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Editorial

Dear Readers

Famous scientist Louis Pasteur had said that even the death will be incomplete without the presence of fungi. So is the importance of the fungi in human life. The Microorganisms have unbreakable linkage parallel to human life, with fungi even more prominent in viable-microbial processes associated with human. In agriculture also its importance is in tune with the thoughts of Louis Pasteur. It has an extended role in nutrient cycle and their availability to the plants. From solubilization to mobilization, absorption to transportation and itself being the source of nutrient, fungi has multifarious role in nutrition transmission between soil and plants. A number of mechanisms involving the role of fungi have been explained by scientists. Yet there are many ones to be explained to harvest its desired effect to meet the burgeoning requirements. A number of genus and species are still to be identified which certainly contribute in nutrient management but has no recognition. This issue in first paper reports new invention in mycorrhizal developments. Hope this pragmatic approach would help in efficient use of mycorrhizal cultures to obtain better yields and also would explore new dimensions in ongoing quests.

Certain microorganisms may become antagonistic to fellow microbes limiting their population in a given medium. Therefore assessment of the behavior of composite microbes in a given medium has remained an interesting subject. In this series the microorganisms of nutrient importance have a great scope of their mapping in various forms and conditions in both isolated and coexisting situations to obtain the target results. In the second paper of the issue authors have shown the way for effective use of microbes in combination. It is high time, the manufacturing sector adopt and promote such technologies so that their benefit can be reaped by the end users.

The permanent columns and features of the issue are accomplished with maximum latest inputs of relevant field. Hope the issue turns useful to its readers to their maximum satisfactions. We pay sincere thanks to authors of papers and valued readers for their continued interest attached with us.

Ravinder Kumar
Editor and Assistant Director
RCOF, Bangaluru

Piriformospora indica: The Model Microbe for Organic Green Revolution

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Introduction

The most complex area within the soil environment is the region of soil surrounding a plant root known as the "rhizosphere" or more appropriately "mycorrhizosphere". The region differs from the surrounding soil in many physico-chemical factors and this soil along with the rhizoplane (i.e., the root surface) is colonized by a wide range of microorganisms representing the site with the highest microbial biomass and activity. It is here that interaction between plants and microorganisms are most intense, variable and mostly symbiotic. Nitrogen fixing root-nodule bacteria (*Rhizobium*), actinomycetes (*Frankia*) and mycorrhizal associations are some of the best studied examples of symbiosis.

The Background

Mycorrhiza refers to associations or symbioses between plants and fungi that colonizes the cortical tissue of roots during periods of active plant growth. Generally, these symbioses are often characterized by bi-directional exchange of plant-produced carbon (photosynthates) to the fungus and fungal-acquired nutrients to the plant thereby providing a critical linkage between the plant root and soil called arbuscules (Fig.1A, see inside of back cover, p-25). The term mycorrhiza (modern Latin of Greek words *mykes* + fungi, *rhiza* + root) which literally means "fungus-root" was first applied to fungus-tree associations described in 1885 by the German forest pathologist A.B. Frank (Fig. 1B see inside of back cover, p-25). About 90 per cent land plants, no matter where they grow are mycorrhized. The benefits accorded to the plants from mycorrhizal symbioses can be characterized agronomically by increased growth, yield, providing regulation against biotic and abiotic stress and ecologically by improved fitness (i.e., reproductive ability).

Fig. 2 (inside of back cover, p-25) shows typical view of diverse mycorrhizal spores and they survive in the soil many years under adverse conditions. The key functions of AM symbiosis can be summarized as follows: (1) improving rooting and plant establishment, (2) enhancing plant tolerance to stresses, (3) improving nutrient cycling, and (4) enhancing plant community diversity. Besides, they are helpful in increased photosynthesis efficiency, increased water conducting capacity, enhanced nutrient uptake, enhanced plant tolerance to environmental stresses including drought, cold, salinity and pollution, providing protection from harmful soil borne pathogens, changing the supply of mineral nutrients from soil thereby modifying soil fertility, mycorrhizosphere and aggregation of soil particles and in promoting growth, fitness and conservation of endangered plants.

Despite numerous researches the fungus cannot be cultured in the laboratory condition in the absence of living root system. Arbuscular mycorrhizal fungi (AMF) are obligate symbionts and do not grow in the absence of living host. Despite the significant importance of the AMF to soil health and plant productivity, their biotechnological applications could not be exploited to the level they deserve due to this particular factor.

A Silver Lining in Mycorrhizal Research-Novel Discovery

Authors have discovered a new and novel cultivable mycorrhizal fungus and named it as *Piriformospora indica* (Fig 3 see inside of back cover, p-25). It is cultivable on synthetic media and is suitable for large scale multiplication and conversion into a commercial agro-biological product. Till date authors have found positive impact of the fungus on the diverse group of plants, including agriculture, horticulture, arboriculture, floriculture crops including medicinal plants. Experiments were performed in tissue culture laboratory, green house, under field trials in the cultivator fields in National Capital Region (NCR), Haryana, Himachal Pradesh and Uttarakhand.

Biotechnological Applications

Mutualistic interactions between microbes and agro-forestry, horticultural and medicinal plants have been attractive for many years, since mutualists can improve the growth, biomass and seed production on poor soil with little input of chemical fertilizers and pesticides. *P. indica* is strong candidates for supporting the mass scale production of cereals, medicinal plants and flori-horticulture. They also combat virus and fungal pathogens.

Case Studies

I. Economic Importance

Sugar Cane: *P. indica,* increased the survival rate of micro-propagated sugarcane plantlets when inoculated at hardening stage (Fig. 4, see inside of back cover p-25). *P. indica* increased root and shoot dry weight of the plants by increasing water and nutrient uptake thereby making them healthier and hence increased their survival rate in the glasshouse and field. The increase in survival rates by 10% is of much significance and thus *P. indica* can be commercially exploited for bio-hardening of micro-propagated sugarcane plantlets to boost the sugarcane industry.

Sugarcane variety CoJ 83 and CoJ 88 were treated with *P. indica.* There was significant increase in tiller number (36%), cane number (51%), cane height (13%) and cane yield (10%) as compared to control. In the second crop (ratoon crop), iron deficiency was observed in the untreated plants. The treated plants also exhibited increased iron (39%) and copper (120%) acquisition (Table 1). Significantly higher tiller numbers (85%), cane numbers (96%), cane yield (13%) and sugar content (17%) was also observed (Table 2).

Table 1. Effect of *P. indica* on nutrient acquisition of sugarcane ratoon crop

Treatments	Fe (ppm)	Mn ppm)	Cu (ppm)	Zn (ppm)	K (%)	P (%)
Control	202.2	25.0	4.9	1.87	0.24	0.086
P. indica	281.4	30.2	10.8	1.31	0.40	0.088
CD (5%)	47.07	NS	0.88	0.33	NS	NS

Table 2. Effect of *P. indica* inoculation on yield attributing characters and yield of sugarcane ratoon crop

Treatments	Tiller number/ clump	Cane number/ clump	Cane height (cm)	Cane girth (cm)	Sugar content*	Weight per clump (kg)	Weight per plot (kg)
Control	9.27	8.10	179	2.22	18.35	6.50	122.2
P. indica	17.2	15.90	191	2.21	21.40	7.34	138.3
CD (5%)	2.59	2.51	NS	NS	1.99	NS	2.39

***Brix Value**

Zea mays: The experiments were conducted to study the role of *Piriformospora indica* in maize (*Zea mays* L) var PMH-1. The results suggest that inoculation of *P. indica* works efficiently especially in P-deficient soil and helps in P acquisition in P-deficient soils. It may be used as a potential biofertilizer in the areas where the soil is either phosphorus deficient or the areas where the practice of under application of phosphorus fertilizer is prevalent among the resource poor and marginal farmers.

P. indica **Accelerates the Early Profuse Flowering** in a large number of plants of economic importance. Typical examples in Tobacco, Brassica and the medicinal plant Coleus (see Fig. 5a, b and c):

Piriformospora indica **a Boon for Pharmaceutical Industries**

Authors have found *P. indica*'s intense interaction with a large number of medicinal plants like *Bacopa monniera* (Brahmi), *Artemisia annua* (Sweet wormwood), *Azadirachta indica* (Neem), *Tridex procumbans* (Coat Buttons), *Abrus precatorius* (Rosary Pea), *Coleus forskohlii* (Makandi), *Ocimum sanctum* (Tulsi), *Stevia rebaudiana* (Sweet Leaf), *Chlorophytum borivilianum* (Safed Musli), *Withania somnifera* (Ashwagandha), *Spilanthes calva* and *Adhatoda vasica* increasing their biomass and their active ingredients. Table 3 gives the name of medicinal plant with which *P. indica* has positively interacted.

