AMITY UNIVERSITY
A RESEARCH & INNOVATION DRIVEN UNIVERSITY

AMITY- OVER 2 DECADES OF EXCELLENCE IN EDUCATION

Amity is India's leading education group where 100,000 students are pursuing over 250 programmes in 6 Universities and over 150 Institutions of higher learning.

Amity has established world-class universities in Noida, Lucknow, Jaipur, Gurgaon, Gwalior and Mumbai with plans to have University in every state of India. Making a name in education globally, Amity has campuses in London, Dubai, Abu Dhabi, Singapore, USA, Mauritius, China, South Africa & Romania.

With over 2 decades of excellence in education, Amity today has a team of 4,500 faculty, researchers and scientists who make up a brilliant academic and professional learning environment. In just one year, Amity scientists & researchers have filed 542 patents in diverse areas, besides being credited with 500 books and 6,000 research papers.

Amity University Noida is India's top ranked not-for-profit pvt. University established through state legislature, with powers to award degrees under the UGC Act.

As a leading University of the Country, Amity University's institutions including those of Management, Law, Engineering, Biotechnology, Insurance, Fashion, Mass Comm., Telecom etc., have been ranked amongst the top in India's most respected surveys by India Today, Business Today, Economic Times, Outlook etc.

What's more, Amity has also got prestigious national and international recognitions & accreditations including 'Grade A' by NAAC, 'Premier status by ASIC (UK)', 'Accreditation by IET (UK) for B.Tech degrees' & 'Accreditation by ACBSP (USA) for Management degrees'

Amity University Noida has the most hi-tech 60 acre campus in New Delhi NCR with live transmission of classes & events, web-cast of lectures, access to over 17,000 online journals. Students enjoy the privileges of a self-reliant, fully air-conditioned campus with 8,000 hostel seats, multi-cuisine cafeterias (Dominos, Cafe Coffee Day, Subway, Dosa Plaza & Cocoberry), L'Oreal Salon, Tekson's Bookstore and a 15 acre sports complex that includes Olympic size swimming pool, tennis and squash courts and horse riding academy.

60 acre Amity University Campus, Noida (New Delhi NCR)

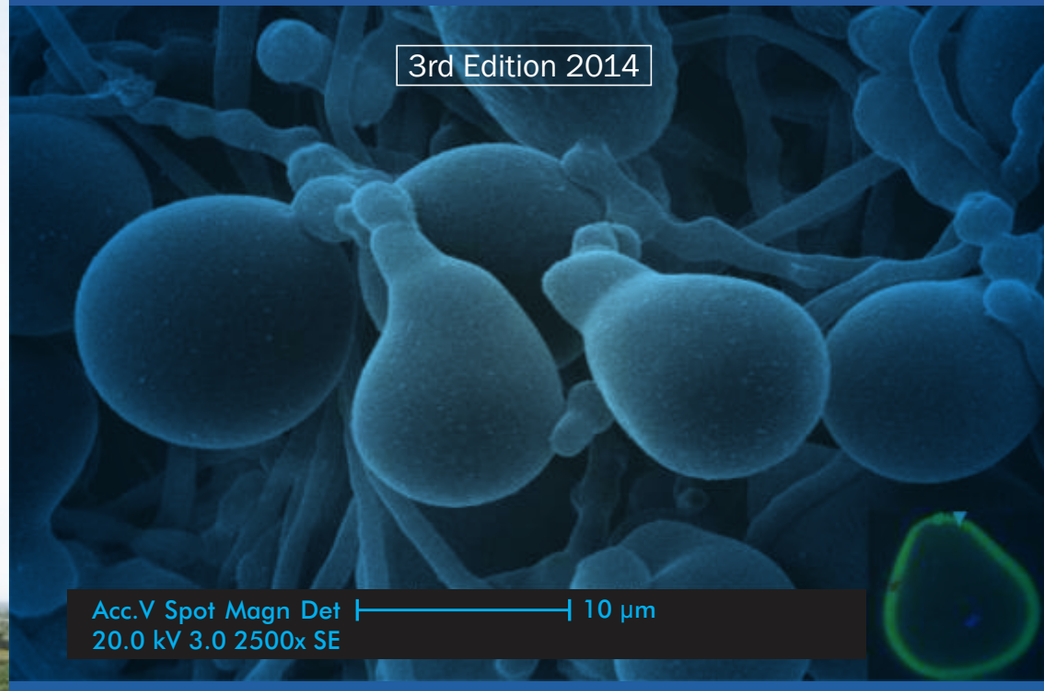
AMITY UNIVERSITY
A RESEARCH & INNOVATION DRIVEN UNIVERSITY

MEMOIRS OF 'ROOTONIC'-THE MAGIC FUNGUS

PROMOTES AGRICULTURE, HORTICULTURE AND FOREST PRODUCTIVITY

बॉयो खाद - जड़ों की ताकत

3rd Edition 2014



Compiled by | Ajit Varma* | Ruchika Bajaj | Aparna Agarwal | Shobhit Asthana | Kartikeya Rajpal | Aparajita Das | Ram Prasad | Amit C Kharkwal

*For further correspondence: ajitvarma@amity.edu

Amity Institute of Microbial Technology
Amity University Uttar Pradesh, India

Contents

Foreword

It was per chance that I attended Dr. Ajit Varma's talk on *Piriformospora indica* (henceforth referred to as PFSI) and that too only for the last 15 minutes. Even in that short time, I could feel the potential of this symbiotic mycorrhizal fungus. I met him immediately after the talk. My short question to him was that if it had all the properties mentioned in the talk why we have kept it confined only to lab experiments and limited controlled field trials. Why do we not do something about it? This short talk led to a revolution, howsoever minuscule, it may have been.

Soon I arranged a meeting with Sardar Prakash Singh Badal, Chief Minister of Punjab, Dr. Ajit Varma and myself went for this meeting. He personally welcomed us. We gave a detailed presentation about PFSI. His Agriculture Minister and all the senior officials of department concerned with entire agriculture and forest sectors, as also representatives of Punjab Agriculture University, Ludhiana were present. Sardar Badal's reaction was very positive towards it and he invited us to visit various parts of the state, interact with the farmers, and organized field trials. He instructed all the departmental officers concerned to arrange these meetings with the farmers. During the next few months thereafter we toured extensively in Punjab. We also gave presentation to Shri P.K. Dhumal, the then Chief Minister of Himachal Pradesh who similarly welcomed us and gave suitable instructions to all those, including Agriculture and Horticulture Ministers for taking necessary action for field work. He further encouraged us that apart from the lower areas of state we should carry out trials at higher altitude also.

We gave presentations to Shri Omar Abdullah, Chief Minister of Jammu & Kashmir and also at many other fora.

That done Dr. Ajit Varma, Dr. Amit C. Kharkwal and myself extensively toured Punjab and Himachal Pradesh, held meetings with the farmers, gave them presentations and distributed PFSI for field trails on a variety of crops.

Our second round of meetings with the farmers was an eye opener, even for us, about the potential of PFSI. The results had been way beyond our expectations. Apart from increase in productivity and improved quality of the produce they discovered many other positive qualities. One farmer claimed that it had even kept away the rodents from attacking his Brinjal crop.

The final word was from a farmer from Bhogpur, district Jalandhar, who interrupted the proceedings, got up and made the sweetest and shorted speech 'Sirs, it is Karishma'.

Rest as they say is history. The information travelled much faster and wider that we had anticipated. Within a year we had demand not only from these state but from all over the country and abroad. One Norwegian MNC dealing with fertilizers, pesticides etc approached us for joint production and marketing.

Farmers keep on trying it on more and more crops and sending us further information about the wonders it can do. One farmer in Yamuna bed sowed Methi, essentially a winter crop, in the month of June and made a fortune by being in the market much in advance of any other. What normally happens in agriculture sector in years it happened here in months.

PFSI has now been christened as ROOTONIC. The handbook 'Memoirs of Rootonic' is a very good thought on the part of Dr. Varma. It explains in a simple way all that Rootonic stands for and can do. I am glad he has avoided unnecessary technicalities and given it a form which can be easily understood and followed by technicians as well as by farmers.

I am sure that the third edition of "Memoirs of Rootonic" will prove to be of immense help in bringing up the entire agriculture sector and, God willing, prove to be the long awaited input which the country has waited for long, since Green Revolution for quantitative jump in agricultural production.

K.S. Bains, IAS (Retd.)
Padma Shri
Advisor & Head,
Amity Institute of Training & Development

Preface

In 1990, while holidaying in hot scenic sand dunes of Jaisalmer area of Rajasthan, I was astonished to see some very healthy green plants and few of them were flowering. A number of persons, artist and camels were seen on the sand dunes. I was told that the film shooting is going on, in which actress Madhuri Dixit was playing an active role. When the crowd left the site, with the help of local people, we dug open the vicinity of the plants, and after 2-3 meters depth, some wet sand lumps were observed. The samples were brought to the laboratory. Under stereoscopic binocular microscope, we saw some spores. Efforts were made to culture them on a nutrient broth. A few spores germinated, which were transferred at-least ten times to obtain the axenic culture. Later they were treated with young seedlings and roots were seen colonized. On careful examination, the colonized organism was same. In 'nutshell', Koch's Theory of Recapitulation was confirmed. I was confident that it is a typical mycorrhiza fungus, however, none accepted the break through discovery.

In 1997, I was invited by then Director of Max Plank Institute of Terrestrial Microbiology (MPI), Marburg, Germany, Prof. RK Thauer to retake up the joint collaborative work with their scientists. Soon it was confirmed that it is a typical plant promoting mycorrhiza fungus. The plant growth properties were patented in Europe and the first scientific documentation was done in a reputed journal in United States of America.

Since then the success story started. Several scientists all over the world conducted a large number of experiments on basic and applied research. Our data was always reproducible both in laboratory experiments and field trials. A new family Sebacinales and the new order Sebacenales were established. Subsequently another species *Piriformospora williansii* was created.

This 'Memoirs' is the outcome of green house experiments and field trials conducted in North India and extreme cold

deserts of Leh-Ladakh. The most astonishing part was that the fungus was screened from the extreme hot deserts which functioned well in extreme cold deserts. I particularly hope that this compilation will serve as a useful focal point for further studies and would render benefit to the hardcore agriculturists, horticulturists and foresters.

It has been the pleasure to compile the Memoirs, primarily due to the stimulating cooperation of colleagues and students. To name a few, Shri KS Bains, Dr. Abha Agnihotri, Mr. Anil Chandra, Dr. K Sowjanya Sree, Dr. Neeraj Shrivastava, Dr. Jasroop Kaur Aneja, Mr. Narendra Kumar, Madhunita Bakshi, Monika Arora, Priyanka Sharma, Neha Chadha, Neha Singh, Manjita Mishra, Prasun Bandhopadhyay and Divya Kilam. Services received from Niraj Kumar, Dharmendra Kumar and Ram Naresh Yadav are to be appreciated.

