti, I., Tripathi, S., Prasad, R., Das, A. *et al*., 2012. The Symbiotic Fungus *Piriformospora indica*: Review. In: Hock B (ed) Fungal Associations, 2nd edn, The Mycota, vol IX, (Springer-Verlag Berlin Heidelberg, New York), pp. 231-254.

Verma, S., Varma, A., Rexer, K. H., Hassel, A., Kost, G., Sarbhoy, A., Bisen, P., Bütehorn, B. and Franken, P. 1998. *Piriformospora indica*, gen. nov. sp. nov., a new root-colonizing fungus. Mycologia, 90:896–903.

****