Table 3. Medicinal plants and its active ingredients

Medicinal Plants	**Common name**	**Active Ingredients**
Bacopa *monniera*	Brahmi	Bacosides
Coleus forskohlii	Makandi and Mayani	Forskolin
Withania somnifera	Ashwagandha, Indian ginseng	Tropine, cuscohygrine, withanolides
Adhatoda vasica	Malabar nut	Vasicine, vasicol
Tridax procumbens	Ghamra , Coat buttons and tridax daisy	
Podophyllum hexandrum	Himalayan May apple	picropodophyllin, podophyllotoxin, quercetin
Spilanthes calva	'toothache plant' or 'virus blocker'	spilanthol
Abrus precatorius	Wild Liquorice	abrin, abraline, choline, precatorine, abricin, abridin
Chlorophytum borivilianum	safed musli	saponins and alkaloids are the source of its aphrodisiac properties
Ocimum sanctum	Holy Basil	tannins, alkaloids and volatile oil (eugenol, ursolic acid, rosmarinic acid, thymol)
Artemisia annua	Sweet Wormwood, Sweet Annie	artemisinin
Stevia rebaudiana	Sweet Leaf, Sugar Leaf,	stevioside and rebaudioside
Linum album	White Flax	podophyllotoxin and 6-methoxypodophyllotoxin
Trigonella foenum graecum	Fenugreek	Saponins (diosgenin, yamogenin, gitogenin, tigogenin)
Curcuma longa	Turmeric	Curcumin
Azadirachta indica	Margosa Tree, Neem	nimbin, nimbinene, azadirachtin, azadirachtol, azadirachnol
Foeniculum vulgare	Fennel	Essential oil (a-pinene, myrcene, fenchone, limonene)
Solanum nigrum	Black Night Shade, Makoy	solanine

Some case studies on medicinal plants

Bacopa monniera: *Bacopa* a well-known memory booster is a classic brain and nervine tonic known as "Brahmi" in Ayurveda that benefits both the mind and improve the intellect and consciousness. The interaction of the fungus *P. indica* with *Bacopa* plant resulted in an unprecedented increase in the plant biomass, photosynthetic pigments, metabolic regulators and active ingredients like antioxidant and bacosides many fold (unpublished data).

***Artemisia annua*:** Artemisinin (an anti-malaria drug) has shown very strong potential as a non-conventional antimalarial drug and recommended by WHO in combination therapies as one of the ingredients for the treatment of drug resistant and cerebral malaria. Several attempts have been made for the selection and breeding of high-yielding strains of *A. annua* plant resulting in significantly increased production of artemisinin (1.1%), but still far behind to meet the demands of market. Hence, to increase artemisinin content in *Artemisia annua* plant the most feasible alternative for the commercial production of this compound is to facilitate interaction of live cells of *Artemisia annua* with symbiotic fungus *P. indica*.

Spilanthes calva: *Spilanthes calva*, commonly known as toothache plant or virus blocker, is well known for enhancing immunity. Because of its high medicinal value, it is costly and there is much demand of this plant in the market. This plant has antiageing properties and cures various diseases of tooth and gums including pyorrhoea. It is antimicrobial in nature and economically very useful as tooth powder, which is prepared from this plant. Its leaves stimulate salivation, which is due to the presence of an active chemical spilanthol. The influence of *P. indica* on the antifungal principle of *S. calva*, was studied. Antifungal efficacy was shown by aqueous and petroleum ether extracts of *S. calva* against *Fusarium oxysporum* and *Trichophyton mentagrophytes*. The petroleum ether extract of *S. calva* was more effective than the aqueous extract in inoculated as well as uninoculated plants. The antifungal activity of the plant was enhanced due to the increase in spilanthol content after inoculation of *P. indica*.

Safed musli: Scientifically known as *Chlorophytum borivilianum*, is endowed with Rasayana (antiageing and immunoboosting), Balya (performance-boosting) and Vrishya (aphrodisiac) properties to keep one young and healthy with a well-tuned body for better handling of stress. Phytochemicals like saponins, carbohydrate and proteins are present in the root. They are normally used to maintain the equilibrium of all the systems of the body and keep the “Body-Mind-Soul Complex” in a state of harmony. Biotization of *Chlorophytum borivilianum* with *P. indica* and *Pseudomonas fluorescens* increased resistance of plants to biotic and abiotic stresses at the time of transplantation thus protect them from ‘transplantation shock’.

Culture filtrate of *P. indica* Promotes Growth

The culture filtrate of this fungus has been treated with various seeds like Bengal gram, rajma, wheat, mustard etc. It has been shown this fungus is capable of enhancing early seed germination, plant growth and yield like biomass.

Authors have conducted large scale field trials in vegetable crops, cereals, sugarcane, horticultural plants etc. in states of Punjab, Himachal Pradesh and Delhi NCR. In general 10-40% increase in plant productivity was observed with concomitant reduction of pesticide usage in the treated plants (Table 4).

P. indica is considered a magic fungus which promotes plant productivity at various agro climatic conditions. At the same time it obviates the pathogenesis.

Table 4. Performance of *Piriformospora indica* Trial on Vegetables and other Crop plants in Punjab.

Crop	Place	Yield in Q/ha		% change	Remarks
Cotton	Muktsar	6	7.2	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
Maize	SAS Nagar	24	28	16.67	On an average a yield of 14.64 % is reported after application of *P. indica*.
Maize	SAS Nagar	23	26	13.04	Results Authenticated by Dr. Rajesh Kumar, ADO
Maize	SAS Nagar	24	27	12.50	Results Authenticated by Dr. Rajesh Kumar, ADO
Maize	SAS Nagar	22	25	13.64	Results Authenticated by Dr. Rajesh Kumar, ADO
Maize	SAS Nagar	23	27	17.39	Results Authenticated by Dr. Rajesh Kumar, ADO
Wheat	Amritsar	14	14.5	3.57	Results Authenticated CAO
Wheat	Bathinda	18.72	19.04	1.71	Results Authenticated CAO
Wheat	Faridkot	19.04	19.68	3.36	Results Authenticated CAO
Wheat	Fatehgarhsahib	18.66	19.52	4.61	Results Authenticated CAO
Wheat	Ferozpur	18.25	18.65	2.19	Results Authenticated CAO
Wheat	Hoshiarpur	19.8	20.8	5.05	Results Authenticated CAO
Wheat	Jallandhar	17.93	18.2	1.51	Results Authenticated CAO
Wheat	Mansa	17.6	18	2.27	Results Authenticated CAO
Wheat	Sangrur	18.61	20.24	8.76	Results Authenticated CAO
Wheat	Tarn Taran	17.01	17.32	1.82	Results Authenticated CAO
Wheat	Muktsar	18.5	19.51	5.46	Results Authenticated CAO
Tomato	Kapurthala	225	250	11.11	Growth Better in Treated, Pesticide Spray kept constant
Tomato	Kapurthala	210	230	9.52	Stem borer and whitefly infestation less in treated; Pesticide (Midda and Coragen) usage reduced by 50 % in treated
Egg Plant	Kapurthala	50	100	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	70	90	28.57	Results Authenticated by Horticulture Assistant
Bottle Gourd	SBS Nagar	150	200	33.33	Results Authenticated by HDO
Bottle Gourd	Kartarpur, Amritsar	80	100	25.00	Blight and Aphd attack in control; Pesticide spray M-45, Metalex reduced by 50% in treated; Treated fruit more sweet in taste
Pumpkin	Kartarpur, Amritsar	70	80	14.29	White fly infestation less in treated; Pesticide (M-45) weekly spray in treated and every four day in control
Onion	Kartarpur Amritsar	5	6.	20.00	Only for seed production

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Further Reading

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Burkholderia a New biofertiliser for Improvement in Cereal Productivity

Biofertilizers are rhizosphere microorganisms inoculated to reduce the need for N or P fertilizer application and maximize plant growth and nutrition, resulting in greater grain yield and N or P content. A study was carried out to evaluate the effectiveness of diazotrophic bacteria isolated from the rhizosphere of wheat in Victoria, Australia and results are presented in the form of a thesis. This thesis shows that N_2 fixing Burkholderia species have great potential as biofertilizers on wheat productivity. The thesis is divided into 5 chapters with first chapter being introduction.

In Chapter 2, strains of bacteria were isolated from wheat-growing soils in main Victoria wheat belt at Horsham and Birchip in North West Victoria. Strains were identified as Burkholderia spp. by their closest matches in the 16S DNA and by morphology and physiology.