I am thankful to The Founder President Dr. Ashok K. Chauhan, Chairperson Dr. Amita Chauhan, President RBEF and Chancellor AUUP Shri Atul Chauhan, Additional President Shri Aseem Chauhan, Our Industrial Partner Shri Ajit Chauhan, The Group Vice Chancellor Maj. Gen. KJ Singh and Acting Vice Chancellor Dr. Balvinder Shukla for providing encouragement and support. The positive remarks received from our colleagues Deputy Vice Chancellor Lt. Gen. PD Bhargava, Drs. Sunil Saran, AK Shrivastava, KC Upadhayaya, BB Dhar, BB Singh, VPS Arora, BP Sehgal, SN Raina and Ashish Verma are duly acknowledged. Special thanks to Dr Ram Pundir for providing us photographs of the field trials. Logo was prepared by Diksha Bhola, M.Sc. (MT) 2013-2015 batch which is highly appreciable and also thanks to Ms. Swati Gupta from M/s Imagic Communications for designing the book.

Dr. Ajit Varma
Distinguished Scientist & Professor of Eminence
Amity Institute of Microbial Technology

MESSAGE

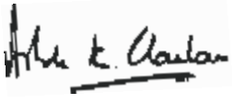
from Hon'ble Founder President

Dr. Ashok K. Chauhan

Soil is our ecological capital and erosion is robbing us of it. It took the earth nearly 400 million years to clothe itself with soil which forms the base on which land plants originated, flourished and provided shelter and sustenance to a wide range of microbes, plants and animals. In the course of evolution, a perfect harmony was achieved between the flora and fauna of this planet with the undisturbed soil system providing an ideal and sustainable environment. The productive soil environment began to change with the origin of agriculture about 9-10 thousand years ago in which tillage of land became an essential component for cultivation of crop plants. The soil with its micro-flora was marked by an abundance of essential nutrients which began to deplete due to their mining by crop plants and loss of top fertile soil. If the top soil is eroded, the seeds do not take roots and, if the soil is not remedied suitably, degradation of land sets in. The prevailing domain of modern agriculture presents a common global scenario: farmers spray pesticides and herbicides, spread artificial fertilizer, and irrigate the land in dry weather as a routine, plough, plant and harvest with machinery run on fossil fuel. It is estimated that we are losing on an average two to eight tonnes of topsoil per year from one acre of ploughed land. It is believed that the earth has already lost one-third of its hardest earned asset, the topsoil in the last one millennium and if urgent steps are not taken, we may lose a very heavy chunk of the fertile upper layer by the end of this century.

'Rootonic'- The Magic Fungus *Piriformospora indica* was discovered by Prof. (Dr.) Ajit Varma way back in 1990. Since then he and a large number of scientists all over the world have made serious endeavors to understand its physiology, biochemistry and biotechnological applications. Several patents have been filed and granted. It is high time that the fungus is commercialized for the benefit of the society, particularly the cultivators, farmers, horticulturists and foresters.

Amity University Uttar Pradesh is proud to bring out a memoir on this fungus which highlights the importance of the unique microorganism at a glance. I am more than confident that through this book, the message for better crop production and sustainable soil fertility would benefit the cultivators, not only from India, but also at the global level.



Dr. Ashok K. Chauhan
Founder President
Ritnand Balved Education Foundation (RBEF)
(Sponsoring Body of Amity Universities)

MESSAGE
from Acting Vice Chancellor
Prof. (Dr.) Balvinder Shukla

The modern scientific research has reached to a level which has created a lot of interest in the study of mycorrhiza. This knowledge has resulted into the awareness of the bio scientist that the productivity of plants and quality of leaves, flowers, fruits and seeds are determined by the activities of root systems and their associated physical, chemical and biological environment. As a result of which the symbiotic fungi have become important subjects of tests to evaluate some of the new opportunities being developed in microbial biotechnology. Though the use of fungi is old to stabilize eroded soils and forests but, the novelty in recent years has given increased recognition, that biological processes can be manipulated genetically. These researches have opened up numerous unexplored opportunities for the optimization of plant productivity in both managed and natural ecosystems, with minimum damage to the environment. These "memoirs" documented herein, contain the current state of knowledge and theories on the structure, function, molecular biology and biotechnological applications of symbiotic fungi. It will thus be of great interest to a diverse researchers and instructors, especially biologists, biochemists, agronomists, foresters, horticulturists and associated scientists in varied disciplines.

Prof. Ajit Varma who has been an architect of the 'Fungi - *Piriformospora indica* (The Magic Fungus -"Rootonic") way back in 1990, and has filed several patents in this area of research. And now it is high time to commercialize this fungus for the benefits of the society in general and cultivators, farmers, horticulturists and foresters in particular.

Amity University Uttar Pradesh is proud to bring out "Memoirs" on this fungus which gives the importance of this unique micro-organism at a glance. I am confident that this book will be beneficial to the Indian Cultivators, and may be to the world at large as well.

Prof. (Dr.) Balvinder Shukla
Acting Vice Chancellor
Amity University Uttar Pradesh, India

Letter from Dr. AK Yadav

सत्यमेव जयते

भारत सरकार Government of India
कृषि मंत्रालय Ministry of Agriculture
कृषि और सहकारिता विभाग Department of Agriculture & Cooperation

राष्ट्रीय जैविक खेती केन्द्र

National Centre of Organic Farming

२०४-बी खण्ड, सी.जी.ओ. काम्प्लेक्स द्वितीय 204-B Wing CGO Complex-II
कमला नेहरू नगर, गाजियाबाद - 201 002 Kamla Nehru Nagar, Ghaziabad-201 002

No. 10-9/2007-NCOF/ 8023 Date 18.10.2011

To
Dr Ajit Verma
Director General
AMITY institute of Microbial Technology
Amity University, Noida, UP

Sub:- Regarding *Piriformospora indica* endophytic fungus for plant growth promotion.

Sir,

In reference to your letter No. AIMT/01/October 2011 Dated 14.10.2011, I have to clarify as follows:

1. The *Priformaspora indica* is a well known plant growth promoting endophyte with far extending hyphae.
2. Although its mechanism of action is very close to mycorrhiza, but being laboratory culturable, it is different from Mycrorrhiza included in FCO and currently do not have defined quality standards.
3. Currently the said fungus is not included in FCO, therefore not subjected to any requirement of registration or licensing.
4. In my opinion the Piriformaspora indica is a promising plant growth promoting fungus and need to be promoted for efficacy assessment on all crops. Based upon the literature available on its efficacy it is highly efficient in phosphorus and other micronutrient mobilization.
5. Besides nutrient mobilization the said fungus is also reported to harvest moisture for the plant from faraway niches not accessible to roots, thus helping plants to withstand draught and survive well at low moisture availability.
6. As it is not host specific and can infect all plants, it is an ideal choice for use in all crops, especially the grain crops, horticultural plants, sugarcane, vegetables etc.

I strongly recommend its promotion in all crops

Yours faithfully

(A.K. Yadav)
Director

Introduction

On uprooting a plant, a bulk amount of soil (mud) remains adhered to the rooting system (Fig 1a). They contain diverse microorganisms like bacteria; fungi and actinomycetes. On washing the mud, clean beautiful root architecture is seen (Fig. 1b). The root system of all the land plants are colonized by a special group of fungi named as mycorrhiza. They produce spores of diverse shape, size and colour, either singly or in aggregates (Fig. 2).

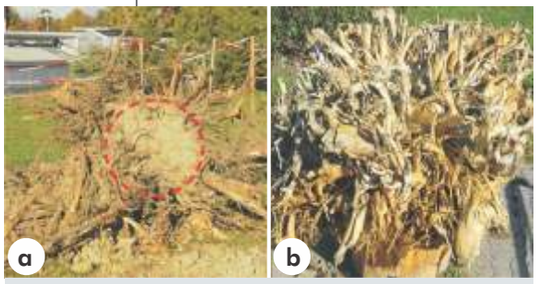


Fig. 1: Rhizosphere of plant system
a) Pre-washed; b) Washed

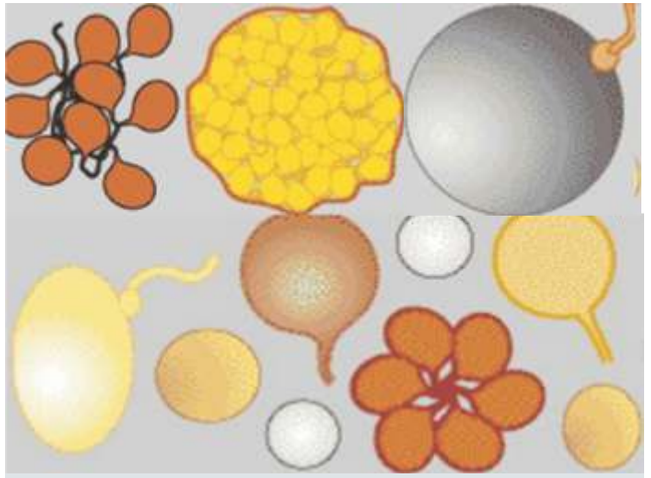


Fig. 2: Spores of Arbuscular Mycorrhiza Fungi

They promote plant growth, enhance the active ingredient, increase seed production and protect plants against disease. One of the most characteristic features is the production of arbuscules into the living cortical cell (Fig. 3). Although this group of fungus (Arbuscular Mycorrhizas) was discovered by German scientist, Prof. KB Frank way back in 1885, their biotechnological applications could not be exploited to the level they deserve because they cannot be cultured in the absence of living root system. Spores / fungal hyphae do not multiply on detaching the living root system.

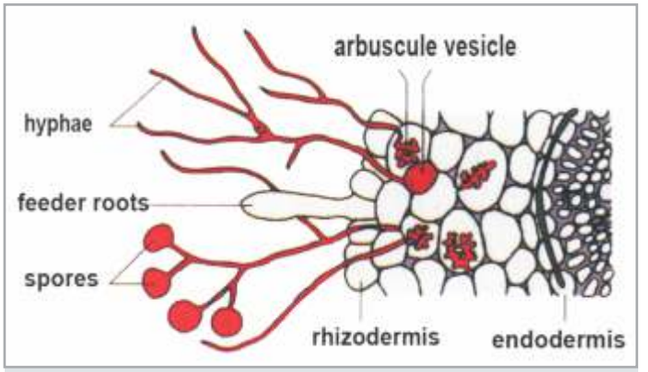


Fig. 3: Anatomy of the root indicating the arbuscule, vesicle and spores.
Note: the extra matrical hyphae in the soil and the root cortex

What is 'Rootonic'?

Piriformospora indica was discovered by Prof. (Dr.) Ajit Varma and his colleagues from Thar Deserts of Western India in 1992 from the root system of several xerophytic plants. Way back in 1997, the properties of the fungus were patented in Germany (European Patent Office, Muenchen, Germany. Patent No. 97121440.8–2105, Nov. 1998). So far, more than 148 plants have been interacted with the fungus and data were documented. The results are positive. Subsequently a new family Sebacinaceae and new order Sebacinales were erected. In 2011 another species, *P. williamsii* was reported. Test plants were members of Bryophytes, Pteridophytes, Gymnosperms and Angiosperms (both mono-dicots), including orchids. This is very unique symbiotic fungus which not only promotes plant growth but also has multifunctional activities (Fig. 4).

