

# जैव उर्वरक सूचना पत्र

## BIOFERTILIZER NEWSLETTER

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## **From Editor's Desk**

Dear Readers

First decade of 21<sup>st</sup> century is witnessing the complete transformation of microbial inoculant industry. Changes are being brought in, not only in carriers, shape of the product, new microorganisms but also in production process with greater emphasis on process automation and sterility maintenance.

Emerging technologies and increasing commercialization has also necessitated the requirement of quality control regime and their regulatory mechanism. Four biofertilizers namely Rhizobium, Azotobacter, Azopsirillum and PSB were already under FCO since December 2009. New emerging biofertilizer, Mycorrhiza has been brought under the same mechanism recently.

A brief description on specifications, requirements and testing protocols for mycorrhizal biofertilizers, as notified under FCO (1985) is presented in this issue. On production front also changes are apparent and the industry is shifting its attention from biofertilizers to bio-pesticides on commercial grounds. Current status of biofertilizers and bio-pesticides during the year 2009-10 is also an attraction of this issue.

Shift in attention of industry from biofertilizers to other inoculants is a worrying trend, and scientists need to address the issue. Over the years biofertilizers have proved their potential and their usefulness in nutrient mobilization, plant growth promotion and soil microbial health is unquestionable, but deficiencies in technology and improper adoption of technology has adversely affected the reputation of biofertilizers as commodity. I think we all need to assess the scenario and work strongly to ensure the survival and sustainability of the proven technology.

For the benefit of readers, especially the scientific community abstracts of large numbers of research papers published during last one year has also been provided. I hope the readers will find the compilation very useful for enrichment of their bibliographical records.

Dr. A.K. Yadav  
Editor

# Quality Control Mechanism for Mycorrhiza Biofertilizer under Fertilizer Control Order

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## Preamble

Mycorrhiza is a known biofertilizer since long and has been found to be mobilizing various nutrients, including phosphorus, iron and zinc in adequate quantities. Mycorrhiza is also known for its potential in ensuring water and nutrients availability from deeper layers of soils, far away from the reach of roots and even in poor and depleted soils. But being obligate parasite it was difficult to produce mycorrhizal inoculants in commercial quantities at competitive costs. With the development in tissue culture based production technologies, now it has become possible to produce sterile mycorrhizal inoculants in commercial quantities with acceptable limits of total viable propagules.

Keeping in view of the acceptance of such technology by the industry and growing availability of product in the market it was felt necessary to institute a quality control mechanism to ensure consistently quality product to the farmers. Recently Govt of India vide Gazette notification Dated 8<sup>th</sup> November 2010 has notified the inclusion of Mycorrhizal biofertilizer under Fertilizer Control Order 1985. Salient features of the notification and

quality control mechanism are discussed here.

## Definition

Mycorrhiza is covered under the same definition specified for other biofertilizers which reads "*Biofertilizers means the product containing carrier based (solid or liquid) living microorganisms which are agriculturally useful in terms of nitrogen fixation, phosphorus solubilization or nutrient mobilization, to increase the productivity of the soil and/or crop*".

## Requirement of registration for manufacture

As per the requirement of FCO 1985, no person can manufacture any Bio-fertilizer including mycorrhiza unless such Bio-fertilizer conforms to the standards set out in the part A of schedule-III. Also no person can carry on the business of preparing any Biofertilizers till he obtain a certificate of manufacture under clauses 15 of FCO.

Any person desiring to obtain a Certificate of Manufacture for preparation of biofertilizer, shall make an application in Form D, in duplicate, together with a fee prescribed thereof

under clause 36, to Registering authority. On receipt of application under Clause 14 the registering authority shall, by order in writing, either grant or refuse to grant the certificate of manufacture in respect of biofertiliser and shall, within 45 days from the date of receipt of application, furnish to the applicant a copy of the order so passed. Where an application for a certificate of manufacture for biofertilisers or organic fertilizers is not refused under sub-clause (1), the registering authority, shall within 45 days from the date of receipt of the application, grant a certificate of manufacture in Form F.

#### **Requirement of registration for sale/ marketing**

Under these rules no person can sell, offer for sale or carry on the business of selling of biofertilizer at any place as wholesale dealer or retail dealer except under and in accordance with clause 8 of FCO and after obtaining necessary certificate for sale from issuing authority notified under FCO.

#### **Registering authority**

The State Government by notification in the Official Gazette, appoints the registering authorities for the purpose of this Order for industrial dealers, and may, in any such notification define the limits of local area within which each such registering authority shall exercise his jurisdiction. Generally Commissioner or Director of Agriculture of the concerned state act as Registration authority

### **Schedule III, Part A**

#### **5. Specifications of Mycorrhizal Biofertilizers**

i.	Form/base	Fine Powder/ tablets/ granules/ root biomass mixed with growing substrate
ii.	Particle size for carrier based powder formulations	90% should pass through 250 micron IS sieve (60 BSS)
iii.	Moisture content percent maximum	8 -12
iv.	pH	6.0 to 7.5
v.	Total viable propagules/ gm of product, minimum	100 /gm of finished product
V.	Infectivity potential	80 infection points in test roots/gm of mycorrhizal inoculum used

## **Part-B**

### **Tolerance limit of Biofertilizers**

In case of Mycorrhizal Biofertilizers, the viable propagules shall not be less than 80/ gm of product.