In Chapter 3, one selected strain from each of Birchip and Horsham were used to inoculate wheat in a pot trial in a glasshouse during winter-spring. Soil was collected on site from wheat fields. Pots were inoculated with these strains to evaluate the effects of Burkholderia inoculum as Biofertilizer on the plant growth and yield. Different nitrogen sources (urea 46% N and ammonium sulphate 21% N) were used as fertilizer at one of four levels (0, 50, 100 and 150 kg N/ ha).

In Chapter 4, field-grown wheat was inoculated with the same strains of Burkholderia. Three experiments were carried out in plots at two sites, dry land and irrigated fields at Horsham and a dry land field at Birchip, during the winter wheat season of 2006, to evaluate the effect of Burkholderia species inoculum and different types of nitrogen source at one of four levels of added N (0, 50, 100 and 150 kg N/ha) on wheat growth and yield. The effects of both bacterial inoculation and N fertiliser on growth promotion and grain yield were observed. Grain %N as well as total N content in grain per area in the Horsham irrigated field increased with increasing N fertiliser levels up to 100 kg N/ha.

In Chapter 5, acetylene reduction (ARA) activity was measured in the pots for both inoculated and un-inoculated plants at various growth stages and populations of nitrogen-fixing bacteria associated with the wheat roots and bulk soil were measured in addition to biomass and N content of plants and grain. Molecular tracing using specific primers showed that the inoculum was present only in inoculated treatments. Up to 60% of the increased N content of the grain in inoculated plants was potentially derived from nitrogen fixed by the inoculum in the rhizosphere.

It was concluded that the most significant result due to inoculation was the consistent maximal increase of N content in grain in inoculated treatments with ammonium sulphate fertilizer at 100 kg N/ha. Inoculation with Burkholderia consistently increased %N in wheat grain, with the potential benefit of decreasing the production cost and reducing use of chemical fertilizers.

(Source - Ben Mahmud, M 2008, *Effect of Burkholderia as biofertiliser on cereal productivity*, PhD Thesis, School of Applied Sciences, RMIT University)

Three New Biofertilizer Formulations Being Commercialised

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Introduction
Starting from early seventies, biofertilizers have gone a long way and are accepted as important nutrient inputs under both integrated nutrient management strategy and organic management approach. The journey started with Rhizobium has now been diversified, and many new microorganisms have been identified and are being exploited commercially as microbial inoculants. While Rhizobium, Azotobacter, Azospirillum, Phosphate solubilising biofertilizers and Myccorrhizal biofertilizers have been accepted as regular inputs and their quality is being monitored through Fertilizer Control Order (1985), other biofertilizers in spite of being covered under the definition of biofertilizers are not being regulated in the absence of quality standards and testing protocols.

On survey of the market it was found that in the name of Biofertilizers following microbial formulations, which in spite of being qualified as Biofertilizers as per the definition but not covered by FCO in want of specifications, are being sold in adequate quantities:

1. Potash solubilising/ mobilizing biofertilizers
2. Zinc solubilising biofertilizers
3. Consortia of biofertilizer organisms consisting of two or more of nutrient mobilizing microorganisms.

To ensure the coverage of all commercially available biofertilizer formulations under statutory quality control mechanisms and finally bringing them under the ambit of FCO (1985), efforts were made at National Centre of Organic Farming to define quality standards of such products. Abstract findings of the study with suggested parameters are being described here.

Potash solubilising/ Mobilizing Biofertilizer
Through research many bacterial and fungal organisms have been screened for their ability to solubilise mineral potash rocks such as K-binding soil silicate particles, quartz, keolinite, Mica, Illite, feldspar, muscovite etc. Among various microorganisms screened for the purpose following bacterial strains have been found to be most effective and are being widely used for inoculant production.

a. *Bacillus edaphicus*
b. *Bacillus mucilaginous* and
c. *Fraturia aurantia*

Mechanism of action
All the organisms identified as K-solubilizers are reported to be organic acids, siderophores, organic ligands and exo-polysaccharide producers. All these active ingredients are known to decompose or solubilise natural silicates and help in removal of metallic ions from the rocks and soils.

As a rule although all microorganisms producing organic acids are good P-solubilizers but all good P-solubilizers are not K-solubilizers. Mechanism of K-solubilisation, although not fully understood is slightly different from P-solubilisation in the way that all organic acids do not act on potassic mineral rocks and silicates. It is only the combination of organic acids, organic ligands and exo-polysaccharides which collectively acts on such fixed particles and release metallic ions from silicate particles.

As per the hypothesis proposed by Liu et al, 2006, the leaching of K^+ and SiO_2 from silicate minerals occurs as a result of both organic acids and exo-polysaccharides.

Organic acids such as oxalates and citrates can form bidentate complexes with metal ions and which tend to be more effective in enhancing dissolution than monodentate ligands such as those formed by acetate or propionate.

Exo-polysaccharides secreted by these bacteria combine with the minerals and form bacterial-mineral complexes. These polysaccharides strongly adsorb the organic acids and an area of high concentration of organic acids is formed near the minerals.

Under the effect of organic acids the minerals are partially degraded. On the other hand the polysaccharides also absorb SiO_2. The resulting alteration of the concentration of SiO_2 affect the equilibrium between the mineral and fluid phase leading to reaction towards SiO_2 and K^+ solubilisation, which finally leads to further degradation of the minerals.

Effect of K-Solubilizing biofertilizers on crop growth and yields

Studies conducted by different workers on effect of KSB inoculation on crop yields indicate increase in yields by 10-20% with increased K-uptake by the crop.

Chandra et al, 2005 (RCOF, Bangalore) reported 15-20% increase in yield of yam and tapioca by application of KSB.

Supanjani et al 2006 reported that integration of P and K rocks with inoculation of PSB and KSB increased P availability from 12 to 21% and K availability from 13 to 15%. Yield of Capsicum green pods was increased by 23-30%.

Badar et al 2006 also reported that by application of PSB and KSB with P and K rocks increased dry matter yield and uptake of P and K by sorghum.

Recently, Basak and Biswas 2010 in their studies at IARI concluded that Co-inoculation of KSB and N-fixing biofertilizers (Azotobacter) improved K availability from waste mica and stimulated the growth of Sudan grass.

Status of K-solubilizing biofertilizers (KSB) in market

Although quantum of KSB being produced in the country is not known, but large number of biofertilizer producers are producing and marketing it. Even some of the states have approved the products and they are purchasing the KSB for their supplies under different schemes.

To study the ingredients and microbial strength, lot of KSB samples were taken for study. While some of these samples were picked up from the market and from manufacturers some samples were also sent by different State Governments for quality assessment.

Based upon the studies conducted on these samples it was revealed that while majority of such samples were made with Fraturia aurantia as the target organism, some producers were also found to be using Bacillus species (probably B. edaphicus). Total viable counts were found to be varying from $5x10^7$ to 5×10^8.

Based upon the studies and keeping in view of the specifications of other biofertilizers, following specifications are proposed for K-solubilizing biofertilizers.

Specifications of Potash Solubilizing/ Mobilizing biofertilizers

S.No.	Parameter	Requirement
1.	Base	Carrier* based in form of moist powder or granules or liquid based
2.	Viable cell count	CFU minimum $5x10^7$ cells/g of powder, granules or carrier material on dry weight basis or $1x10^8$ cell/ml of liquid
3.	Contamination	No contamination at 10^{-5} dilution
4.	pH	6.5 – 7.5 for carrier based in form of powder or granules and 5.0 – 7.5 for liquid based
5.	Particle size in case of carrier based moist powder	All material shall pass through 0.15 to 0.212 mm IS sieve
6.	Moisture content percent by weight in case of carrier based	30 – 40%
7.	Efficiency character	Minimum 10 mm solubilization zone in prescribed media having at least 3mm thickness.

Type of carrier – The carrier material such as peat, lignite, peat soil, humus, wood charcoal or similar material favouring growth of microorganisms

Zinc solubilising biofertilizers

In the recent years some microorganisms have been identified which are not only having potential for insoluble zinc solubilisation but can also tolerate high concentrations of zinc. Species of Pseudomonas and Bacillus have been identified as the potential target organisms and can solubilise zinc sulphide, zinc oxide and zinc carbonate. Initial screening on media provided with insoluble zinc showed zinc solubilisation zone ranging from 10 to 30 mm. In quantitative assay these strains have been found to be solubilizing 13 to 16 mg of zinc per lit of broth in 15 days. Many studies conducted by private industry indicate that zinc deficiency can be overcome in several cases by the application of Zinc solubilizing biofertilizers.

Mechanism of action of Zinc solubilizers

Although exact mechanism for zinc solubilisation is not well known but the potential of these organisms for secretion of specified organic acids is widely believed to be the reason for zinc solubilization. As per Shahab and Ahmed (2008) zinc solubilization can be accomplished by a range of mechanisms, which include excretion of metabolites such as organic acids, proton extrusion or production of chelating agents.