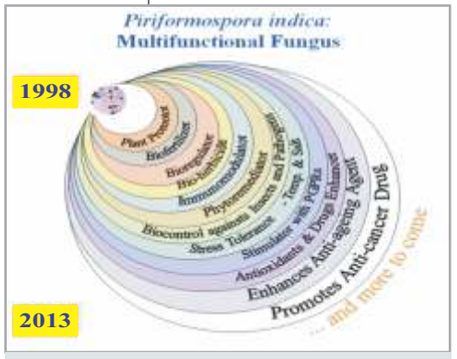


Fig. 4: Functional characteristics of fungus

Aneura pinguis L., *Cicer arietinum, Adhatoda vasica* L., *Aristolochia elegans, Daucus carota* L., *Arachis hypogaea* (groundnut or peanut), *Petroselinum crispum* L., *Medicago sativa, Centella asiatica, Glycyrrhiza glabra, Cuminum cyminum, Abrus precatorius* L., *Foeniculum vulgare, Mimosa pudica, Carum capticum, Vigna*

Table 1: Performance of Rootonic trial on vegetables and other crop plants in Punjab

Crop	Place	% Change Enhancement	Remarks
Cotton	Muktsar	20.00	The treated plants were healthier and were less affected by insects and pests as observed by farmer. The results were authenticated by Dr. Jallore Singh, ADO, Lambi, Punjab
Maize	SAS Nagar	16.67	On an average a yield of 14.64 % is reported after application of Rootonic
Maize	SAS Nagar	13.04	Results Authenticated by Dr. Rajesh Kumar, ADO
Maize	SAS Nagar	12.50	Results Authenticated by Dr. Rajesh Kumar, ADO
Maize	SAS Nagar	13.64	Results Authenticated by Dr. Rajesh Kumar, ADO
Maize	SAS Nagar	17.39	Results Authenticated by Dr. Rajesh Kumar, ADO
Wheat	Amritsar	3.57	Results Authenticated CAO
Wheat	Bathinda	1.71	Results Authenticated CAO
Wheat	Faridkot	3.36	Results Authenticated CAO
Wheat	Fatehgarhsahib	4.61	Results Authenticated CAO
Wheat	Ferozpur	2.19	Results Authenticated CAO
Wheat	Hoshiarpur	5.05	Results Authenticated CAO
Wheat	Jalandhar	1.51	Results Authenticated CAO

Crop	Place	% Change Enhancement	Remarks
Wheat	Mansa	2.27	Results Authenticated CAO
Wheat	Sangrur	8.76	Results Authenticated CAO
Wheat	Muktsar	5.46	Results Authenticated CAO
Rice	Hoshiarpur	7.0	Results Authenticated ADO
Tomato	Kapurthala	11.11	Growth better in treated, pesticide spray kept constant
Tomato	Kapurthala	9.52	Stem borer and whitefly infestation less in treated; Pesticide (Midda and Coragen) usage reduced by 50 % in treated
Brinjal	Kapurthala	100.00	Stem borer and whitefly infestation 50% less in treated; Pesticide (Midda and Coragen) usage reduced by 50% in treated; Fruit quality good in treated with no white spot
Muskmelon	Jalandhar	28.57	Results Authenticated by Horticulture Assistant
Bottle Gourd	SBS Nagar	33.33	Results Authenticated by HDO
Bottle Gourd	Kartarpur	25.00	Blight and Aphid attack in control; Pesticide spray M-45, Metalex reduced by 50% in treated; Treated fruit more sweet in taste
Pumpkin	Kartarpur	14.29	White fly infestation less in treated; Pesticide (M-45) weekly spray in treated and every four day in control
Onion	Kartarpur	20.00	Only for seed production
Wheat	Amritsar	11.02	Higher yield per acre & higher 100 seed weight as compared to control

*ADO- Agriculture Development Officer; CAO- Chief Agriculture Officer; HDO- Horticulture Development Officer

unguiculata, Coriandrum sativum, Glycyrrhiza glabra, Artemisia annua L., *Acacia catechu, Spilanthes calva, Nilotica, Stevia rebaudiana, Prosopis chilensis Stuntz sys., P. juliflora, Calendula officinalis, Arnica* spp. etc. are the plants with which Rootonic interacted positively.

Taxonomic Position

P. indica, a basidiomycete, resembles in many aspects to Arbuscular Mycorrhizal Fungi (AMF) which, however, belongs to new family Sebacinaceae and new order Sebacinales Glomeromycota. In contrast to AMF, *P. indica* can grow axenically. Similar to AMF, this fungus promotes plant growth, increases resistance of colonized plants against fungal pathogens, tolerance to abiotic stress and shows further benefit to plants. It not only alters the secondary metabolites of many plants of economic importance but also promotes overall growth and seed production. In contrast to AMF *P. indica* colonizes *A. thaliana*, a model plant for which a multitude of well characterized mutants is available.

P. indica genome is assembled into 1,884 scaffolds (size: 1 kb; N50: 51.83 kb) containing 2,359 contigs with an average read coverage of 22 and a genome size of 24.97 Mb. The estimated DNA content of *P. indica* nuclei ranges from 15.3 to 21.3 Mb. To assess the genome completeness of *P. indica* a BLAST search was performed with highly conserved core genes present in higher eukaryotes. A genetic transformation system is established using a fragment of the TEF promoter region for construction of vectors carrying the selectable marker hygromycin B phosphotransferase. It is already shown that *P. indica* can be stably transformed by random genomic integration of foreign DNA and that it possesses a relative small genome as compared to other members of the Basidiomycota.

Cultivation and Morphological Characteristics

The fungus has very simple morphology containing hyphae and pear shaped large spores (Fig. 5).

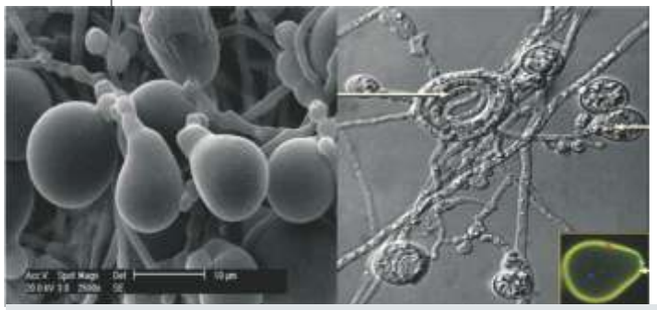


Fig. 5: A view of the morphology of the fungus indicating pear shaped spores. A view of the SEM of the spore (left)

Fig. 6: The fungal colony incubated on rotatory shaker at 27°C

The fungus can be cultivated on simple defined medium both in solid and liquid broth. Optimum conditions for growth are temperature 27°C ± 2, pH 6.8, carbon engery 1.5% glucose. Incubation is done on the rotatory shaker (120 rpm). Best growth is obtained after 7 days incubation where colonies can be large or small (Fig. 6).

Plant Promotion

Rootonic promotes the plants of forestry, horticulture and agriculture importance (Figs. 7-9).

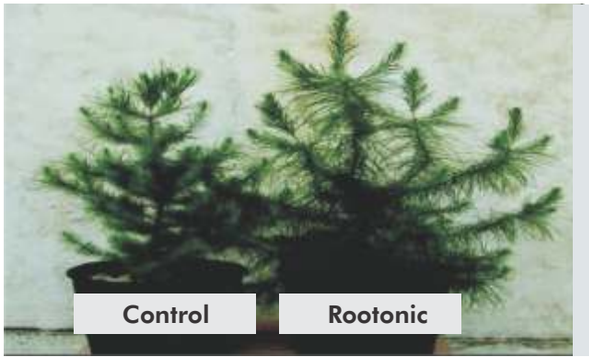


Fig. 7: The interaction of Rootonic on *Pinus* species grown in the rocky sand in Cyprus

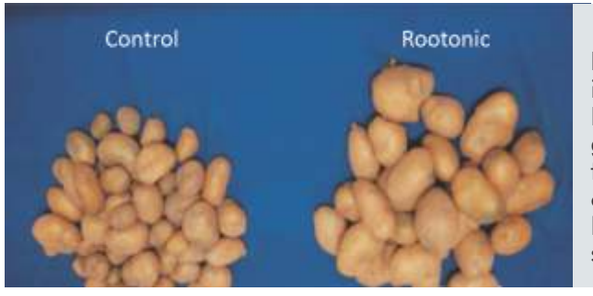


Fig. 8: The positive influence of Rootonic on Potato grown in the farmers' field of Punjab.
Note: the enlarged shining tubers

Fig. 9: The positive influence of Rootonic on Sugarcane (*Saccharum officinarum*) grown in the farmers' field in Punjab.

Note: In the ratoon crop plants not receiving the fungus turned yellow indicating the iron deficiency. In contrast, leaves were dark green where fungus was applied. It was found that this fungus transports iron. Almost 16 % increase was recorded in sugar contents (Table 2)

Table 2: The value addition in the sugarcane as a result of interaction with fungus

Iron (ppm)	Control	202.2	18.35
Sugar (°Bx*)	Rootonic	281.4	21.4

* ° Bx (degree Brix) is the sugar content of an aqueous solution. One degree Brix is 1 gram of sucrose in 100 grams of solution.

Fig. 10: Effect of Rootonic, Bio-Zinc, F. solani and different combination treatments on growth parameters of P. glaucum plants at 60 days after sowing

Interaction with Plants used as Spices

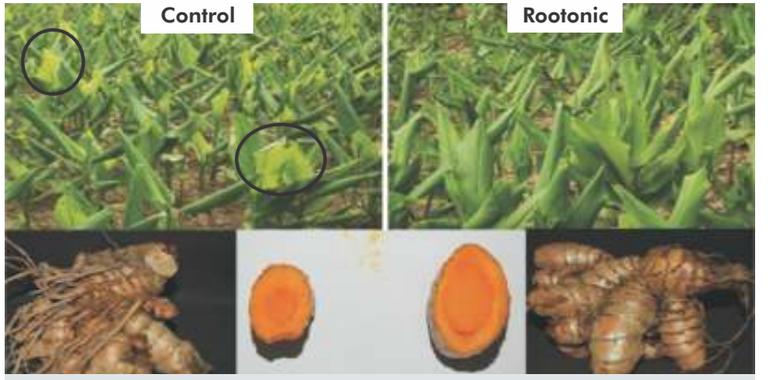


Fig. 11: Co-cultivation with *Curcuma longa* (haldi) in Punjab fields. Note: Control had yellow leaves

Table 3: Interaction with *Curcuma longa* (haldi)

Parameters	Percent increase over control
Volatile Oil	21.09
Curcumin	19.00
Rhizome Yield	12.69

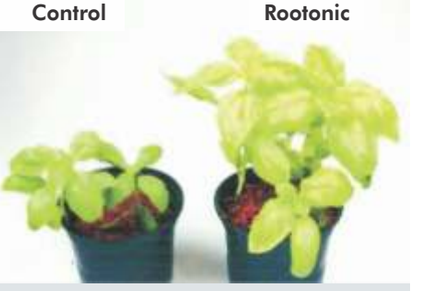


Fig. 12: Interaction of *Ocimum basilicum* with Rootonic enhanced the growth

The yield of Rootonic treated fields enhanced by 12.69%. An overall increase of 32.6 quintal/hectare. In India 1 quintal (100 kg) of normal rhizome costs (in wholesale market)a sum of Rs 500.00 (US $ 10.00). Based on rough calculation, a farmer can earn an extra amount of Rs 16,000.00 (a sum of US $ 280.00) per hectare. There is a strong possibility that the healthy, shiny looking rhizome with enhanced secondary metabolites may fetch a better price.