## **Part C**

### **Procedure for drawl of samples of Mycorrhizal Biofertilizers**

#### **1. General Requirements of Sampling**

- 1.0 In drawing, preparing and handling the samples, the following precautions and directions shall be observed.
- 1.1 Sampling shall be carried out by a trained and experienced person as it is essential that the sample should be representative of the lot to be examined.
- 1.2 Samples in their original unopened packets should be drawn and sent to the laboratory to prevent possible contamination of sample during handling and to help in revealing the true condition of the material.
- 1.3 Intact packets shall be drawn from a protected place not exposed to dampness, air, light, dust or soot."

#### **2. Scale of Sampling**

##### **2.1 Lot**

All units (containers in a single consignment of type of material belonging to the same batch of manufacture) shall constitute a lot. If a consignment consists of different batches of the manufacture the containers of the same batch shall be separated and shall constitute a separate lot.

##### **2.2 Batch**

All inoculant prepared from a batch fermenter or a group of flasks (containers) constitute a batch.

2.3 For ascertaining conformity of the material to the requirements of the specification, samples shall be tested from each lot separately.

2.4 The number of packets to be selected from a lot shall depend on the size of the lot and these packets shall be selected at random and in order to ensure the randomness of selection procedure given in IS 4905 may be followed."

#### **3. Drawal of Samples**

- 3.1 The Inspector shall take three packets as sample from the same batch. Each sample constitutes a test sample.
- 3.2 These samples should be sealed in cloth bags and be sealed with the Inspector's seal after putting inside Form P. Identifiable details such as sample number, code number or any other details which enable its identification shall be marked on the cloth bags.
- 3.3 Out of the three samples collected, one sample so sealed shall be sent to in-charge of the laboratory notified by the State Government under clause 29 or to National Centre for Organic Farming or to any of its Regional Centres. Another sample shall be given to the manufacturer or importer or dealer as the case may be. The third sample shall be sent by the inspector to his next higher authority for keeping in safe custody. Any of the latter two samples shall be sent for referee analysis under sub-clause (2) of clause 29B.
- 3.4 The number of samples to be drawn from the lot

### Lot/Batch Number of Samples

Upto 5,000 packets	03
5,001-10,000 packets	04
More than 10,000 packets	05

### Methods for analysis of mycorrhizal biofertilizers

#### 1. Estimation of pH

- Make 25 g of biofertilizer into a suspension in 50 ml of distilled water and shake on a rotary shaker for 2 hours.
- Filter through Whatman No. 1 or equivalent filter paper under vacuum using a Buchner funnel.
- Determine pH of the filtrate by pH meter.

#### 2. Estimation of moisture contents

Weigh to the nearest mg about 5 gm of the prepared sample in a weighed clean, dry Petri Dish. Heat in an oven for about 5 hours at 65°C ±1°C to constant weight. Cool in a dessicator and weigh. Report percentage loss in weight as moisture content.

#### Calculation:

Moisture percent by weight =

$$\frac{100(B-C)}{B-A}$$

A = Weight of the Petri Dish

B = Weight of the Petri dish plus material before drying

C = Weight of the Petri Dish plus material after drying

#### 3. Estimation of total viable propagules

##### 3.1. Harvesting of spores from finished product

### By sieving

#### Equipment and Reagent

Stalking sieves with nylon or stainless steel mesh and a large range of pore sizes for isolating spores from the carrier or soil sample

- 40-50 micron (0.04 mm) seive for small sized spores
- 100 micron (0.10 mm) sieve for medium sized spores
- 250 micron (0.25 mm) sieve for very large spores and sporocarps
- Wash bottles containing water
- Jars for collecting the sieving
- Stereo zoom (stereomicroscope)
- Petri dishes ( 11 cm ) for observing the sieving under stereomicroscope
- Micropipettes for spore picking
- Centrifuge

**Procedure** - Mix the Soil in a substantial volume of water and decant through a series of sieves arranged in descending order of mesh size. Roots and coarse debris are collected on a coarse (60-ISS) sieve, while spores are captured on one or more finer sieves. Vigorous washing with water is necessary to free spores from aggregates of clay or organic materials. Collect the sieving in jars. Transfer the sieving onto the grided petri dishes/plate and observe under stereomicroscope. Count the number of spores in plate/dish and express it as spores/g of the sample.

### By sucrose gradient

- i. Collect the sieving by the method described above. Transfer the sieving into centrifuge tubes and centrifuge for 5 minutes at 1750 rpm in a horizontal rotor.

- ii. Decant the supernatant liquid carefully and resuspend pellet in 60% sucrose solution. Again centrifuge for 2 –5 minutes
- iii. Pour the supernatant (with spores) onto a 300BSS sieve size and rinse with water to remove the sugar. Transfer the sieving onto the grided petri dishes/plate and observe under stereomicroscope. Count the number of spores in plate/dish and express it as spores/g of the sample.