Effect of zinc solubilizing biofertilizers on crop growth

Studies conducted by Tariq et al (2007) indicate that mixed inoculation of Azospirillum, Zn solubilizing bacteria Pseudomonas and Agrobacterium alleviated the deficiency symptoms of Zn and invariably increased the total biomass (23%), grain yield (65%) and harvest index as well as Zn concentration in the grain. The inoculation had a positive impact on root length (54%), root weight (74%), root volume (62%), root area (75%), shoot weight (23%), panicle emergence index (96%) and showed the highest Zn mobilization efficiency as compared with the un-inoculated control.

Defining quality standards – Based upon microbiological and physiological studies, analysis of commercial samples and research findings published in literature, studies were carried out at NCOF and practically achievable quality norms are proposed as follows:

Specifications of Zinc solubilizing biofertilizers (ZSB)

S.No.	Parameter	Requirement
1.	Base	Carrier* based in form of moist powder or granules or liquid based
2.	Viable cell count	CFU minimum $5x10^7$ cells/g of powder, granules or carrier material on dry weight basis or $1x10^8$ cell/ml of liquid
3.	Contamination	No contamination at 10^{-5} dilution
4.	pH	6.5 – 7.5 for carrier based in form of powder or granules and 5.0 – 7.5 for liquid based
5.	Particle size in case of carrier based moist powder	All material shall pass through 0.15 to 0.212 mm IS sieve
6.	Moisture content percent by weight in case of carrier based	30 – 40%
7.	Efficiency character	Minimum 10 mm solubilization zone in prescribed media having at least 3mm thickness.

Type of carrier – The carrier material such as peat, lignite, peat soil, humus, wood charcoal or similar material favouring growth of microorganisms

Consortia of biofertilizer organisms consisting of two or more of nutrient mobilizing microorganisms

Since the development and commercialization of Azotobacter, Azospirillum and phosphate solubilizing biofertilizers voluminous data has been generated on the synergistic effects of these micro-organisms when they are applied together. Now it has been established beyond doubt that combined application of N-fixing, P-solubilizing and growth promoting bacteria delivers best results. Since last many years combined application of at least one N-fixing and PSB is being promoted and recommended. Keeping the advantages of combined application various industries have developed their own mixed inoculants combining either of two (one N-fixer+PSB), three (one N-fixer+PSB+K-solubilizer) or four biofertilizer organisms (one or two N-fixer +PSB+K-solubilizer+Pseudomonas fluorescens).

Under Network Project on Biofertilizers also such mixed formulations have been developed and are being promoted. Technology for one such formulation was also transferred to NCOF, which is being provided to industry. Now various combinations of such biofertilizer organisms are being formulated in different commercial forms and are being sold in the market. As the mixed inoculants are now established commercial inputs, therefore there is a need for regulation of their quality through specified specification requirements.

Based upon the studies carried out at NCOF under a Research Project specifications were worked out. As all the microorganisms being used in such mixed formulations are the same organisms/strains recommended for single inoculant production, only their compatibility with each other in single formulation and total and individual counts were studied and a specification frame work was developed. Methodology used for their individual population enumeration, total population enumeration and individual organism's efficiency determination was the same as that of single inoculant.

Proposed specifications for consortia of biofertilizers organisms are given below with individual organism's efficiency requirement. Although Trichoderma viride (a decomposting microorganism) and Pseudomonad fluorescens are not recognised biofertilizers, but as they are invariably be the components of mixed formulations, therefore they have been included in the proposed specifications.

Specifications of Consortia of biofertilizers (Mixed Biofertilizer inoculants)

S.No	Parameter	Requirement
1.	Base	Carrier based in form of moist powder or granules or liquid based
2.	Individual Viable count in carrier based on dry weight basis	CFU minimum in a mixture of any 2 or more of following microorganisms: Rhizobium > $1x10^6$ per g Azotobacter > $1x10^6$ per g Azospirillum > $1x10^6$ per g PSB > $1x10^6$ per g KMB >$1x10^6$ per g ZSB > $1x10^6$ per g Pseudomonas fluorescens > $1x10^6$ per g Trichoderma viride > $1x10^6$ per g
3.	Individual Viable count in Liquid based	CFU minimum in a mixture of any 2 or more of following microorganisms Rhizobium >$1x10^7$ per ml Azotobacter >$1x10^7$ per ml Azospirillum >$1x10^7$ per ml PSB >$1x10^7$ per ml KMB > $1x10^7$ per ml ZSB > $1x10^7$ per g Pseudomonas fluorescens >$1x10^7$ per ml
4.	Total viable count of all the biofertilizer organisms in the product	CFU minimum $5x10^7$ cells per gm of carrier/ powder or $5x10^8$ cells per ml of Liquid based
5.	Contamination	No contamination at 10^{-5} dilution
6.	pH	6.5 – 7.5 for carrier based in form of powder or granules and 5.0 – 7.5 for liquid based
7.	Particle size in case of carrier based moist powder	All material shall pass through 0.15 to 0.212 mm IS sieve
8.	Moisture content percent by weight in case of carrier based	30 – 40%
9.	Efficiency character Rhizobium Azotobacter Azospirillum PSB KMB ZSB Pseudomonas fluorescens	 Should show effective nodulation on all the legume species listed on the pack 10 mg N fixation /g of C-source utilized 10 mg of N-fixation/ g of malate applied >10 mm zone of solubilization on PSB media having at least 3 mm thickness >10 mm zone of solubilization on KMB media having at least 3 mm thickness >10 mm zone of solubilization on ZSB media having at least 3 mm thickness Indole positive reaction as per the method prescribed

Type of carrier – The carrier material such as peat, lignite, peat soil, humus, wood charcoal or similar material favouring growth of microorganisms.

Suggested media for enumeration of TVC and efficiency character tests for K-solubilizing, Zinc solubilizing and consortia of biofertilizers

While standard method of serial dilution and plating is suggested for enumeration of total viable count (TVC) for all the organisms under study, but medium for growth are organisms specific and are suggested as below:

Medium for analysis of TVC, contamination and for studying zone of solubilization for Potassium mobilizing bacteria (KMB) (Aleksandrov Medium)

(Ingredients g/lit)

Glucose	5g
Magnesium sulphate	0.005g
Ferric chloride	0.1 g
Calcium carbonate	2.0 g
Potassium alumino silicate (mica powder)	2.0g
Calcium phosphate	2.0 g
Distilled water	1000ml

Mix all the ingredients in about 750 ml of water, except glucose and autoclave. Filter sterilized Glucose dissolved in 250 ml water is mixed with above solution after autoclaving.

Medium for analysis of TVC, Contamination and zone of solubilization for Zn solubilizing biofertilizer

(Ingredients g/lit)

Glucose	10g
Zinc oxide	1.0 g
Amm sulphate	0.5 g
Potassium chloride	0.2 g
Yeast extract	0.5 g
Ferrous sulphate	0.01 g
Manganese sulphate	0.01 g
Di Pot Hyd.phosphate	0.5 g
Distilled water	1000 ml

Medium for analysis of TVC, Contamination and for efficiency character testing methods for consortia of Biofertilizers (Mixed inoculants)

i. Methods for analysis of Rhizobium – Yeast Extract manitol agar medium

ii. Methods for analysis of Azotobacter – N- free Jenson's agar

iii. Methods for analysis of Azospirillum – NFB semi-solid medium

iv. Methods for analysis of PSB – Pikovaskya agar medium

v. Methods for analysis of KMB – Same as of KMB as above

vi. Methods for analysis of ZSB – Same as of ZSB as above

vii. Methods for analysis of Pseudomonas fluorescence – King'B medium

King's B medium

Proteose peptone	20 g
Glycerol	10 g
K_2HPO_4	1.50 g
$MgSO_4.7H_2O$	1.50 g
Agar	15.0 g
Distilled water	1000 ml

Adjust pH at 7.2

Method for Indole reaction assay
Medium for growing bacteria

a) Add 0.25 gm/lit of Tryptophan into Kings B medium as described above

Growing bacteria in specified medium

b) Fill 125 ml medium in six conical flasks and autoclave at 121^0C for 15 min. After cooling inoculate 3 flasks with loopful of bacteria (from a colony of Pseudomonas fluorescens grown on Kings B medium) and incubate for 5 days under continuous shaking at 28 ± 2^0C. Keep 3 unioculated flasks as control.
c) After 5 days of incubation centrifuge the broth at 15000 rpm for 30 min and supernatant is collected.
d) Use this clear centrifuged solution for indole reaction.