Interaction of Plants of Economic Importance

In a field trial with Guar seeds at Rajasthan, Rootonic showed the positive results which were confirmed by Sunita Hydrocolloids Ltd. Guar is an important commercial crop in India. Percentage increase was about 12% after treatment with Rootonic (Fig. 13)

In a pot experiment with Bajra, greener leaves were observed after 40 days of treatment. (Fig. 14)

Fig. 13 : Interaction of Rootonic with *Cyamposis* sp. (a) Afield experiment. A plant with fruiting bodies (b) seeds c) endosperm d) Gum powder

Fig. 14 : Rootonic treated Bajra leaves were greener and taller compared to control

Co-cultivation with Fruits

Mango and Litchee were interacted with Rootonic and showed the early fruiting. The leaves of both the trees were greener compared to control (Fig. 15).

Fig. 15 : Interaction of Rootonic with Mango.

Note: Early flowering and fruiting. New leaves appeared much earlier in treated plants

Fig. 16 : Treatment of Rootonic with Litchee resulted in early flowering and fruiting (right). In control plants, the delay of 2-3 weeks was noted.

Interaction with Cereals

Trials in farmers field at Ambala and Yamuna bed in New Delhi on wheat and paddy respectively, resulted a positive response.

Fig. 17 : Co-cultivation of Rootonic with Wheat (Left) and Paddy (Right) showed the positive effect.

Note: the treated plants were taller in both the cereals

Table 4 : Percentage yield enhancement of wheat after co-cultivation with Rootonic over control and different doses of chemical fertilizers.

Chemical Fertilizer	100%	75%	25%
Percentage yield enhancement over control	35	23	27

Recent trial conducted on wheat (variety PB 2967) in Amritsar lead to an enhancement of 11.02% increase in yield per acre and 3.24% increase in 100 seed weight.

Value Addition to Plants of Pharmaceutical Importance

Rootonic was interacted with a large number of medicinal plants like *Spilanthus calva, Artemisia annua, Tridax procumbens, Abrus precatoriu, Bacopa monnieri, Coleus forskohlii, Adhatoda vasica, Withania somnifera, Chlorophytum tuberosum, Foeniculum vulgare, Linum album, Podophyllum* sp., and many more both in the laboratory and field conditions. Interestingly besides plant promotion, the active ingredients like curcumin, artemisnin, podophyllotoxin and bacoside were enhanced leading to value addition to medicinal plants.

Fig. 18: Interaction of *Aloe vera* increased the shoot and root length

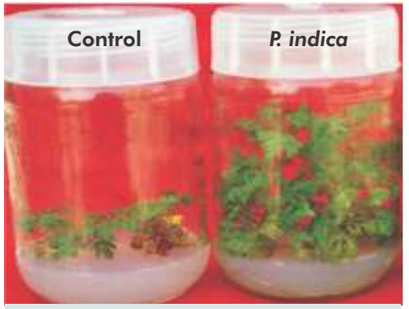
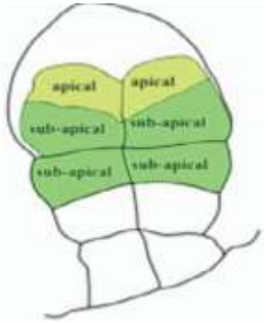
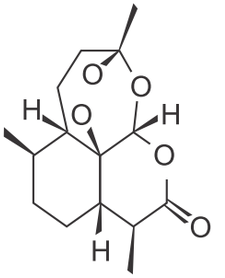


Fig. 19: Co-cultivation of *Artemisia annua* in plant tissue culture laboratory

Fig. 20: Field trial of *Artemisia annua* in Central India. Note: dark green leaves in the plants treated with Rootonic. The artemisinin content was increased by 1.6 fold as compared to control



Glandular secretary trichome of *Artemsia annual*



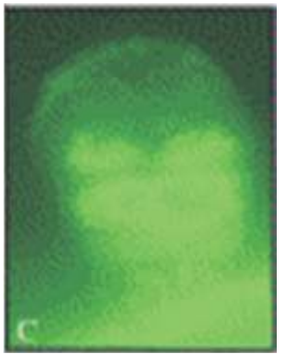


Fig. 21: A view of the glands storing the oil (artemisinin) in the apical leaves

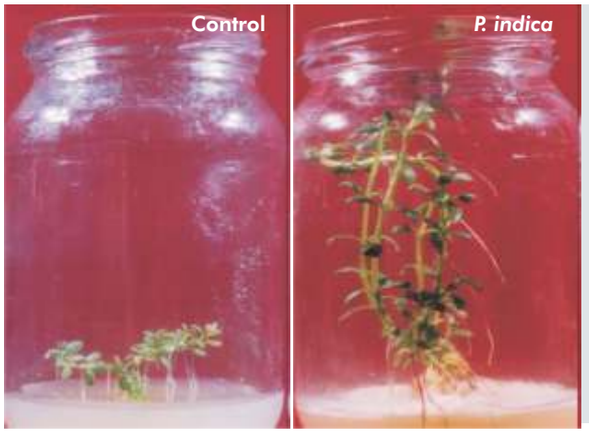


Fig. 22: Interaction with *Bacopa monniera* led to vigorous growth and 3.5 fold increase in the bacoside

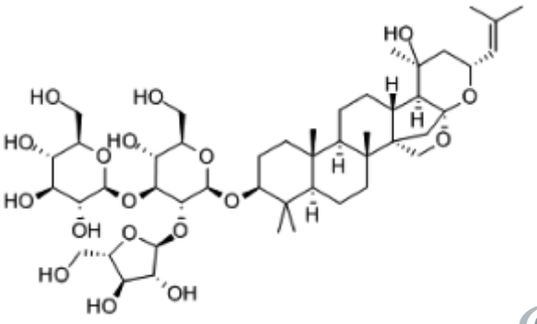


Fig. 23: Chemical structure of the bacoside

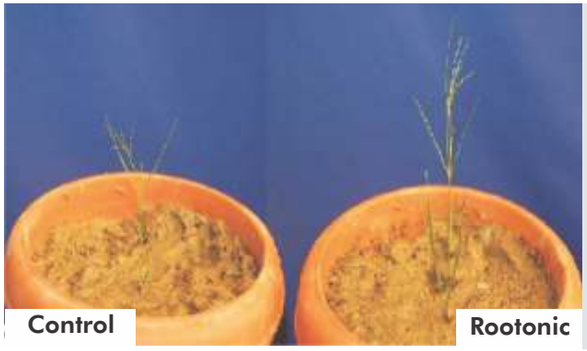


Fig. 24: Interaction of *Ephedra ciliata* led to enhanced growth of treated plants.
Note: *Ephedra* sp. is used for the treatment of cough, cold, hay fever and asthama

Fig. 25 Interaction with *Chlorophytum borivilianum* (safed musli) in field trial

Fig. 26: A field trial of *Spilanthes calva* (akkalkara) in central India

Rootonic Rejuvenates Fruiting

Kinnow *(Citrus reticulata)* plant was introduced in India in recent past. A vigorous fruiting was recorded. After a lapse of some years, flowering and fruiting considerably dropped in an orchard in Punjab. Addition of inoculum near the root led to nearly normal fruiting (Fig. 27).

Fig. 27: Formulated cultures were placed in the vicinity of plant roots. After 6 months, fruiting was regained near normal

Co-cultivation Promotes Early Flowering

This fungus possesses unique characteristic feature of promoting early flowering in plants tested viz., tobacco, *Coleus, Brassica,* bottle guard and cotton (Figs. 28-30).

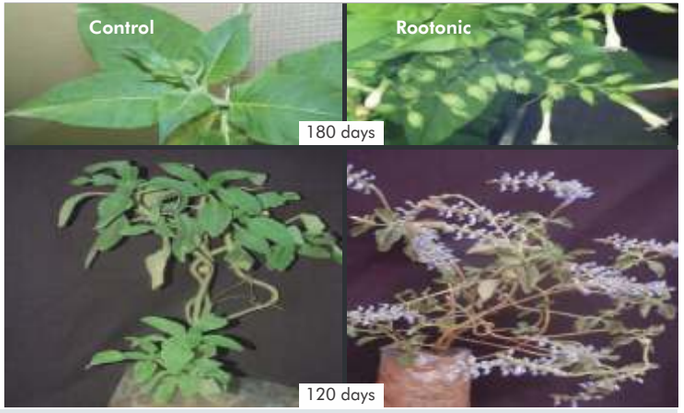


Fig. 28: Co-cultivation of tobacco and *Coleus* with Rootonic

Fig. 29: Co-cultivation of sponge gourd and cotton

Fig. 30: Interaction with *Brassica napus*. Note: Treated plants produced early pods and seed settings

Rootonic acts as a Bioprotectant

The fungus has suppressed the growth of a large number of pathogens like *Geaumannomyces graminis, Alternaria* sp., *Colletotrichum falcatum, Fusarium oxysporum, F. udum, Rhizoctonia bataticola, R. solani, Sclerotinum rolfsii, Verticillium* sp and many more.