### 3.2. Spore staining Equipment and Reagent

1. Equipments and reagents for spore extraction as described previously.
2. 2,5-diphenyl-2N-tetrazolium bromide (MTT).
3. Distilled water
4. Eppendorf
5. Stereomicroscope
6. Petri-dishes

### Procedure

- Prepare 0.25% solution of MTT (2,5-diphenyl-2N-tetrazolium bromide).
- Avoid exposure of MTT solution to light, as the stain is light sensitive.
- Add freshly collected AMF spores (approximately 100 in number) collected by any of the two methods described above, to the staining solution and incubate at 27°C in sterile eppendorf in dark.
- Observe the spores for different colour reactions using stereomicroscope under dark field after 24 hours, 48 hours and 72 hours of incubation.
- Spores, which stained red or pink, are treated as viable.

### % Spore viability =

$$\frac{\text{No. of spores which stained red or pink}}{\text{Total Number of spores}} \times 100$$

### 4. Assessment of Infectivity Potential

The bioassay is used to determine the number of infective propagules present in the product. Once the infective propagules (spores, mycelia and vesicles in the root fragments) come in contact with the host roots they give out a turgid mycelial structure - the appressoria, which is the initial step in the penetration event. This appressoria enters the root through an 'entry point'. This entry point can be visualized by staining and enumerated as a measure of the infectivity of the inoculum. Host plants are grown from pre germinated seeds and a known weight of the inoculum is applied to experimental host plant in pots. These pots are maintained for 14 days after which they are harvested, the root length measured and then stained. The resulting entry points are counted to ascertain the infectivity potential.

#### (A) Equipments and Reagents

- a. Pots (5 x 7 cm in size)
- b. Sorghum seeds (*Sorghum vulgare*)
- c. Scissors and needles
- d. Petri dish ( grided )
- e. Water bath
- f. Glass slides and cover slips
- g. Compound microscope
- h. Coarse sieve to prevent root loss during washing/changing solutions

- i. Plastic vials with tight-sealing lids for storage of stained samples in 50% glycerol
  - j. Potassium hydroxide solution (5-10%)
  - k. Alkaline H<sub>2</sub>O<sub>2</sub> (25% Ammonia solution: 3 ml + 10% H<sub>2</sub>O<sub>2</sub>: 30 ml + Distilled water 67 ml)
  - l. 1% HCl
  - m. 50% glycerol-water (v/v) solution for de-staining and storage of stained roots.
  - n. Lactoglycerol (Lactic acid: 876 ml + Glycerine: 64 ml + Distilled water: 60 ml)
- Clear the roots in KOH solution and stain the root pieces (described below)
  - Count the number of infection points/entry points formed on randomly picked 100 segments
  - Calculate the average number of entry points formed in 1 cm segment
  - Calculate the total number of infection points/infective propagules (IP) by multiplying the average number of entry points formed in 1 cm segment by the total root length.
  - Extrapolate the IP present as numbers per gram of substrate/inoculum

### **(B) Staining solutions**

- a. 0.01 % acid fuchsin: 0.01 g acid fuchsin in 100ml acetoglycerol.
- b. 0.05% trypan blue: 0.05 g trypan blue in 100ml acetoglycerol.
- c. 0.03% Chlorozol black E ( CBE) in lactoglycerol ( 1:1:1 lactic acid, glycerol and water ).
- d. Dissolve CBE in water before adding equal volumes of lactic acid and glycerol.

### **(C) Procedure**

- Place 100 g test sample in a pot
- Dilute the inoculum with sterilized sand if the inoculum is very rich
- Plant 10-12 pre germinated seeds of Sorghum and grow for 14 days
- Harvest the pots and recover roots (fine roots can be rescued using sieve) completely.
- Chop the roots equally 1 cm in length.
- Measure and record the root length (using grid line intersect method described below) from each sample/dilution.

### **(D) Estimation of root length (Tennant D 1975)**

#### **(1) Equipment**

- a. Scissors
- b. Petri dish (9 cm in size consisting 1.33 cm x 1.33 cm grids )
- c. Wash bottle
- d. Stereo zoom microscope

- (2) The lines intersect (Tennant 1975) method is used to estimate the length of hyphae and roots. Root length is measured by dispersing roots against a grid of squares on the bottom of a tray. The roots are spread apart from one another over a grid in 2 mm to 10-mm depth of water. The eyes of the observer are cast along all the horizontal and vertical lines of the grid and root is counted using a hand held click counter.

The root length is calculated as follows:

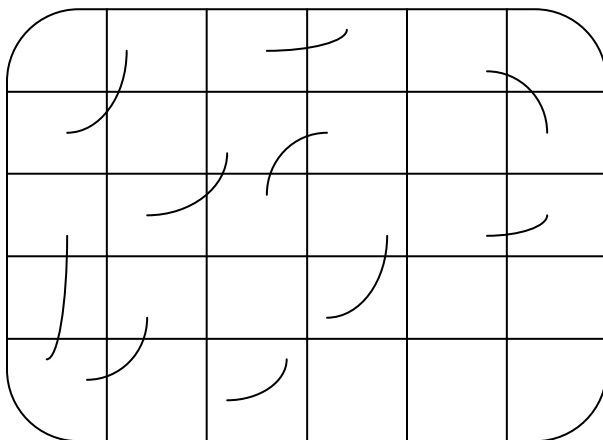


**Root length = No. of intersects x 11/14 x grid size**

Where, 11/14 is a constant, and the size of the grid is the length of one side of one square of the grid.