Indole reaction

Material required

- Stock solution of 0.5M ferric chloride ($FeCl_3$)
- Stock solution of 35% per-chloric acid ($HClO_4$)
- Ortho-Phosphoric acid

Procedure

a. Preparation of Salkowski reagent – Suspend 1 ml of 0.5M $FeCl_3$ in 50 ml of $HClO_4$ (this test reagent needs to be prepared fresh every time)
b. Take 2 ml of centrifuged broth in a test tube, add 2-3 drops of orthophosphoric acid followed by 4 ml of freshly prepared salkowski reagent.
c. Incubate the tubes at room temperature for 25 min.
d. Observe the colour.
e. Development of pink colour indicates presence of indole and is treated as indole positive.
f. Uninoculated broth shall show no colour development

viii. Medium for estimation of TVC for Trichoderma viride

Potato Dextrose Agar

Potato extract	300 gm
Dextrose	20 gm
Triton X 100	2 ml
Chloramphenicol	80 mg
Agar	20 gm

The potatoes are placed in gauge bag, boiled in 1 liter of water for 30 min, and then filtered through cotton. Filtrate is mixed with Dextrose and agar and made up to 1 lit and autoclaved. Filter sterilized Triton X100 and Chloramphenicol is added after autoclaving just before plating when medium is still in molten condition.

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A review on the role of *Azospirillum* in the yield improvement of non leguminous crops

Rise in human population always demands a rapid and sustainable increase in cereal production. As a result nitrogenous fertilizers were used constantly in excess, which resulted in a number of problems such as green house emissions (particularly N_2O) and leaching to ground-water. Moreover they are expensive. So long term sustainability in agriculture can only be obtained with the use of low cost fertilizer which should also be ecologically safe. In this regard biological nitrogen fixation by microbes, that is, biofertilizer, plays an active role helping in better maintenance of crop nutrient as well as soil health. *Azospirillum*, an associative symbiotic nitrogen fixing bacterium has a higher nitrogen fixing potential in non-legumes in comparison to other nitrogen fixing bacterium, by the formation of *para* nodules. However further investigation is needed to find possible avenues for the exploitation of this bacterium. The current review emphasizes the central issues of *Azospirillum* and its application either alone or in combination with other plant growth promoting rhizobacteria for the benefit of the non leguminous crops. (Source – Saikia et al 2012, African Journal of Microbiology Research Vol. 6(6), pp. 1085-1102)

Research Notes

Productivity and Soil Health of Potato (Solanum tuberosum L.) Field as Influenced by Organic Manures, Inorganic Fertilizers and Biofertilizers under High Altitudes of Eastern Himalayas - Field experiments were conducted in three consecutive summer seasons of 2005 to 2007 to study the effect of integrated nutrient management on soil health and productivity of potato (*Solanum tuberosum* L.) under rainfed condition. The experiment was laid out in a split plot design with eight nutrient management practices (combinations of organic manures viz, farm yard manure (FYM), poultry manure (PM), vermicompost (VC) and inorganic fertilizers in main plots and seed tuber treatment with three biofertilizers (*Azotobacter*, phosphorus solubilizing bacteria (PSB) and *Azotobacter* + PSB) in sub plots. The results showed that 50% of the recommended dose of NPK through inorganic + 50% recommended dose of nitrogen (RDN) through organic manures (FYM, PM or VC) or 100% recommended dose of NPK through inorganic fertilizers alone favorably influenced the tuber yield, nutrient uptake, soil fertility and paid higher returns compared to other treatments. Seed treatment with *Azotobacter* + PSB proved better in tuber yield, nutrient uptake and recorded higher returns as compared to sole treatment of either *Azotobacter* or PSB. Three years pooled result revealed that integrated application of 50% of recommended NPK through inorganic and 50% RDN through PM recorded significantly highest tuber yield (22.73 t/ha) closely followed by 100% recommended NPK through inorganic (22.20 t/ha) which were 228% and 223% respectively, higher than control. Integrated application of inorganic and organic fertilizers and seed treatment with *Azotobacter* +PSB biofertilizers improved tuber yield, nutrient uptake, and gave higher return as compared to other treatment combinations. Total organic carbon (TOC), soil microbial biomass carbon (SMBC), available N, P, and K status of the soil after 3 years were maximum when 50% recommended dose of NPK were applied through inorganic and remaining 50% RDN through PM. (Source - Kumar et al 2012, J Agricultural Sciences Vol 4, No 5).

Effect of bio-fertilizers on growth and productivity of wheat (*Triticum aestivum*) - Field experiment was conducted during the rabi season of 2003-04 and 2004-05 at the farmer fields in village Bhiuraha, district Jaunpur to study the efficacy of bio-fertilizers on growth and productivity of wheat (*Triticum aestivum*). Leaf and shoot dry weight increased up to 90 days after sowing (DAS) and then gradually declined. Shoot dry weight also gradually increased up to 120 DAS and then decreased. However, dry matter in panicle increased rapidly from 120 DAS till harvesting. At early stage (30-60 DAS), dry matter production was slow and the rate of dry matter accumulation increased from 60 DAS onwards. About 21.5 per cent of dry matter of whole plant was observed at 60 DAS, 61.2 per cent at 90 DAS and 94.4 per cent at 120 DAS. The result showed significant response of bio-fertilizers on growth and productivity of wheat. Combined application of bio-fertilizers caused considerable increase in plant height over all the treatments. Tillering enhanced significantly due to application of bio-fertilizers either alone or in combination. Greater tillering was noticed when the crop received combined treatments than other treatments. Similar trend of results was also observed in case of yield components of wheat i.e. ears/m^2, grains/ear and 1000 grain weight increased significantly when the crop received bio-fertilizers either alone or combined. (Source – Singh and Prasad 2011, International Journal of Farm Sciences, Vol 1, No 1).

Pseudomonas sp. strain AKM-P6 enhances tolerance of sorghum seedlings to elevated temperatures - A thermo-tolerant strain AKM-P6 of Pseudomonas sp. possessing plant growth-promoting properties was isolated from rhizosphere of pigeon pea grown under semiarid conditions in India. The effect of inoculation withAKM-

P6 on survival and growth of sorghum seedlings at elevated temperatures (ET) was investigated under sterile and non-sterile soil conditions. Inoculation with strain AKMP6 helped sorghum (var CSV-15) seedlings to survive and to grow at elevated temperatures (47–50°C day/30–33°C night) up to 15 days while uninoculated plants died by the fifth day of exposure to elevated temperature. Under sterile and non-sterile conditions, significantly higher root and shoot biomass were recorded in inoculated seedlings as compared to uninoculated control at ET, but this difference was non-significant at ambient temperature. Inoculation induced the biosynthesis of high-molecular weight proteins in leaves under elevated temperature, reduced membrane injury, and improved the levels of cellular metabolites like proline, chlorophyll, sugars, amino acids, and proteins. Scanning electron microscopy studies confirmed the colonization and establishment of the organism on the root surface. The 16SrDNA sequence of the strain AMK-P6 showed 97% homology with that of Pseudomonas aeruginosa in the existing database. The results indicate that Pseudomonas sp. strain AKM-P6 can enhance tolerance of sorghum seedlings to elevated temperatures by inducing physiological and biochemical changes in the plant. (Source – Ali et al 2009, Biol Fertil Soils, 46:45–55)

Alleviation of drought stress effects in sunflower seedlings by the exopolysaccharides producing Pseudomonas putida strain GAP-P45 - Production of exo-polysaccharides (EPS) can be used as a criteria for the isolation of stress tolerant microorganisms. In the present study, EPS-producing fluorescent pseudomonads were isolated from alfisols, vertisols, inceptisols, oxisols, and aridisols of different semiarid millet growing regions of India and were screened in vitro for drought tolerance in trypticase soy broth supplemented with different concentrations of polyethylene glycol (PEG6000). Out of the total 81 isolates, 26 could tolerate maximum level of stress (–0.73 MPa) and were monitored for the amount of EPS produced under maximum level of water stress. The strain GAP-P45, isolated from alfisol of sunflower rhizosphere, showed the highest level of EPS production under water stress conditions, was identified as Pseudomonas putida on the basis of 16S rDNA sequence analysis, and was used as seed treatment to study its effect in alleviating drought stress effects in sunflower seedlings. Inoculation of Pseudomonas sp strain GAP-P45 increased the survival, plant biomass, and root adhering soil/root tissue ratio of sunflower seedlings subjected to drought stress. The inoculated bacteria could efficiently colonize the root adhering soil and rhizoplane and increase the percentage of stable soil aggregates. Scanning electron microscope studies showed the formation of biofilm of inoculated bacteria on the root surface and this, along with a better soil structure, might have protected the plants from the water stress. (Source – Sandhya et al 2009, Biol Fertil Soils 46:17–26)