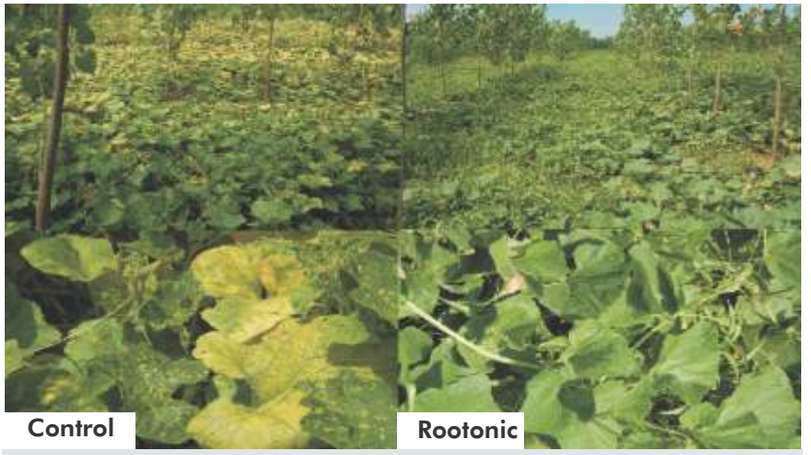


Fig. 31: The field trial on bottle gourd has greatly suppressed the infestation by plant pathogens including viruses (upper); a magnified view of the same (lower)

Protection against Insects

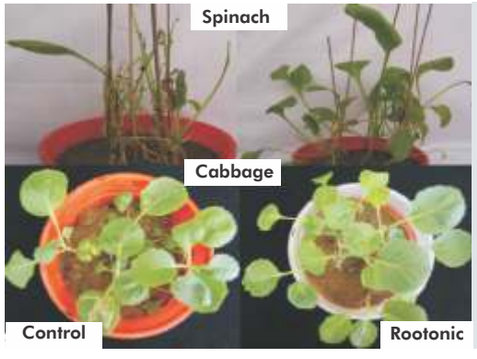


Fig. 32: Fungus treated plants did not receive insect infection

Interaction with Flowering Plants

Fig. 33: Interaction with Marigold
Note: Control plant with folded leaf due to mite infestation; no such symptoms were observed on treated plants

Challenges for the Cultivation of Orchids

Fig. 34: Seeds infested by fungus (upper); infested seedlings attained mature growth and produced flowers (lower)

Cultivation of orchids is an expensive proposition. They produce million of tiny seeds, characteristically, 99% do not germinate, transforming into fully grown and mature plants unless they establish contact with mycorrhiza like fungus.

Interaction with Energy Plants

Rootonic interacts with energy plants like *Jatropha* and *Populus*. The positive influence is observed (Figs. 34-36).

Jatropha seeds obtained from AFRI (Jodhpur)

Photographed after 14 days

Fig. 35: Interaction of *Jatropha* showed early seed germination

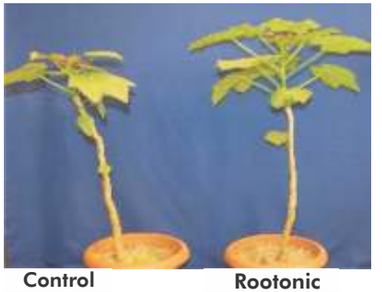


Fig. 36: Fungus treated plants had shown better growth in *Jatropha*

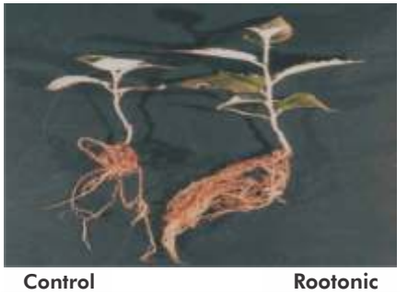


Fig. 37: Co-cultivation with *Populus*, showed positive influence

Culture Filtrate as the Liquid Biofertilizer

One of the unique features of the fungus is that the culture filtrate also acts as an excellent source for plant promotion. In an independent experiment, the fungus was grown in broth. After 10 days the biomass was removed. The culture filtrate caused early seed germination and flowering (Figs. 38-42).

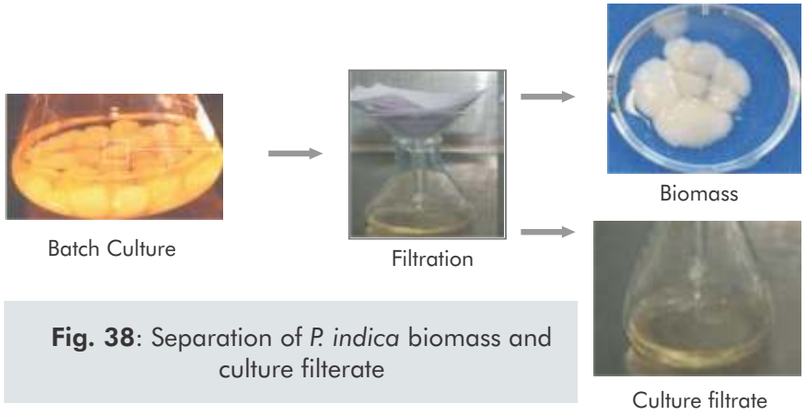


Fig. 38: Separation of *P. indica* biomass and culture filterate

The fungus biomass secretes some unknown chemicals which stimulated early seeds germination, better plant growth and early flowering. They also alter the pathway to improve the secondary metabolites. Obviously, a promising liquid biofertilizer.

Culture Filtrate Impact on Seed Germination

Fig. 39: Interaction of *Phaseolus vulgaris* (rajma) with *P. indica*

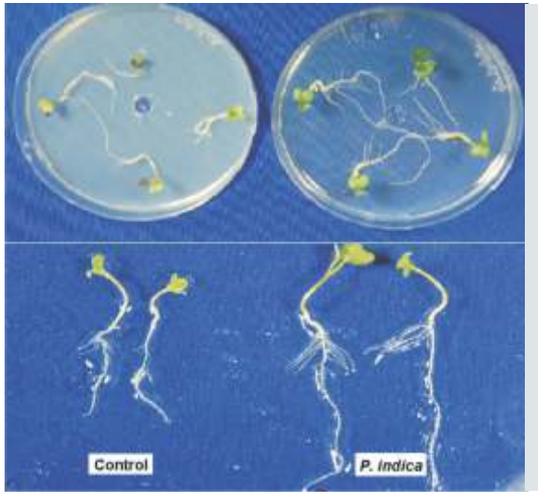


Fig. 40: Co-cultivation of *Brassica oleracea* (broccoli) with *P. indica*

Plant Growth

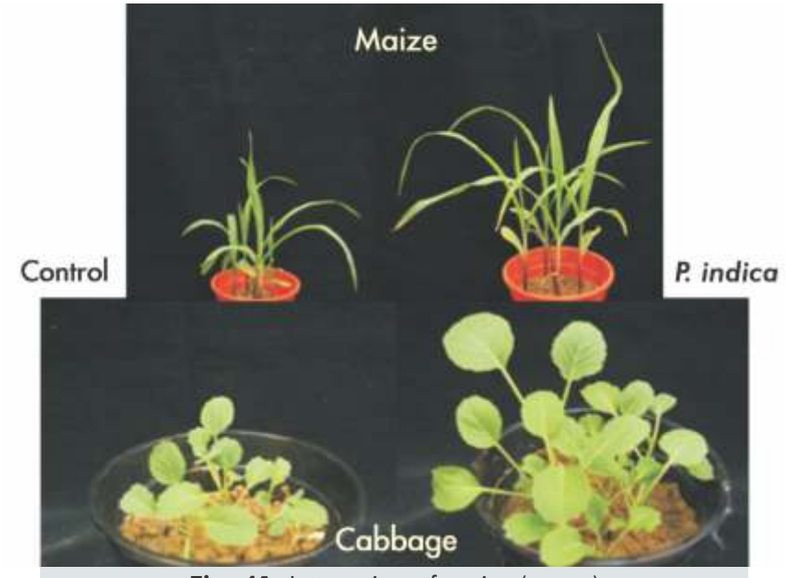


Fig. 41: Interaction of maize (upper) and cabbage (lower) with *P. indica*

Fig. 42: Interaction of sunflower with *P. indica*

Unique Features

The scientists from Amity University had conducted field trials in north, central India and in extreme cold deserts like Leh-Ladakh. The performance was significantly high in Rootonic treatments. *P. indica* cultures were mixed with frozen Leh soil (temperature -30°C). All the fifteen Varieties of seeds germinated within 12-25 days at such extreme low temperature (Table 5). In contrast not a single seed broke the dormancy in absence of the fungus at this low temperature. Uninoculated seeds germinated at the later date when temperature was high. The germinated seedlings were transferred to macro-plots. Treated seedlings attained the full growth and imparted better productivity than the controls (Fig. 44). Fungus propagules did not survive in harsh cold conditions for the next seasons.

Fig. 43: Germinated seedlings of *Cichorium endivia*

Fig. 44: Seedlings germinated at low temperature transferred to macronutrients had matured faster than those receiving the fungus (upper untreated control; lower Rootonic treated)

Table 5: Leh-Ladakh natural frozen soils were mixed with fungus. The day temperature was 4°C and night temperature was -18°C. Seeds receiving fungus germinated even at such low temperature

Note: The fungus was isolated from arid desert of Rajasthan (temperature +40°C). It is very unique microorganism that works on extreme climatic conditions

Hosts	Days after	% Increase
Cabbage	25	100
Endive	25	100
Swisschord (Palak)	25	100
Swisschord (Red)	25	100
Radish – 100% (25 days)	25	100
Onion	25	100
Carrot	21	84
Cauliflower	21	84
Beetroot	20	80
Peas	15	60
Snowpea	12	48

The Secret of Plant Promotion,Value Addition and Early Flowering!!!

Fungus interactions are characterized by a more efficient nutrient uptake from the soil due to a better hyphal penetration into the soil compared to the penetration of the thicker root hairs. The plant delivers phosphor-assimilates to the fungus. Mycorrhizal associated, plants acquire phosphate from the extensive network of fine extra radical hyphae of fungus, which extend beyond root depletion zones to mine new regions of the soil. Several studies have indicated that the interaction alters the pathway for nitrogen metabolism whereby transferring more nitrogen nutrients to the plants. Preliminary studies indicate that *P. indica* influences the sulphate reduction leading to formation of sulphur proteins and glutathione contents. This in turn, influences the resistance against water deficiency and drought. The supply of the fungus with carbon (C) sources, and the faster growth of colonized plants require the breakdown of starch which is deposited in the root amyloplasts. Thus, it is not surprising that one of the major starch degrading enzymes, the glucan-water dikinase is activated by the fungus.

The fungus regulates the uptake and transportation of important macronutrients like iron, zinc, manganese, copper etc. Interaction of plants results in synthesis of important phytohormones.

The cumulative effect of macro-micronutrients and phytohormones influences the plant metabolism-the value addition, early flowering and plant growth. Massive proliferation of useful rhizospheric microorganisms sustains soil fertility (Organic Farming).

Step forward towards Commercialization

To enhance the usage so that the benefits of the fungus are used by common farmer, it was formulated with magnesium sulphite which acts as a carrier. For this, 2% (w/w) formulation served as effective and stable carrier. On an average the colony forming unit (CFU) count was maintained as 10^9 and moisture 20%. The protocol for formulation is given in Fig. 45.