**Counting root intersections**

- a. Randomly disperse root in dish with grid lines.
- b. Count the intersects on roots across the horizontal and vertical lines.
- c. An example of 10 root segments is presented to show how the root length is calculated



Horizontal intersects (HI)
2
2
2
2
2
(Total 8 HI)

Vertical intersects (VI) (Total 7 VI)				
2	1	2	0	2

Total number of intersects = HI+VI = 8 + 7 (example) = 15

Thus, the root length = 11/14 X 15 X 1 (as the grid size is 1cm) = 11 cm (example)

**(3) Clearing and staining root specimens**

Clearing and staining procedures requires root samples that should be washed free of soil. It is important that KOH and staining solution volumes are sufficient for the amount of roots being processed and that, roots are not tightly clumped together for uniform contact with solutions. To ensure uniform staining, the roots should

be chopped in to smaller (1-2 cm) segments.

- (a) Wash root specimens under running tap water thoroughly. Place them in beaker containing 5-10% KOH solution for about 15-30 minutes. The concentration of KOH and time of incubation of roots depend upon the age and tenderness of the roots.

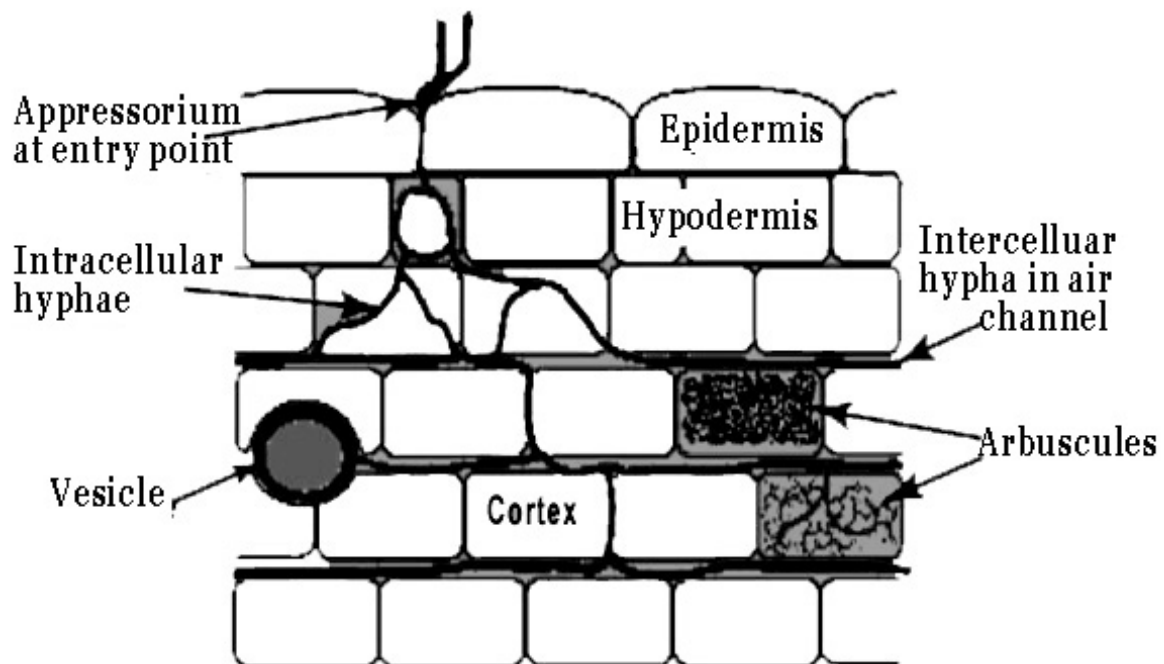
- (b) Pour off the KOH solution and rinse the roots well in a beaker using at least three complete changes of tap water or until no brown colour appears in the rinse water.
- (c) Cover the roots with alkaline H<sub>2</sub>O<sub>2</sub> at room temperature for 10 minutes or until roots are bleached.
- (d) Rinse the roots thoroughly using at least three complete changes of tap water to remove the H<sub>2</sub>O<sub>2</sub>.
- (e) Cover the roots with 1% HCl and soak for 3-4 min. And then pour off the solution. DO NOT rinse after this step because the specimens must be acidified for proper staining.
- (f) Incubate the roots with staining solution (0.01% acid fuchsine in lactoglycerol or 0.05% trypan blue in lacto phenol) and keep them overnight for staining.
- (g) Place the root specimens in glass petriplate /multiwell plate for destaining. The destaining solution

(50% glycerol) is the standard used in step 4, but of course, without the stain.

### (3) Sample storage and slide preparation

If clearing and staining is not possible immediately then fresh roots can be kept moist and stored at 5 °C (for several days), or may be preserved in 50% ethanol for months together in tightly sealed vials.