***Pseudomonas* sp. strain P45 protects sunflower seedlings from, drought stress through improved soil structure** - An attempt was made to study the effect of an exo-polysaccharide (EPS) producing fluorescent *Pseudomonas* strain P45 on soil aggregation and growth of sunflower seedlings subjected to drought. *Pseudomonas* sp. strain P45 could efficiently colonize the rhizosphere of sunflower resulting in stable soil aggregates formation and increased root adhering soil/root tissue (RAS/RT) ratio under drought stress conditions. Inoculation improved seedling biomass, total chlorophyll and relative water contents (RWC)in the leaves thus helping the seedlings to survive under drought stress. (Sandhya et al *J. Oilseeds Res 2009,* 26 *(Speciai /ssue),* 600-602)

Evaluation of Biofertilizers in Irrigated Rice: Effects on Grain Yield at Different Fertilizer Rates - Biofertilizers are becoming increasingly popular in many countries and for many crops, but very few studies on their effect on grain yield have been conducted in rice. Therefore, authors evaluated three different biofertilizers (based on *Azospirillum*, *Trichoderma*, or unidentified rhizobacteria) in the Philippines during four cropping seasons between 2009 and 2011, using four different fertilizer rates (100% of the recommended rate [RR], 50% RR, 25% RR, and no fertilizer as Control). The experiments were conducted under fully irrigated conditions in a typical lowland rice environment. Significant yield increases due to biofertilizer use were observed in all experimental seasons with the exception of the 2008-09 DS. However, the effect on rice grain yield varied between biofertilizers, seasons, and fertilizer treatments. In relative terms, the seasonal yield increase across fertilizer treatments was between 5% and 18% for the best biofertilizer (*Azospirillum*-based), but went up to 24% in individual treatments. Absolute grain yield increases due to biofertilizer were usually below 0.5 $t \cdot ha^{-1}$, corresponding to an estimated additional N uptake of less than 7.5 kg N ha^{-1}. The biofertilizer effect on yield did not significantly interact with the inorganic fertilizer rate used but the best effects on grain yield were achieved at low to medium fertilizer rates. Nevertheless, positive effects of the biofertilizers even occurred at grain yields up to 5 $t \cdot ha^{-1}$. However, the trends in results seem to indicate that biofertilizers might be most helpful in rain-fed environments with limited inorganic fertilizer input. However, for use in these target environments, biofertilizers need to be evaluated under conditions with abiotic stresses typical of such systems such as drought, soil acidity, or low soil fertility. (Source – Banayo et al *Agriculture* 2012, *2*(1), 73-86)

Impact of Application of Biofertilizers on Soil Structure and Resident Microbial Community Structure and Function - Biofertilizers are believed to be an eco-friendly alternative to chemicals that have been used extensively in agriculture thereby contaminating the environment. The mechanism by which biofertilizers lead to positive effect on plant growth is not completely understood. Target effects of biofertilizers have been investigated before their field release to determine their efficacy. However, a largely ignored aspect in the development and release of biofertilizers has been studies on their impact on the indigenous microbial community. The introduction of biofertilizers, in numbers which largely exceed their normal populations, can change the microbial community structure and function in both positive and negative ways. It is, therefore, important to study the microbial ecology of resident microbial communities post biofertilizer application. A thorough understanding of the interactions between bioinoculants and other soil components would help in improvement of their survival and competitive ability in the rhizosphere of crops. The chapter primarily focuses in discussing the reports of impact of biofertilizers on soil structure and microbial community dynamics. (Source – Shilpi Sharma et al Bacteria in Agrobiology: Plant Probiotics, 2012, 65-7)

Strategies for the Exploration and Development of Biofertilizer - Microbial agents have been recognized as biofertilizers and used in the crop production in recent years. These biological agents serve as an alternative way to promote plant growth meanwhile reducing the chemical fertilizer requirements, which move toward a more sustainable management in agriculture. This article gives overall concepts and strategies in the exploration and development of biofertilizers. By elucidating the mechanisms involved in the plant growth promotion and conducting bioassay after inoculation with the microorganisms, the superior bacteria may be screened and isolated. Besides, the identification and tracking for the microbial fertilizer through molecular approaches provide useful information regarding their bio-safety level and adaptabilities in the soil. The integrated approaches are proposed here to increase the efficiency of the biofertilizers in the rhizosphere: inoculation from a single bacterium to bacterial consortium; from sole microbial agents to a combination of microbial agents, organic fertilizer, or chemical fertilizer (biotech-fertilizer); from fast nutrient-releasing liquid fertilizer and slowly nutrient-releasing solid fertilizer to control-releasing biofertilizer (biowise-fertilizer); and from microbial agents with fertilization activity to multifunctional microbial agents with both fertilization and bio-control activities. The biofertilizers will be brought into the markets and show huge benefits to the farmers and environments once the limitations in the research, production, and commercialization of these microbial agents have been overcome in the near future. (Source – Young et al Bacteria in Agrobiology: Plant Probiotics, 2012, 127-139)

Bacterial Inoculants for Field Applications under Mountain Ecosystem: Present Initiatives and Future Prospects - While microorganisms are ubiquitous in nature, their distribution is governed by environmental specificities. The use of biological fertilizers, in recent times, has received well deserved attention mainly due to increased global preference for natural "organic" products, as well as to reduce the load of chemical fertilizers on the environment. Two major benefits associated with this eco-friendly microbe-based technology are: (1) improved plant nutrition and (2) bio-control of a wide range of pathogens. Success of this technology, however, depends on the availability of ecologically competent microbes in user friendly formulations. One of the prerequisites for developing this technology is proper understanding of the diversity of microorganisms in a given ecosystem, with particular reference to their functional efficiency. Isolation of microorganisms, screening for desirable characters, selection of efficient strains, pot and field trials, and finally the production of inoculum in easy to store formulations in a cost-effective manner are the main steps toward the development of this microbe-based technology. The present review highlights some of the initiatives taken up by various laboratories located in the Indian Himalayan Region, with particular attention to elucidate the potential of this technology in low temperature environments of the mountain ecosystem, with comments on the future prospects in this area of research with the introduction of modern molecular tools. (Source – Trivedi et al Bacteria in Agrobiology: Plant Probiotics, 2012, 15-44)

Consortium of Plant-Growth-Promoting Bacteria: Future Perspective in Agriculture - The term "plant-growth-promoting rhizobacteria" (PGPR) include soil bacteria that colonize the roots of plants following inoculation onto seed and enhance plant growth. The bacteria useful to plants were proposed to be characterized in two general types: bacteria forming a symbiotic relationship with the plant and another the free-living ones found in the soil but are often found near, on, or even within the plant tissues. The PGPR are known to enhance growth by several direct mechanisms—like nitrogen fixation provide N-nutrient, phyto-stimulators directly promote the growth of plants by the production of hormones, and several other metabolites like siderophore, ACC deaminase, etc., are produced by PGPR strains for plant growth enhancement. Also, bio-control agents that are able to protect plants from soil borne infection by deleterious microorganisms also offer environment-friendly strategy for pest control. Recently, application of two or more PGPR as consortium is gaining ground in field application worldwide. This offers multifarious approach of promoting plant growth and improve yield. In this review, the various strategies for consortium formulation are described. In fact, use of rhizobia with free-living nitrogen fixers or with phosphate solubilizers including VAM fungi has been widely reported. Also, application of bio-control agents along with direct growth promoters is also observed as holistic approach for sustainable agriculture. Further, tailor-made consortium is sometimes designed to include other benefits like improving soil health. (Source – Pandey et al Bacteria in Agrobiology: Plant Probiotics, 2012, 185-20)

Improvement of growth and nutritional quality of *Moringa oleifera* using different biofertilizers - *Moringa* seeds were cultivated in polyethylene bags (1 kg capacity) filled with clay loamy soil. Bags were treated with microorganisms using three methods of inoculation i.e. soil inoculation (single or mixed cultures); leaf inoculation (single culture), and soil and leaf inoculation (mixed inoculation). Plants were harvested after 3 months of cultivation. Shoot and root lengths, shoot and root dry weights, leaves fresh and dry weights, vitamin C g/g fresh leaf, protein g/g leaves dry weight and mineral contents (Mg, P, K, Zn, Mn, Fe and

Cu) were recorded. Biofertilization by different inoculation methods increased most of the parameters tested. The highest records of shoot and root lengths, and shoot and root dry weights were obtained with soil inoculation with mixed cultures of (*Azotobacter chroococcum* and *Saccharomyces cerevisiae*) and (*A. chroococcum* and *Bacillus circulans*). The same trend in respect of Vitamin C was obtained. But, the highest protein contents (g/g dry weight leaves) were obtained with soil inoculation with (*Azot. chroococcum* and *B. circulans*), (*Bacillus megatherium*) and (*Azot. chroococcum* and *S. cerevisiae*), which gave 0.73, 0.59 and 0.58 g protein/g leaves dry weight respectively. Generally, soil inoculation with either *B. megatherium*, *B. circulans*, (*Azot. chroococcum* and *Pseudomonas fluorescens*), (*Azot. chroococcum* and *B. circulans*),*Azot. chroococcum*, and (*Azospirillum brazilense* and *B. megatherium*) gave the highest records of Mg, P, K, Zn, Mn, Fe and Cu respectively. (Source – Zayad 2012 Annals of Agricultural Sciences, Online edition)