Fig. 45: Steps for the preparation of formulation

Protocol for Seed Treatment

Fig. 46: Protocol for seed treatment

Table 6: Quantity of formulation required for the seed treatment

Crops	Seed Treatment (gm/acre)
Tomato, Chillies, Brinjal, Capsicum, Cabbage, Cauliflower	25.00
Muskmelon, Watermelon, Long Melon, Cucumber, Bottle Gourd, Bitter Gourd, Sponge Gourd, Round Melon	50.00
Sunflower, Ladies Finger, Onion, Spinach, Fenugreek, Mustard, Cotton	100.00
Maize, Paddy and Millets (Barley, Pearlmillet, Sorghum)	300.00
Pulses (Gram, Pea, Lentil, Mungbean, Uradbean, Pigeonpea, Cowpea, Soyabean)	500.00
Potato, Wheat, Sugarcane	1,000.00

1000 grams = 1kilogram

Important Books

- Varma A, Abbott L, Werner D and Hampp R (2008). Plant Surface Microbiology. Springer-Verlag, Germany (Revised).
- Varma A (2008). Mycorrhiza. Third Edition, Springer-Verlag, Germany.
- Ajit Varma and Amit C Kharkwal (2009). Symbiotic Fungi: Principles and Practice. Springer-Verlag, Germany.
- Ralf Oelmüller, Gerhard Kost and Ajit Varma (2013). *Pirifomospora indica*. Springer-Verlag, Germany.
- Asun Morte and Ajit Varma (2014) Root Engineering. Springer-Verlag, Germany.
- Dilfuza Egamberdieva, Smriti Shrivastava, Ajit Varma (2014). PGPR and Medicinal Plants Springer-Verlag, Germany).

Important Publications

1. Bagde US, Prasad R and Varma A (2011). Influence of culture filtrate of *Piriformospora indica* on growth and yield of seed oil in *Helianthus annus*. Symbiosis **53**: 83–88.
2. Bagde US, Prasad Ram and Varma A (2010). Characterization of culture filtrates of *Piriformospora indica*. Asian Journal of Microbial Biotech. Env. Sc. **12** : 805 – 809.
3. Bagde US, Prasad Ram and Varma A (2010). Mass cultivation of *Piriformospora indica* in New Brunswick Fermenter and its formulation as biofertilizer. Asian Journal of Microbial Biotech. Env. Sc. **12**: 911 – 916.
4. Camehl Iris, Sherameti Irena, Venus Y, Bethke G, Varma A, Lee Justin and Oelmueller R (2010). Ethylene signalling and ethylene targeted transcription factors are required for balancing beneficial and non-benificial traits in the symbiosis between the endophytic fungus *Piriformospora indica* and *Arabidopsis thaliana*. New Phytologists DOI: 10.1111/j.1469-8137.2009. 03149.x (added doi).
5. Das Aparajita, Kamal Shwet, Shakil Najam Akhtar, Sherameti Irena, Oelmüller Ralf, Dua Meenakshi, Tuteja Narendra, Johri Atul Kumar and Varma Ajit (2012) 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. **7**: 103-112.
6. Dolatabadi Hossein Kari, Goltapeh Ebrahim Mohammadi, Moieni Ahmad, Jaimand Kamkar, Sardrood Babak Pakdaman and Varma Ajit (2011) Effect of *Piriformospora indica* and *Sebacina vermifera* on plant growth and essential oil yield in

Thymus vulgaris in vitro and *in vivo* experiments. Symbiosis 53:29-35 DOI 10.1007/s13199-010-0104-0.

7. Dolatabadi Hossein Kari, Goltapeh Ebrahim Mohammadi, Jaimand Kamkar, Rohani Neda and Varma Ajit (2011) Effects of *Piriformospora indica* and *Sebacina vermifera* on growth and yield of essential oil in fennel *(Foeniculum vulgare)* under greenhouse conditions. Journal of Basic Microbiology, **51**, 33–39.
8. Dolatabadi KH, Goltapeh EM, Varma A, and Rohani N (2011) In vitro evaluation of arbuscular mycorrhizal-like fungi and *Trichoderma* species against soil borne pathogens. An International Journal of Agricultural Technology **7**: 73-84.
9. Ghahfarokhy MR, Goltapeh EM, Purjam E, Pakdaman BS, SAM Modarres Sanavy and Varma A (2011). Potential of mycorrhiza-like fungi and Trichoderma species in biocontrol of take-all disease of wheat under greenhouse condition. An International Journal of Agricultural Technology **7**: 185-195.
10. Jogawat A, Saha S, Dayaman V, Kumar M, Bakshi M, Dua M, VArma A, Oelmuller R and Johri AK (2013). Piriformopsora indica rescues growthdiminution of rice seddlings during high salt stress. Plant Signalling Behaviour (in press).
11. Kaldorf M, Koch B, Rexer K-H, Kost G and Varma A (2005). Patterns of Interaction between *Populus* Esch5 and *Piriformospora indica*: A Transition from mutualism to antagonism. Plant Biology, Germany. **7**: 210-218.
12. Oelmüller R, Peškan-Berghöfer T, Shahollari B, Trebicka A, Sherameti Irena and 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. Physiologia Plantarum **124**:152-166.

13. Oelmüller R, Sherameti I, Tripathi S and Varma A (2009). *Piriformospora indica*, a cultivable root endophyte with multiple biotechnological applications. Symbiosis **19**: 1-19.
14. Peskan-Berghoefer T, Shahollari B, Giang P.H., Hehl S, Markent C, Blank V, Kost G, Varma A and 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 endocytoplasmic reticulum and in the plasma membrane. Physiologia Plantarum **122**: 465-471.
15. Pham G H, Kumari R, Singh An, Sachdev M, Prasad R, Kaldorf M, Buscot F, Oelmuller R, Tatjana P, Weiss M, Hampp R and Varma A (2004). Axenic Cultures of *Piriformospora indica*. In: Plant Surface Microbiology (eds Varma A, Abbott L, Werner D and Hampp R), Springer-Verlag, Germany pp. 593-616.
16. Prasad R, Bagde US, Pushpangadan P, Varma Ajit (2008). *Bacopa monniera* L: Pharmacological aspects and case study involving *Piriformospora indica*. International Journal of Integrative Biology, Singapore 3: 100-110 (Available online: http://www.classicrus.com /IJIB/Arch/2008/1074.pdf).
17. Prasad R, Kamal S, Sharma PK, Oelmueller R and Varma A (2013) Root endophyte *Piriformospora indica* DSM 11827 alters plants morphology, enhances biomass and antioxidant activity of medicinal plant *Bacopa monniera*. Journal of Basic Microbiology (Accepted).
18. Prasad R, Pham GH, Kumari R, Singh A, Yadav V, Sachdev M, PeskanT, Hehl S, Oelmuller R, Garg AP and Varma A (2005) Sebacinaceae: culturable mycorrhiza–like endosymbiotic fungi and their interaction with non-transformed and transformed roots. In: *In vitro* Culture of Mycorrhizas (ed. Declerck S, Strullu DG, Fortin A) Soil Biology Series, Springer-Verlag, Germany 4: 291-312.

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

20. Shahollari B, Varma A and Oelmüller R (2007). A leucine rich repeat protein is required for growth promotion and enhanced seed production mediated by the endophytic fungus *Piriformospora indica* in *Arabidopsis thaliana*. Plant Journal, USA.

21. Sherameti I, Shahollari B, Venus Y, Altschmied L, Varma A and Oelmüller R (2005). 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 which binds to a conserved motif in their promoters. Journal of Biological Chemistry. **280**:26241-7.

22. VM, Nitz I, Varma A, Grundler FM, Oelmüller R (2008). PYK10, a ß-glucosidase located in the } endoplasmatic reticulum, is crucial for the beneficial interaction between *Arabidopsis thaliana* and the endophytic fungus *Piriformospora indica*. The Plant Journal doi: 10.1111/j.1365-313X.2008.03424.

23. Sherameti Irena, Tripathi Swati, Varma Ajit and Oelmüler Ralf (2008). The root-colonizing endophyte *Pirifomospora indica* confers drought tolerance in *Arabidopsis* by stimulating the expression of drought stress – related genes in leaves. Mol Plant – Microbe Interaction **21**: 799 – 807.

24. Suman, Prasad R, Jain VK and Varma A (2010). Role of nanomaterials in symbiotic fungus growth enhancement. Current Science **99**: 1089-1091.

25. Vadassery Jyothilakshmi, Ritter Claudia, Venus Yvonne, Camehl Iris, Varma Ajit, Shahollari Bationa, Novák Ondřej, Strnad Miroslav, Ludwig-Müller Jutta and Oelmüller Ralf (2008). The Role of Auxins and Cytokinins in the Mutualistic Interaction Between Arabidopsis and *Piriformospora indica* Molecular Plant Microbe Interaction 21(10): 1371–1383.

26. Vadassery Jyothilakshmi, Tripathi Swati, Prasad Ram, Varma Ajit and Oelmüller Ralf (2009). Monodehydroascorbate reductase 2 and dehydroascorbate reductase 5 are crucial for a mutualistic interaction between *Piriformospora indica* and *Arabidopsis* J Plant Physiol **165**:60–70.

27. Varma A, Bakshi Madhunita, Lou Binggan, Hartmann Anton and Oelmüller R (2012). Functions of novel symbiotic fungus- *Piriformospora indica*. Agricultural Research NAAS **1**: 117-131.

28. Varma A, Kharkwal A, Bains KS, Agarwal Aparna, Bajaj R and Prasad R (2012). *Piriformospora indica*: The model microbe for organic green revolution. Biofertilizer Newsletter **20**(1): 3-8.

29. 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, Sherameti I, Chen Y, Ma J, Lou B, and Oelmuller R (2012). The Symbiotic Fungus *Piriformospora indica*: Review. In: The Mycota XXL, Hock B (ed), Springer, Berlin Heidelberg New York, pp. 231-254.

30. Manisha Sharma, Preeti Panthari, Palpu Pushpangadan, Ajit Varma and Harsha Kharkwal (2014) Phytochemical analysis of Glycosides from leaves of Triogonella foenum graecum. International Journal of Pharmaceutical Sciences Review and Research [In Press]

31. Katare DP, Kharkwal AC and Sharma P (2014) Effect of ZnSO4 and CuSO4 on regeneration and xanthotoxin content in *Ammi majus* and *Agrobacterium* mediated transformation. World Journal of Pharmacy and Pharmaceutical Sciences 3 (10)

32. Sharma P, Kharkwal AC, Kharkwal H, Abdin MZ and Varma A (2014) A review on pharmacological properties of *Aloe vera*. International Journal of Pharmaceutical Sciences Review and Research. 29 (1) [In press]

33. Zarea MJ, Hajinia S, Karimi N, Goltapeh EM, Rejali F and Varma A (2012). Effect of *Piriformospora indica* and *Azospirillum* strains from saline or non-saline soil on mitigation of the effects of NaCl. Soil Biology & Biochemistry. doi:10.1016/j.soilbio.2011.11.006. 45: 139–146.

34. Komal Gupta, Shashank Gerg, Joginder Singh and Manoj Kumar (2014). Enhanced Production of Napthoquinone metabolite (Shikonin) from cell suspension culture of *Arnebia* sp. and its up-scaling through Bioreactor. 3 Biotech, Springer, 4:263-273.

35. Joginder Singh, Manoj Kumar and Anil Vyas (2014). Healthy Response from Chromium survived Pteridophytic Plant-*Ampelopteris prolifera* with interaction of Mycorrhizal fungus *Glomus deserticola*. International Journal of Phytoremediation; Taylor and Francis; V 16 (5); 524-535.