Staining quality is subsequently improved by destaining roots in 50% glycerol for several months prior to observation to allow excess stain to leach from roots. Semi-permanent slides of stained roots can be made with PVLG mountant. For temporary slide the stained roots can be observed in plain lactoglycerol



# Biofertilizers Production Scenario 2009-10

A Compilation By  
National and Regional Centers of Organic Farming under  
National Project on Organic Farming

## Background

Biofertilizer industry in India now-a-days is passing through a phase of transformation and various changes are being introduced both at production and quality control front. In marketing also many new efforts are being made and a small quantity of biofertilizers has also started leaving Indian shores and is being used in some other countries. Implementation of FCO (1985) was finally enforced after many policy hiccups and representations by the industry.

On quality front industry now seems to be more serious and is trying to bring in really international class products in the form of liquid inoculants. In production infrastructure also modernization and process automation is finding its place and are contributing to the better quality of the product.

One of the most important features, the biofertilizer industry has witnessed in the recent times, which has boosted their economic viability, is the introduction of microbial pesticides (popularly known as Bio-pesticides). Now practically more than 75% of commercial biofertilizer units are integrated production units of both biofertilizers and bio-pesticides. Surprisingly in spite of being recent entrants, bio-pesticides popularity has grown much faster than biofertilizers

and they alone accounts for nearly 70% of total agriculturally useful microbial products.

## Biofertilizer industry in 2009-10

As per the information gathered by NCOF/RCOFs, the installed production capacity of all microbial inoculants (Biofertilizers + bio-pesticides) production units in India has increased by 25% and as on March 2010 stood at 86078.00 tons. Phosphate solubilizing bacterial (PSB) biofertilizer, *Azotobacter*, *Azospirillum* and *Rhizobium* continue to be important biofertilizers followed by Mycorrhiza, and *Trichoderma* compost enhancers. Among bio-pesticides important ones are *Trichoderma viride*, *T. harzianum*, *Pseudomonas fluorescens*, *Metarhizium anisopliae*, *Bauveria bassiana* and *Verticillium lacini etc.* Although, overall production of Biofertilizers seems to have declined during the year 2009-10 compared to previous year, but the total production of inoculants remained static at above 65,000 MT. The reduction in biofertilizer production is probably due to better acceptance of bio-pesticides in the market, better profit margins and better price realization compared to biofertilizers. Implementation of FCO and introduction of mandatory registration may also have added to some shift.

Overall production of different biofertilizers, including other inoculants during the year 2009-10 is given in Table 1. State-wise details in respect of actual production of different biofertilizers during the year 2008-09 are given in Table 2. State wise details in respect of installed production capacity and actual production of total biofertilizers and other inoculants are given in Table 3.

**Table 1. Overall Production of Different Inoculants in the Country during the Year 2009-10**

Sr. No.	Name of Biofertiliser/ Inoculants	Production (in tones)	% share in total production
1.	<i>Azotobacter</i>	3196.62	4.87
2.	<i>Azospirillum</i>	1667.9	2.5
3.	<i>Rhizobium</i>	2339.32	3.57
4.	Phosphate solubilising Microorganism	12836.4	19.59
	<b>Total Biofertilizers</b>	20040.34	30.58
5.	Other Inoculants*	45475.90	69.41
	<b>Grand Total (all inoculants)</b>	65516.25	100

**Table 2. State Wise Production of different Biofertilizer and other microbial inoculants in 2009-10**

SN.	Name of State	Biofertilizer Production during the year 2009-10						
		Azoto	Azosp	Rhizo	PSB	Total BF	Other Inoculants *	Grand Total
1	Andhra Pradesh	126.03	214.12	34.13	971	1345.28	326.2	1671.48
2	Assam	22.2	22.74	9.2	66.9	121.04	0	121.04
3	Bihar	0	0	0	0	0	0	0
4	Delhi	907.07	35.68	9.73	69.37	1021.85	211.99	1233.84
5	Gujarat	366.59	125.51	99.64	717.45	1309.19	48.69	1357.88
6	Goa	0	0	0	0	0	8.44	8.44
7	Haryana	1.2	0	1.725	3.27	6.195	647.0778	653.2728
8	Himachal Pradesh	1.4	0.6	6	0.5	8.5	0	8.5
9	Jharkhand	0	0	0	15	15	0	15
10	Karnataka	250.165	485.25	358.96	2601.12	3695.5	18109.126	21804.626
11	Kerala	40.53	300.00	170.46	1425.46	1936.451	6736.447	8672.898
12	Madhya Pradesh	173.401	25.364	396.137	992.775	1587.6775	83.5	1671.1775
13	Maharashtra	463.13	92.29	298.65	1007.26	1861.33	250.86	2112.19
14	Mizoram	0.65	0.47	0.68	0.7	2.5	0	2.5
15	Nagaland	4	1.25	2.8	10.2	18.25	0	18.25
16	Orissa	79.616	23.571	122.748	63.931	289.867	12.149	302.016
17	Punjab	1.34	0	0	299.892	301.232	0	301.232
18	Pondicherry	10.64	2.8	53	386.35	452.79	1137.63	1590.42
19	Rajasthan	156.692	0	140.854	508.025	805.571	0	805.571
20	Tamil Nadu	49.4	244.76	531.813	2906.61	3732.5862	17622.492	21355.078
21	Tripura	98.002	90	2	88.4	278.402	0	278.402
22	Uttar Pradesh	375.669	0	89.4004	497.571	962.6417	7.5	970.1417
23	Uttarakhand	7.9	3.5	11.4	9.2	32.00	273.8	305.80
23	West Bengal	61	0	0	195.5	256.5	0	256.5
	Total	3196.62	1667.9	2339.32	12836.4	20040.34	45475.90	65516.25