Assessing the Benefits of *Azotobacter* Bacterization in Sugarcane: A Field Appraisal - Biofertilizers have long been assessed as powerful technology to obtain sustainable enhanced crop production. The present investigation revealed the positive effects of inoculation of *Azotobacter* biofertilizer on growth and yield parameters in sugarcane var. CoJ 83 under field conditions. Application of *Azotobacter* biofertilizer at both the nitrogen levels ($N_{75\%Rec}$ and $N_{100\%Rec}$ levels) resulted in significant increase in the cane yield over the respective controls. Maximum increase in cane yield was recorded by *Azotobacter* inoculation at recommended dose of nitrogen. Inoculation with *Azotobacter* at $N_{75\%Rec}$ level of N fertilizer resulted in cane yield that was observed to be statistically at par with $N_{100\%Rec}$ level. The application of this biofertilizer would not only be beneficial keeping in view the phenomenon of enhanced productivity using environmentally benign technology, but also would be useful to obtain better yield with improvement of the soil microbial ecology/soil food web. (Source – Gosal et al 2012, Sugar Technology, Volume 14, Number 1 (2012), 61-67).

Effects of biofertilizers on grain yield and protein content of two soybean (Glycine max L.) cultivars - Nutrient management is one of the most important factors in successful cultivation of plants. Biofertilizers can affect the quality and quantity of crop. In order to study the effects of biofertilizers on grain yield and protein content of two soybean (Glycine max L.) cultivars, an experiment was conducted using a factorial arrangement based on randomized complete block design with four replications, at the Mahidasht Research Station of Kermanshah in 2010. The factors were soybean cultivar (Williams and Line no. 17) and fertilizer application (b1= N + P, b2= Bradyrhizobium japonicum + P, b3= N + Bacillus and Pseudomonas + 50% of P, b4= B. japonicum + Bacillus and Pseudomonas + 50% of P, b5= B. japonicum + 50% of N + Bacillus and Pseudomonas + 50% of P). Results show that Line no. 17 with 2911.2 kg/ha had higher seed yield than Williams with 2711 kg/ha. Also, fertilizer levels of b3 with 3058.2 and b2 with 2643.8 kg/ha produced the highest and the lowest seed yield, respectively. Plants treated with fertilizer levels of b1, b2 and b5 in comparison with other fertilizer levels significantly produced lower thousand seed weight. In Line no. 17 fertilizer level of b3 with 2.88 produced the highest seed per pod. Results show that fertilizer levels had a significant effect on the number of pod per plant and treatments containing biological fertilizers in terms of the number of pods per plant were equal or superior to chemical fertilizer. It was also observed that fertilizer levels of b1, b3 and b5, produced the highest protein percentage. It therefore seems that biofertilizers can be considered as a replacement for part of chemical fertilizers in soybean production. (Source – Zarei et al 2012, African Journal of Biotechnology Vol. 11(27), pp. 7028-7037)

Assessing the in vitro zinc solubilization potential and improving sugarcane

growth by inoculating *Gluconacetobacter diazotrophicus* - *Gluconacetobacter diazotrophicus* is an endophytic diazotroph of sugarcane that has been reported to possess various plant growth-promoting characteristics. Authors assessed the zinc (Zn) solubilization potential of *G. diazotrophicus* isolates under in vitro conditions (plate and broth assay) with different Zn compounds (ZnO, $ZnCO_3$, and $ZnSO_4$) and by the improvement of sugarcane growth following inoculation. The soluble Zn present in the culture broth was assessed by inductively coupled plasma–optical emission spectrophotometry. All five isolates solubilized the tested Zn compounds, although there were variations in the solubilization potentials, and effectively solubilized ZnO over $ZnCO_3$ or $ZnSO_4$. The solubilization might be due to the production of acids, since the pH of the culture broth shifted during the solubilization of Zn compounds. The nitrogenase activity of the *G. diazotrophicus* isolates ranged between 80.3 and 125.3 C_2H_4 h^{-1} $vial^1$ (ethylene), which was much more efficient than the nitrogenase activity in micro-propagated plants. The GaD-1 isolate was found to be the most efficient isolate in terms of Zn compound solubilization and promotion of sugarcane plant growth when compared to other isolates. The application of *G. diazotrophicus* isolates with efficient nitrogenase activity in sugarcane production systems may possibly result in the reduction of chemical N fertilizer usage and the cost of sugarcane production; there may also be a considerable increase in production of sugarcane. (Source - Natheer and Muthukkaruppan, Annals of Microbiology, Volume 62, Number 1 (2012), 435-441)

Indole acetic acid and ACC deaminase-producing *Rhizobium leguminosarum* bv. *trifolii* SN10 promote rice growth, and in the process undergo colonization and chemotaxis - This study focuses on the chemotaxis, colonization and rice growth promoting ability of indole acetic acid (IAA) and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase-producing rhizobacteria *Rhizobium leguminosarum* bv. *trifolii* SN10, previously isolated from root nodules of *Trifolium alexandrium* L. Authors show here that *R. leguminosarum* bv. *trifolii* SN10 promote the growth of four different rice varieties grown in India in terms of biomass, root branching and N content. In addition, using scanning electron microscopy and viable cell counts, authors provide evidence that the bacteria successfully colonize the root surface of the rice variety which showed maximum growth promotion upon inoculation. Not only this, *R. leguminosarum* bv. *trifolii* SN10 exhibit a strong chemotaxis response towards the rice seed and root exudates despite the presence of a bacteriostatic phenolic compound, 7-hydroxycoumarin (umbelliferone). Further, *R. leguminosarum* bv. *trifolii* SN10 secretion of phytohormones such as IAA and ACC deaminase suggest the potential of the plant growth promoting rhizobacteria to be used as biofertilizer to enhance rice crop production in the subcontinent. (Source – Bhattacharjee et al Biology and Fertility of Soils 2012, Volume 48, Number 2, 173-18)

A perspective towards development and commercialization of potential BGA biofertilizers of Assam, North East India and carrier materials for BGA mass production and inoculum development - Blue green algae are a group of gram negative photosynthetic prokaryotes mostly known as cyanobacteria which has drawn worldwide attention for the nitrogen fixing ability and their use in agriculture. BGA are very common in Indian in rice fields. The present study is focused towards developing eco-friendly technique for mass production and suitable bio-inoculum development for field application of BGA strains. For that purpose three BGA strain namely *Anabaena torulosa*, *Anabaena doliolum* and *Calothrix marchica* were isolated from selected rice field of Assam, North East India. Growth behavior and potentiality of all the three strains were explored in terms of their biomass production, chlorophyll-a and total chlorophyll content, packed cell volume (PCV) and their production of IAA like substances. The Intrinsic antibiotic resistance profile (IARP) test and compatibility study among the three aforementioned BGA isolates were also carried out for their efficient growth and biomass production for application and development of biofertilizer technology. Three bio-wastes namely paddy straw, sugarcane trash and water hyacinth were analyzed for substrate preparation. It was *Anabaena torulosa* which showed maximum biomass yield of 18.33 mg/100 ml and N-content of 10.16% obtained when paddy straw was taken as substrate. Again paddy straw showed best result of 28.16 mg/100 ml and N-content of 20.33%, when composite inoculation of all the three stains was considered. Based on these findings an integrated BGA immobilized inoculum was also formulated with *Luffa cylindrica* which is locally known as bhol and with sugarcane trash. The experiment conducted was aimed to achieve suitable biofertilizer production at a very low cost using cheapest substrate which is far superior to harmful chemical fertilizers available in market. (Source – Malakar and Kalita Annals of Biological Research 2012 Vol. 3 No. 2 pp. 814-828)