Important Patents

- Ajit Varma, P. Franken (1998). The properties of the fungus, Piriformospora indica (97121440.8-2015).
- Ajit Varma, V.K Jain, Suman Ram Prasad (2009). A Nanomaterial Based Culture Medium for Microbial Growth Enhancement (14/DEL/2003).
- Harsha Kharkwal (2009). Binding agents from Cassia Species (676/DEL/2009).
- Geetanjali Chauhan, H. Kharkwal, Manisha Sharma, Ajit Varma, Harsha Kharkwal (2009). Saponin Derivatives (676/DEL/2009).
- Harsha Kharkwal (2009). A formulation from emulsifying agent from native and derivatized grandis gum (812/DEL/2009).
- Amit Kharkwal, A Das, A Varma (2009). A modified medium for co-cultivation of sebacinales fungi with plants of lamiaceae family (902/DEL/2009).
- Manisha Sharma, Harsa, Geetanjali, Ajit Varma (2009). Polygalactomannans and Modified Polygalactomannans from Fenugreek and their uses thereof (1469/DEL/2009).
- Harsha Kharkwal (2009). Suspending agent from cassia species (1051/DEL/2009).
- B.K Behera (2009). Invitro immobilized bacillus megaterium cell for the production of demethylated colchicosides from cochicines and process thereof (1216/DEL/2009).
- Ram Prasad, Jyoti Pande, Ajit Varma (2009). Nano material, their method of production and application thereof. (1612/DEL/2009).
- Ajit Varma, Shashibala Singh, R.B. Srivastava, Madhunita Bakshi, Pal Murugan (2011). Piriformospora indicaobviates extreme temperatures stresses (709/DEL/2011).
- Deepshikha Pande Katare and Amit Chandra Kharkwal (2011). An efficient method of fungal elicitation of callus cultures of Lepidium sativum for enhanced production of lepidine (3251/DEL/2011).
- Monika Arora, Amit Chandra Kharkwal, K.S. Bains and Ajit Varma (2012). Novel and new nutrient for cultivation of Piriformospora indica (944/DEL/2012).
- Harsha Kharkwal, Kumud Bala, Deepshikha Pande Katare, Devi Datt Joshi, Amit Chandra Kharkwal (2012). Polymer based gelatin free capsules (1031/DEL/2012).
- Amit C Kharkwal (2012). Novel Polysaccharide Ethers (2026/DEL/2012).
- Amit C Kharkwal (2012). Carbohydrate based Biodegradable and Hydrobiodegradable Plastics (2140/DEL/2012).
- Ajit Varma (2012). Synthesis of natural saponin through co-cultivation of microorganisms (2491/DEL/2012).
- Preeti Panthari, Harsha Kharkwal, Hina Sanwal, Amit C Kharkwal, Ram Prasad, Devi Datt Joshi (2012). A novel composition for the preparation of herbal tea (2646/DEL/2012).
- Harsha Kharkwal, Devi Datt Joshi, Amit Chandra Kharkwal and Ram Prasad (2012). Thickness agents for food, paint and textile industry(2800/DEL/2012).
- Monika Arora, Malik Zainul Abdin and Ajit Varma (2013). Plant growth promoting root endophyte (2017/DEL/2013) Provisional.
- Monika Arora, Malik Zainul Abdin and Ajit Varma 2014. Plant growth promoting formulation of Piriformospora indica and Azotobacter chroococcum with talcum powder (PCT/IN2014/000447).

Letters from State Government

12AKV(AS) ①

डॉ आर बी श्रीवास्तव
निदेशक,
वैज्ञानिक 'जी'
Dr. R. B. SRIVASTAVA
DIRECTOR,
SCIENTIST 'G'

रक्षा उच्च तुंगता अनुसंधान संस्थान
रक्षा अनुसंधान तथा विकास संगठन
रक्षा मंत्रालय, भारत सरकार
पिन: 901205, द्वारा 56 सेना डाकघर

Defence Institute of High Altitude Research (DIHAR)
Defence Research and Development Organisation
Ministry of Defence, Government of India
Pin : 901 205, C/o 56 APO

दिनांक/Dated 27 Sep 2011

120/1/ARU/DIHAR

To Whom So Ever It May Concern

This is to certify that Symbiotic plant promoting Fungus *Pirifomospora indica* developed by Scientists of Amity University, Uttar Pradesh, Noida, U.P. was inoculated for its symbiotic interactions with seeds of 15 vegetables at Defence Institute of High Altitude Research, DRDO Leh-Ladakh during extreme subzero conditions at winter. The experiments were conducted in Polyench green house of the laboratory. The temperature ranged from -18 to +4^{0}C. Within 12-25 days treated seeds were germinated. (Table No. 1) On transferring the seedlings to microplot led to production of large Cabbage and Cauliflower heads, Beet root, Carrot and Turnip roots. (Figs. 1 to 5) In Contrast, untreated seedlings were lagged behind. A microscopic view of fungal hyphae and spores is given in Fig. 6 a, b.

DIHAR is planning to treat millions of vegetable seeds with *Pirifomospora indica* and supply to farmers of Ladakh to increase fresh food availability at Ladakh region. It is an excellent micro-organism for mass production of green vegetables and will promote the Socio-economic conditions of Ladakh region.

Dr. R B Srivastava
Director, DIHAR

दूरभाष/Telephone (O) 01982-255352, 2475 (A), Res. : 252224, 2482 (A) मोबाइल/Mobile : 9419970177, फैक्स/Fax : 01982-252096
Transit Office : DIHAR Det, Behind 'N' Area Officers Mess, Air Port Road, Chandigarh
ई-मेल/E-mail : dihardrdo@gmail.com

12AKV(AS)

From

The Director of Agriculture, Punjab,
Chandigarh.

To

Dr. K.S. Bains,
Director General
Amity Institute, Noida.

Memo No.357/JDA (Inputs)
Chandigarh, dated: 30-03-2012

SUBJECT: INCLUSION OF FUNGUS MYCORRHIZA IN FERTILIZER CONTROL ORDER, 1985-REG.

Kindly refer to the discussions held in the meeting on 24-02-2012 under the Chairmanship of Hon'ble Chief Minister, Punjab.

During the meeting, it was made clear by Director of Agriculture, Punjab that the fungus Mycorrhiza (PFSI) has not been included in the Fertilizer Control Order, 1985. However, other Mycorrhiza being obligate parasite has been included in the FCO, 1985 recently. Since the fungus isolated i.e. Mycorrahiza (PFSI) is cultured in the laboratory and as yet no standard have been notified by the GoI, therefore, your organization need not to obtain any license under FCO, 1985 in the State of Punjab. This is for your information please.

30/3/12

JDA (Inputs)
for Director Agriculture, Punjab.

DIRECTORATE OF AGRICULTURE
HIMACHAL PRADESH

No.Agr.H(H-Tech)F(7)24/2006. Dated:Shimla-5,the 2 NOV 2011

From

Director of Agriculture
Himachal Pradesh

To

Shri P.Mitra
Addl. Chief Secretary (Agri)
to the Government of Himachal Pradesh, Shimla-2

Dr. K.S.Bains
Director General
Amity Institute, Noida.

Dr. Gurdev Singh
Director of Horticulture
Navbahar, Shimla-2.

Subject: **Minutes of the meeting regarding promotion of Mycorrhiza fungus for plant growth held on 20th October, 2011.**

Sir,

Enclosed kindly find herewith the minutes of the meeting regarding promotion of Mycorrhiza fungus held on 20th October, 2011 under the Chairpersonship of Chief Secretary to the Govt. of Himachal Pradesh for information and necessary action please.

Director of Agriculture
Himachal Pradesh

Endst.No. As above, Dated:Shimla-5,the

Copy forwarded to the Private Secretary to the Chief Secretary, Government of Himachal Pradesh, Shimla-2 for information and necessary action please.

Director of Agriculture
Himachal Pradesh

MINUTES OF MEETING HELD ON 20TH OCTOBER, 2011 AT 1.15 PM UNDER THE CHAIRPERSONSHIP OF CHIEF SECRETARY TO THE GOVERNMENT OF HIMACHAL PRADESH REGARDING PROMOTION OF MYCORRHIZA FUNGUS FOR PLANT GROWTH.

The list of participants is enclosed as Annexure.

At the outset, Chief Secretary recalled the presentation given by Dr. K.S. Bains, Director General, Amity Institute before Hon'ble Chief Minister. Dr. Bains then explained about the performance in trials conducted in respect of Mycrorrhiz (PFSI) at various places including HP. He said that effect of this fungus in crop growth and increased productivity is very good. He also submitted a copy of report given by the National Centre of Organic Farming, Government of India regarding the performance of this product and its use in the grain crops, Horticultural plants, sugarcane and vegetable etc. Dr. Bains wanted to know the administrative requirements of the State for further promotion of this product.

The Director of Agriculture explained that this fungus has not yet been included in the Fertilizer Control Order. Other Mycorrhiza which are obligate parasites have been included. He further explained that Mycorrhiza PFSI can be cultured only in the laboratory and no standards have yet been notified by Government of India for this fungus. Since this product is not covered under the provisions of FCO, therefore, there is no requirement of taking license for its distribution. The Director Agriculture explained to the committee the protocol for getting any product included in the FCO. He suggested that Institute may get this product tested through ICAR/SAUs and on the basis of performance reports and data generated, it can be included in the package of practices of the University and also in the FCO. The Chief Secretary directed the department that clarification be given to the Amity Institute. Dr. Bains informed that they have already appointed Lahaul Potato Growers Society as their promoters and will also appoint more such agencies.

DIRECTORATE OF AGRICULTURE
HIMACHAL PRADESH

No.Agr.H(II-Tech)F(7)24/2006. Dated:Shimla-5,the 2 NOV 2011

From

Director of Agriculture
Himachal Pradesh

To

Dr. K.S.Bains
Director General
Amity Institute, Noida.

Subject: **Inclusion of fungus Mycorrhiza in Fertilizer Control Order- Reg.**

Sir.

Kindly refer to the discussions held in the meeting on 20th October, 2011 under the Chairpersonship of Chief Secretary to the Govt. of Himachal Pradesh.

During the meeting, it was made clear by the Director of Agriculture, Himachal Pradesh that the fungus Mycorrhiza (PFSI) has not been included in the Fertilizer Control Order however other Mycorrhiza being obligate parasites have been included in the Fertilizer Control Order recently. Since the fungus isolated by i.e Mycorrhiza (PFSI) is cultured in the laboratory and as yet no standards have been notified by Govt. of India therefore, your organization need not to obtain license under FCO for sale etc. in the State of Himachal Pradesh. It is for your information please.