**Table 3. State Wise Production Capacity and actual production of Biofertilizer and other microbial inoculants in 2009-10**

S. No.	State	Capacity	Total Biofertilizer Production (MT)	Other Inoculants*	Total Production (in MT)
1	Andhra Pradesh	5825	1345.28	326.2	1671.48
2	Assam	225	121.04	0	121.04
3	Bihar	0	0	0	0.00
4	Delhi	2000	1021.85	211.99	1233.84
5	Gujarat	1550	1309.19	48.69	1357.88
6	Goa	1000	0	8.44	8.44
7	Haryana	775	6.20	647.08	653.27
8	Himachal Pradesh	25	8.50	0	8.50
9	Jharkhand	50	15.00	0	15.00
10	Karnataka	25488	3695.50	18109.126	21804.63
11	Kerala	10400	1936.45	6736.45	8672.90
12	Madhya Pradesh	1750	1587.68	83.5	1671.18
13	Maharashtra	5315	1861.33	250.86	2112.19
14	Mizoram	75	2.50	0	2.50
15	Nagaland	150	18.25	0	18.25
16	Orissa	470	289.87	12	302.02
17	Punjab	575	301	0	301.23
18	Pondicherry	1900	452.79	1137.63	1590.42
19	Rajasthan	1000	805.57	0	805.57
20	Tamil Nadu	25265	3732.59	17622.49	21355.08
21	Tripura	300	278.40	0	278.40
22	Uttar Pradesh	1090	962.64	8	970.14
23	Uttarakhand	550	32.00	274	305.80
24	West Bengal	300	256.50	0	256.50
	<b>Total</b>	<b>86078.00</b>	<b>20040.35</b>	<b>45475.9058</b>	<b>65516.2542</b>

### **Castor De-oiled Cake also notified as Organic fertilizer under FCO (1985)**

Keeping in view of the growing role of various organic fertilizers, Govt of India vide Gazette Notification Dated 03.12.2010, notified the Castor De-oiled cake meal as organic fertilizer under separate category in schedule V along with its specifications.

As per the notification the De-oiled cake fertilizer have been defined as “substance obtained as residue after oil extraction (by expeller and/or through solvent extraction) from crushed seeds of non-edible oilseeds (such as castor, neem) for use in soil as fertilizer.

The specifications include: (a) moisture % by weight maximum – 12, (b) colour – Brown to black, (c) Odour - Typical oily odour specific to the oil of that seed and no foul odour (d) Total ash content % maximum 15, (e) Total organic carbon % by weight minimum – 25, (e) Total NPK in % minimum – 4.5, 1.0 and 1.0 respectively.

## Research Notes

### **Standardization of liquid formulation of *Pseudomonas fluorescens* Pf1 for its efficacy against *Fusarium* wilt of tomato -**

*Pseudomonas fluorescens* strain Pf1 is studied as an effective biocontrol agent for the management of plant diseases and plant growth-promoting bacteria. Previous findings from author's research group demonstrated that talc-based *P. fluorescens* Pf1 formulation effectively reduced several plant diseases in addition to promoting plant growth. The modernization of agro-techniques necessitates the development of a new formulation where liquid inoculants can play a significant role. Different chemicals such as trehalose, polyvinylpyrrolidone and glycerol were tested for the development of liquid formulation. Among these, glycerol amendment maintained the greater population level of *P. fluorescens* Pf1 up to 6 months of storage. Further, a study was conducted to standardize the dose of liquid-based formulation of Pf1 for seed treatment and seedling dip. An application of 10 ml kg<sup>-1</sup> of seeds and 150 ml ha<sup>-1</sup> of seedlings was found to be optimum for seed treatment and seedling root dip, respectively. The growth-promoting and antagonistic activities of Pf1 cultures of different ages were found to be greater up to 180 days of storage without much loss in viability of cells. The combination of seed treatment, seedling dip and soil drenching of liquid formulation recorded the minimum disease incidence of *Fusarium* wilt on tomato

under glasshouse (17.33%) and field (4.81%) conditions. In addition, the liquid formulation increased the tomato fruit yield compared to untreated control under glasshouse and field conditions. Thus, this study offered successful technology for development of a liquid-based bioformulation *P. fluorescens* Pf1. (Source – Manikandan et al 2010 Biological Control Volume 54, (2) : 83-89)