Potential of *Bacillus cereus* strain RS87 for partial replacement of chemical fertilizers in the production of Thai rice cultivars - There is increasing interest in the development of technologies which can reduce the requirement for chemical fertilizers in rice production. The objective of this study was to investigate the efficacy of *Bacillus cereus* strain RS87 for the partial replacement of chemical fertilizer in rice production. A greenhouse experiment was designed using different fertilizer regimes, with and without strain RS87. Six Thai rice cultivars were tested separately. Maximum rice growth and yield were obtained in rice receiving the full recommended fertilizer rate in combination with the strain RS87. Interestingly, all rice cultivars which were treated with strain RS87 and 50% recommended fertilizer rate provided equivalent plant growth and yield to that receiving the full recommended fertilizer rate only. A paired comparison between rice treated with 50% of the recommended fertilizer rate with the bacterial inoculant and the full fertilizer rate alone was further examined in small experimental rice paddy fields. Growth and yield of all rice cultivars which received the 50% fertilizer rate supplemented with strain RS87 gave a similar yield to that receiving the full fertilizer rate alone. Bacterial strain RS87 showed the potential to replace 50% of the recommended fertilizer rate for yield production. Integration of plant growth-promoting rhizobacterial inoculants with reduced application rates of chemical fertilizer appears promising for future agriculture. (Source – Jetiyanon and Plianbangchang, Journal of the Science of Food and Agriculture Special Issue: 2nd International Symposium on Sustainable Agriculture in Sub-tropical Regions Volume 92, Issue 5, pages 1080–1085, 30 March 2012)

The Potential of Rhizosphere Microorganisms to Promote the Plant Growth in Disturbed Soils - The significance of rhizosphere microorganisms, especially mycorrhizal fungi and bacteria, in polluted soils can be enormous, since they are able to increase the tolerance of plants against abiotic stress, stimulate plant growth and contribute in this way to an accelerated remediation of disturbed soils. The majority of known higher plant species is associated with mycorrhizal fungi, which can increase the tolerance of plants against abiotic stress, e.g. by an improved nutrient supply or by detoxification of pollutants. Rhizosphere bacteria can strongly promote the growth of plants solely and in interaction with mycorrhizal fungi. The application of microbial inoculum for the remediation of disturbed soils was tested with several plant species, e.g., fast growing tree species, but mostly on a small scale. Main reasons for the lack of field applications in a larger scale are the lack of suitable time- and cost-effective strategies for a site-specific selection, preparation and application of microbial inoculum and the strong restriction of information on on-site efficiency of inoculated microbial strains. This chapter focuses on fundamental and applied aspects of soil microorganisms associated with the rhizosphere of plants at various disturbed sites. Major objectives are to present strategies for the promotion of phytoremediation of disturbed soils with the use of microbial inoculum and to indicate potentials and limitations of such microbial inoculation in the field. (Source - Katarzyna Hrynkiewicz and Christel Baum Environmental Protection Strategies for Sustainable Development, Strategies for Sustainability, 2012, 35-64)

Survival of endophytic bacteria in polymer-based inoculants and efficiency of their application to sugarcane - Studies have been conducted to evaluate maintenance of cell viability and stability, as well as to select cheap carriers to extend the shelf life of plant beneficial bacterial inoculants for agricultural crops. The purpose of this study was to evaluate the shelf life and the colonization efficiency of novel liquid and gel-based inoculant formulations for sugarcane. The different inoculant formulations were all composed of a mixture of five strains of diazotrophic bacteria (*Gluconacetobacter diazotrophicus*, *Herbaspirillum seropedicae*, *H. rubrisubalbicans*, *Azospirillum amazonense* and *Burkholderia tropica*), which are recognized as sugarcane growth promoters. Different inoculant formulations containing as carrier the polymers carboxymethylcellulose (CMC) and corn starch (60/40 ratio) at five different concentrations (named PIC, for Polymeric Inoculant Carrier) were supplemented, or not, with 2 % MgO, an interfacial stabilizing agent. Bacterial survival in the different formulations during storage was evaluated under controlled conditions, and two experiments with mini-cuttings of sugarcane variety RB72454 were carried out under greenhouse conditions. Laboratory tests showed that in the formulation composed of 0.8 g of the polymeric mixture per 100 g of the final product (PIC 0.8), survival of *G. diazotrophicus* and *A. amazonense* was around 10^9 CFU·mL^{-1} after 120 days of storage, regardless of the supplementation with MgO. The other formulation (2.2 g of polymeric mixture, PIC 2.2) presented survival levels of 10^8 CFU·mL^{-1} for up to 60 days of storage for all the individual strains. In the greenhouse, sugarcane seedlings showed a positive growth response 50 days after inoculation when inoculated with the mixture of five bacteria, with and without PIC 2.2. The polymer carriers described here allowed for the long-term survival of the five different bacterial strains tested. In addition, short-term experiments in the greenhouse showed that their application as part of an inoculant on sugarcane cuttings was at least as effective in terms of bacterial colonization and the promotion of plant growth as that of the bacterial mixture without carriers. (Source – de Silva et al Plant and Soil 2012)

Isolation and Characterization of Bacterial Endophytes from *Lycopersicon esculentum* Plant and their Plant Growth Promoting Characteristics - The study was designed to isolate and characterize bacterial endophytes from root and stem of *Lycopersicon esculentum* plant which was collected from different region of Gujarat. Total 18 isolates of endophytic bacteria were selected in which, all the endophytic bacteria produced one or the other different characteristics involved in plant growth promotion. They either produced phytohormones like indole acetic acid, siderophore, protease, pectinase, organic acid showed antifungal activity, chromium tolerance and solubilized phosphate. Four of the strains among the 18 showed maximum positive results of plant growth promoting regulators (PGPR) test and among them best probable isolate was selected and thus its 16SrDNA was amplified and sequenced. Only HR7 endophyte of tomato turned out to be *Pseudomonas aeruginosa*. It's a gram negative coccobacili, sporeforming motile bacilli and show maximum PGPR activity. The results of present studies indicated that above strains might be endophytic and therefore, were associated with the plant growth (Source – Patel et al 2012, Nepal Journal of Biotechnology , Vol 2, No 1)

Book Reviews

Biofertilisers and Organic Fertilisers -- A Sourcebook-cum-Directory, by Dr HLS Tandon. 2011. pp. 156. ISBN: 81-85116-63-6.Fertiliser Development and Consultation Organisation, New Delhi (India), Price Rs 500 - Biofertilisers and organic fertilizers are two of the most important components of integrated nutrient management (INM). Biofertilisers mainly consist of living microbial inoculants which either make net additions to the nutrient supplies (as in case of N fixation) or as solubiliser/ mobilizer of nutrients already present in the soil (as in case of P/K-solubilizers, mycorrhiza etc). The aim of this publication is (i) to create greater awareness about these vital farm inputs which are now key components of INM (ii) to provide the contact details of producers and products available to the end users farmers and (iii) to stress that biofertilizers and organic fertilizers have a role to play in the entire land under agriculture/horticulture and not restricted to organic farming/production systems. Apart from an overview chapter, this book deals with the (ii) status of production of biofertilizers and organic fertilizers (ii) quality standards (iii) a directory of producers and technology providers followed by a listing of research and development resources, addresses of certification agencies for organic production, a detailed index followed by a short functional glossary of common terminology. This source book has been written for a diverse readership including the producers and marketers of biofertilizers and organic fertilizers, entrepreneurs planning to enter these fields, microbiologists, those engaged in waste recycling for manurial purposes, fortification of low-analysis organic materials/composts, plantation mangers, students and teachers in colleges and agricultural polytechnics, those practicing organic farming, persons interested in environmental aspects, technology transfer centers including *Krishi Vigyan Kendras* (KVKs), independent consultants/farm advisors, technical personnel of the departments of agriculture and horticulture and quality assessment laboratories, among others (AKY).

Biofertiliser Handbook Research Production to Application, by Dr P Bhattacharyya and HLS Tandon . 2012. ISBN: 81-85116- 64-4 Fertiliser Development and Consultation Organisation, New Delhi pp.190. Price Rs 600.00 - The Biofertiliser Handbook written by Dr P Bhattacharyya and Dr HLS Tandon provides a research-based, practically useful account of biofertilizers from production to practical application. The authors have covered all major N-fixers (*Rhizobium, Azotobacter, Azospirillum, Acetobacter*, Blue green algae, *Azolla*), P-solubilising biofertilisers and nutrient mobilisers such as Mycorrhiza. Some aspects of the versatile fungus Trichoderma are also covered. The various aspects dealt with in this handbook are: Biofertilizers – their classification, characteristics, role and mode of action; product characteristics, crop specificity, advantage and limitations; impact of biofertilizers on crops, soils and economics; Biofertilizer production technology; packaging, labeling, handling and storage of biofertilizers; biofertilizers production and consumption; biofertilizer promotion and marketing; biofertilizer application and practical recommendations; quality standards and methods of analysis (as per FCO); R&D efforts and resources; references and additional reading material; and finally a test by which the readers can test their biofertilizer knowledge (self test).The text is supported by a number of tables, diagrams, pictures and appendices. This handbook will be of direct interest and use to all those who are interested in biofertilizers as well as in integrated nutrient management (INM),for augmenting plant nutrient supplies furnished by soil reserves and external sources, in programmes which focus on the need to maintain and improve soil health. (AKY)