Director of Agriculture
Himachal Pradesh

About the Authors

Prof. (Dr.) Ajit Varma
Distinguished Scientist
& Professor of Eminence
AIMT

Born in Allahabad, India on 24th September 1939, Dr. Varma completed M.Sc. (1959) & Ph.D. (1964) degrees from Allahabad University, Allahabad, India. During his professional career, he has also served as Microbiologist (Assistant Professor) IARI, New Delhi (1963-1971), Senior Microbiologist (Associate Professor) IARI, New Delhi (1971-1974), Associate Professor, JNU, New Delhi (1975-1984) and Professor, JNU, New Delhi (1985-2004). Visiting Professorship & Visiting Research Scientist at Technical University, Graz (Austria), University of Tuebingen, Tuebingen (Germany), Friedrich Schiller University, Jena (Germany), Philipps University, Marburg (Germany), Technical University, Muenchen (Germany), Kingstan (Jamaica), Max Planck Visiting Professorship (Germany), HelmholtzZentrum, Muenchen (Germany), Gutenberg University, Mainz (Germany), CSIC, Madrid (Spain), University of Dundee (Scotland), University of Ljubljana (Slovenia), ICGEB (Italy). International Awards/fellowships include Commonwealth Fellowship (Australia), National Research Council (Canada), Alexander von-Humboldt Foundation (Germany), National Science Foundation (USA), Indo-Czechoslovakia Exchange Programme (Prague), DAAD Fellowship (Germany), Deutsches BMFT Programme, George-August University, Gottingen (Germany), RAISA Fellowship for Innovative Research in Biotechnology (Italy), Swiss Federal Research Fellowship (Switzerland), BP Koirala award (Nepal) and DFG-INSA Fellowship (Indo-Germany), FAMI Award - Association of Microbiologists of India and Honorary Diploma, UMF, Cluj-Napoca, Romania. Dr. Varma has guided 54 PhD and 1 D.Sc. students. He has published over 290 research articles, which appeared in National and International, journals of repute and also several major review articles and chapters in books and Published 38 books in the area of Microbial Technology, published by Academic Press, London, CRC Press, Florida, USA, IDRC, Canada and Springer-Verlag, Germany. He has been nominated as Editor-in-Chief by Springer-Verlag (Germany & New York), the leading science-publishing house, to prepare 50 volumes on Soil Biology. He has also been nominated as Editor-in-Chief by IK Internationals to make series of books on Microbial and Biotechnological Research. Membership includes that of National Academy of Agriculture Sciences, International Society of Symbiosis, Boston, USA, Indian Science Congress Association, Executive Council, Amity University Uttar Pradesh, University Research Council, Amity University Uttar Pradesh, Academic Council, Amity University Rajasthan, ASSOCHAM Knowledge Millennium Council, ASSOCHAM Expert Committee on Agriculture and Food Processing, ASSOCHAM Expert Committee on S&T and Innovation, He has a vast experience of organizing national and international training workshop/symposium and congress.

Research Area: The research interest is plant-microbe and microbe-microbe interaction

Dr. Amit C Kharkwal

Dy. Director & Head, AIMT

Over 15 years experience in Research & Analysis with leading research organizations. Currently working as Assistant Professor with Amity University Uttar Pradesh NOIDA.

He did Ph.D. in Botany from Guru Nanak Dev University, Amritsar in the year 2003. While doing Ph.D. Dr. Kharkwal worked at Institute of Himalayan Bioresource Technology, Palampur, Himachal Pradesh as JRF and SRF on CSIR Fellowship from 1997-2002.

Dr. Kharkwal did M.Sc. in Environmental Botany in 1997 from Jamia Hamdard and B.Sc. Life Science from Delhi University in 1995.

Prior to joining Amity group Dr. Kharkwal worked as Research Fellow/Scientist at Reliance Life Sciences, Mumbai from 2002-2005.

Dr. Kharkwal have researched on varied topics in Agricultural Biotechnology including Plant Cell, Tissue and Hairy Root Culture; Transgenic Plant Research, Germplasm Characterization by using Molecular markers and Seed Biology. He is well versed with Plant Tissue Culture, Molecular and Herbarium techniques.

For last 2.5 years Dr. Kharkwal is actively engaged in promoting the Magic Fungus "Rootonic" in Punjab, Himachal Pradesh, Rajasthan and Delhi-NCR with active collaboration of State Governments, Industry, Co-operatives and NGOs.

Apart from all this, Dr. Kharkwal is involved in various in-house and sponsored research projects. The details of the projects are listed below:

- PI for the Project entitled "Phytoremediation of Saline Solis by using a Novel Arbuscular Mycorrhiza Fungus for Value Addition and Growth Promotion of Selected Medicinal Plants" Funded by Ministry of Environment and Forest
- Co-PI for the project entitled "Bioactivity Guided Fractionation of Extracts of Hepatoprotective and Immunomodulatory Medicinal Plants" Funded by National Medicinal Plant Board
- PI for the project entitled "Awareness and Ex-situ Conservation through establishing Demo Medicinal Gardens, distribution of MAPs & Extension activities" Funded by National Medicinal Plant Board
- Co-PI for the project entitled "Commercialisation of a novel biofertiliser" Funded by AUUP (in – house)

Dr. Ram Prasad

Assistant Professor, AIMT

Dr. Ram Prasad is presently working as Assistant Professor, Amity Institute of Microbial Technology, Amity University Uttar Pradesh. He has completed his Ph.D. (Microbiology, 2008) from Department of Microbiology, CCS University-Meerut for which he did the experimental work in School of Life Sciences, Jawaharlal Nehru University (JNU), New Delhi. He did his M. Sc. in Life Sciences (2002) from School of Life Sciences, JNU, New Delhi and has qualified CSIR-NET, ASRB-NET and GATE. He has fifty one publications to his credit which includes research papers and book chapters published in international and national level. He has also filed five patents. Dr Prasad has ten years of research and seven years of teaching experience. He was awarded Young Scientist Award (2007) during International Society for Ecological Communications during International Symposium & Prof. J.S. Datta Munshi Gold Medal (2009). He has also been awarded FSAB fellowship (2010) by Society for Applied Biotechnology in the field of Microbial Biotechnology. Recently he has been nominated as an editor in Academia Journal of Biotechnology. Currently, he is a Post Doctoral Fellow at John Hopkins, USA for one year.

Research Area

In view of multifarious plant growth promoting activities of the model fungus *Piriformospora indica*, it is the need of time to understand the functions and the principles behind the activities so as to exploit this endophytic symbiotic fungus more efficiently for the betterment of humanity, food and nutritional securities and medical applications. The majority of the properties of this fungus are known but even then the principle of the activities has to be studied in order to efficiently use to serve the humanity.

P. indica serves as model fungus to understand the mechanism of molecular aspects of pre-symbiotic and symbiotic phases and their biotechnological applications under the basis of microbe-microbe and plant-microbe interactions.

Recent experiments are underway to understand the influence of nano-particles viz., carbon nano tube (CNT), Ag-nano and TiO_2 on *Piriformospora indica* and biosynthesis & characterization of nanomaterials from different plants and microbes.

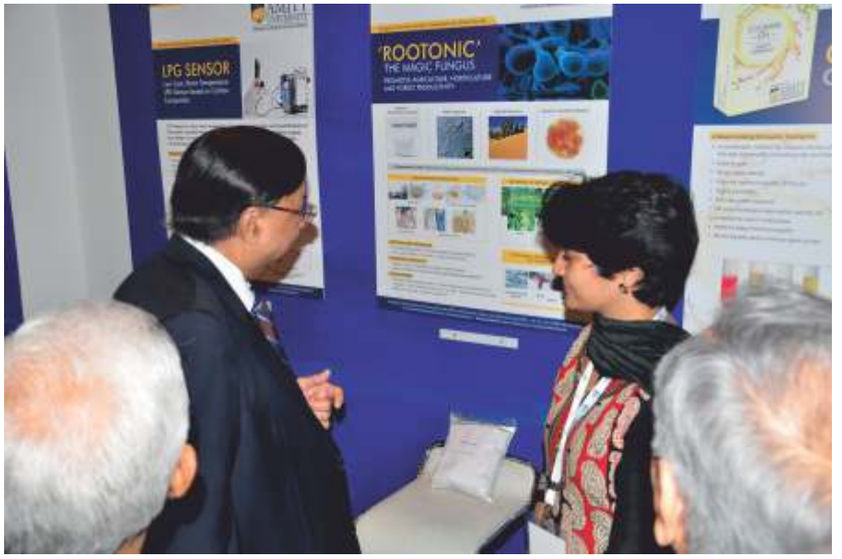
He is a Co-Principle Investigator for the project entitled "Studies on Enhancement of Soil Genesis and Fertility by Microbes in Degraded Land in Semi Arid and Middle Hill Conditions" Funded by Defence Research and Development Organisation (DRDO), India.

Ruchika Bajaj is a Research Assistant in Amity Institute of Microbial Technology working on commercialization of Piriformospora indica. She is currently pursuing Ph.D. from Amity University. She has completed M.Sc. Microbial Sciences from Amity University (2009-2011) and B.Sc. (Microbiology, Biotechnology and Chemistry) (2006-2009) from Rashtrasant Tukdoji Maharaj Nagpur University. She has 3 research papers and 12 abstracts to her credit. Her areas of interest are Plant Tissue Culture and Plant Microbe Interaction.

Aparna Agarwal has completed her B.Sc in Biotechnology (2006) from Ruhelkhand University (U.P) and M.Sc. in Microbial Sciences (2009) from Amity Institute of Microbial Technology (U.P.). Currently she is working as scientific assistant in AIMT in the area of Plant-Microbe Interaction and is pursuing Ph.D. program with on the topic "Performance of *Piriformospora indica* on selected plants of economic importance". She has 2 research papers and 1 book chapter to her credit.

Kartikeya Rajpal has been working at Amity Institute of Microbial Technology in the capacity of Research Assistant. He is working on the commercialization of *Piriformospora indica*. He has done M.Sc. in Biotechnology from Amity University, Noida (2008-2010) and B.Sc. in Biotechnology from Devi Ahilya Vishwavidyalaya, Indore (2005-2008). He had a short work stint at Associated Alcohols & Breweries Ltd. He has qualified GATE 2011. Currently he is pursuing his Ph.D. from Amity University. He has 1 research paper and 1 book chapter to his credit.

Dr. Aparajita Das is a Research Associate at Amity Institute of Microbial Technology (AIMT), Amity University UP Noida. She graduated in Botany (Hons.) in 1994 and completed her post graduation in Botany in 1996 from Delhi University. She has qualified CSIR NET in Life Sciences and has obtained her B.Ed from Meerut University. She has received her Ph.D from Amity University, UP, Noida in 2010. Previously, she was actively involved in research work in the field of plant tissue culture, invitro conservation and cryopreservation for about seven years in National Bureau of Plant Genetic Resources (ICAR), New Delhi in various projects. She has twenty one publications in research journals and several books related to Plant tissue culture and Microbiology. Recently she has received a Research proposal funded under SERB Fast Track Proposals for Young Scientist in the field of plant microbe interactions. She is life member of Association of Microbiologists of India.



Dr. Ashok K Chauhan, Founder President, RBEF interacting at
FICCI Global R&D Summit held at Hotel Ashok, Delhi on July 25-26, 2013