### **Natural compounds enhancing growth and survival of rhizobial inoculants in vermicompost-based formulations -**

The present study demonstrates the usefulness of natural microbial growth-promoting compounds for improving the stability and life of vermicompost-based (both granular and its aqueous extract) bioformulations. Granular vermicompost maintained the number of cells of *Rhizobium meliloti* Rmd 201 up to  $5.9 \times 10^8$  after 180 days at 28 °C compared with  $2.1 \times 10^8$  in charcoal (powdered), while aqueous extract of the vermicompost supported the  $5.6 \times 10^7$  rhizobia numbers even after 270 days. The addition of 25 µL/mL cow urine and 0.01 mM calliterpinone, a natural plant growth promoter, increased the rhizobia number significantly in granular vermicompost and its aqueous extract, respectively. (Source Kalra et al Biology and Fertility of Soils 2010 Volume 46, Number 5, 521-524)

### **Evaluation of shelf life and rock phosphate solubilization of**

**Burkholderia sp. in nutrient-amended clay, rice bran and rock phosphate-based granular formulation** - Five phosphate-solubilizing bacteria (PSB) used in this study were isolated based on their ability to solubilize tricalcium phosphate (TCP) in Pikovskaya's medium. Among the tested bacterial strains *Burkholderia* sp. strain CBPB-HIM showed the highest solubilization ( $363 \mu\text{g}$  of soluble  $\text{P ml}^{-1}$ ) activity at 48 h of incubation. Further, this strain has been selected to assess its shelf life in nutrient-amended and unamended clay, rice bran and rock phosphate (RP) pellet-based granular formulation. The results showed that the maximum viability of bacterium was observed in clay and rice bran (1:1) + 10% RP pellets than clay-RP pellets, irrespective of tested storage temperatures. Further, clay and rice bran (1:1) + 10% RP pellets amended with 1% glucose supported the higher number of cells compared to glycerol-amended and nutrient-unamended pellets. In this carrier solubilization of Morocco rock phosphate (MRP) by *Burkholderia* sp. strain CBPB-HIM was also investigated. The maximum of water and bicarbonate extractable P ( $206$  and  $245 \mu\text{g P g}^{-1}$  of pellet respectively) was recorded in clay and rice bran (1:1) + 10% RP pellets amended with 1% glucose and glycerol respectively on day 5 of incubation. Therefore, this study proved the possibility of developing granular inoculant technology combining clay, rice bran and RP as substrates with phosphate-solubilizing *Burkholderia* (Source - Anandham, et al 2007 World Journal of Microbiology and Biotechnology Volume 23, Number 8, 1121-1129)

**Simultaneous P-solubilizing and biocontrol activity of microorganisms: potentials and future trends** - Phosphate (P)-solubilizing microorganisms as a group form an important part of the microorganisms, which benefit plant growth and development. Growth promotion and increased uptake of phosphate are not the only mechanisms by which these microorganisms exert a positive effect on plants. Microbial mediated solubilization of insoluble phosphates through release of organic acids is often combined with production of other metabolites, which take part in biological control against soil borne phytopathogens. *In-vitro* studies show the potential of P-solubilizing microorganisms for the simultaneous synthesis and release of pathogen-suppressing metabolites, mainly siderophores, phytohormones, and lytic enzymes. Further trends in this field are discussed, suggesting a number of biotechnological approaches through physiological and biochemical studies using various microorganisms. (Source - Nikolay Vassilev et al 2006 Applied Microbiology and Biotechnology 71(2) 137-144)

**Four Materials as Carriers for Phosphate Dissolving *Rhizobium* Sp. Inoculants** - The most commonly used carrier for commercial inoculants, is peat. But its availability is limited and it is a nonrenewable resource. Many other materials have been evaluated as alternatives to peat as carriers of rhizobia, yet seldom have been included in inoculants of phosphate dissolving rhizobia. As accessible and

inexpensive carriers for rhizobial inoculants, corn stalk powder, loessal soil and vermiculite powder were used in the study to compare with peat carrier on the capacities of rhizobial solution absorption, pH value maintaining of microenvironment, viable rhizobial cells and the control of contamination. Completely randomized design and 4 replicates were used in the experiment. Twelve different compositions of selected inoculant carrier with different absorption volumes of rhizobial suspension, were evaluated for the ability of maintaining viable rhizobial cells and undesired microbes during the period of 120 days at room temperature. Thereafter, pH value, viable rhizobial cell number and undesired microbes of inoculants with selected absorption volume of rhizobial suspension that stored at 4°C and room temperature respectively. Viable rhizobial cells in inoculants were examined after 120d and 1year storage by plate counting method, and ratio of undesirable microbes was examined by antibiotic-carrying and normal plates counting method. The result indicated that: for a period of 120days at room temperature, maximum viable rhizobial cells were found in peat, vermiculite powder, corn stalk powder and loessal soil based inoculants when the absorption volume of rhizobial suspension of inoculants were 450, 500, 1000 and 200ml/kg, respectively; viable rhizobial cell numbers were better maintained in corn stalk powder than in peat, loessal soil and vermiculite, but undesired microbes contamination was a severe problem. In the study, viable rhizobial cell numbers in loessal soil was found the highest, followed by peat, while the most serious contamination was found

in peat inoculants. Corn stalk powder and vermiculite could not be used as inoculant carrier because fewer viable rhizobial cells existed in these inoculants. The greatest pH change was found in peat and loessal soil based inoculants during 1year storage because of enhanced acidification caused by metabolism of phosphate dissolving rhizobia; more viable rhizobial cells were found in the 4 carriers that stored at 4°C than at room temperature after 1year storage. As carriers of phosphate dissolving *Rhizobium* inoculants, viable rhizobial cells of corn stalk powder after short time storage (120d) and of loessal soil after long time storage (1year) were found better than that of peat, and was also found more cost effective compared with peat, commercially. Both of the two carriers could be used as inoculant carriers at room temperature, but corn stalk powder could only be used as carrier with short shelf life. (Source - Jian Feng Li et al., 2010, Advanced Materials Research, 156-157, 919-928)

#### **Effect of Ampicillin as Bacteriostats on the Performance of Phosphate Solubilizing *Rhizobium meliloti* Inoculant**

The aim of this study was to evaluate the effect of Ampicillin as bacteriostats on the number of P solubilizing and antibiotic-anti-*Rhizobium meliloti* cells and contamination in inoculants during one year storage, and plant promotion ability of subgeneration cells of antibiotic-resistant phosphate-solubilizing *R. meliloti* strain LW107. Growth on YMA plates containing different concentrations of ampicillin were also investigated; in the first experiment, 100 mg liter<sup>-1</sup> ampicillin



was added in inoculants, number of *R. meliloti* cells and contamination in inoculants were investigated at two temperatures, using both liquid and peat based solid inoculants in experiment. Statistical design was complete randomized block in a factorial 2×2×2 experimental arrangement with 4 replicates. Results show huge variability in viable cells and contamination levels in inoculants. The ampicillin increased *R. meliloti* LW107 viable cells and inoculants stored at low temperature with lesser contamination when ampicillin has been added. To clarify whether ampicillin affect the main character of *R. meliloti* LW107 cells, the ability of calcium phosphate solubilization and IAA production in ampicillin-containing conditions was determined. Results indicated that the ability of calcium phosphate solubilization and IAA production have no significant difference in 6th generation cells of *R. meliloti* LW107 when grown with or without ampicillin. In the third experiment, the plant growth promotion of the ampicillin-containing inoculants on alfalfa seedlings was determined in sterile sand conditions in a temperature-controlled growth chamber at 20 -25°C for 35 days. Authors also found no significant difference in nodule numbers between diluted liquids ampicillin-containing inoculants and the common liquid inoculants without ampicillin, but plant growth and efficiency of nitrogen-fixing responded to undiluted liquids inoculants containing ampicillin with a decrease in biomass and nitrogenase activity indicating that the five-fold dilutions of the ampicillin-containing inoculants are necessary for applications (Source - Jian Feng Li et

al., 2011, Advanced Materials Research, 201-203, 1023)

### **Polymers as carriers for rhizobial inoculant formulations**

- The aim of this work was to evaluate the efficiency of carboxymethyl cellulose (CMC) and starch blends as carrier materials of rhizobial inoculants regarding their capacity to maintain viable cells and promote cowpea (*Vigna unguiculata*) nodulation. The experimental design adopted was completely randomized, with three replicates. Forty different compositions of carboxymethyl cellulose (CMC) with starch, compatibilized or not with different proportions of MgO or ZnO, were evaluated regarding their ability of maintaining rhizobial viable cells during the storage period of one month at room temperature, in an initial screening. Thereafter, selected inoculant carrier blends were evaluated regarding their ability to maintain viable rhizobial cells for a period of 165 days, and their performance as inoculant carriers was compared to a peat-based inoculant carrier under greenhouse conditions. Rhizobial cells were better maintained in blends containing 50-60% CMC. Compatibilizing agents did not increase survival of rhizobial cells for 30 days of storage. The cowpea nodulation of polymer blends was statistically the same as of peat-based inoculants. CMC/starch polymer blends are efficient carriers to rhizobial inoculants for up to 165 days of storage, when compatibilized with MgO (1%). (Source - Paulo Ivan Fernandes Júnior et al 2009, Pesq. agropec. bras. vol.44 no.9 Brasília Sept. )

### **Alternatives to peat as a carrier for rhizobia inoculants: Solid and**

**liquid formulations** - Many of the microbial inoculants all over the world are based on solid peat formulations. This has been mostly true for well developed legume inoculants based on selected rhizobial strains, due to peat bacterial protection properties. Six carriers (bagasse, cork compost, attapulgite, sepiolite, perlite and amorphous silica) were evaluated as alternatives to peat. Compost from the cork industry and perlite were superior to peat in maintaining survival of different rhizospheric bacteria. Other tested materials were discarded as potential carriers for soybean rhizobia. Also, different liquid culture media have been assayed employing mannitol or glycerol as C sources. Some media maintained more than  $10^9$  cfu ml<sup>-1</sup> of *Sinorhizobium (Ensifer) fredii* SMH12 or *Bradyrhizobium japonicum* USDA110 after 3 months of storage. Rhizobial survival on pre-inoculated seeds with both solid and liquid formulations previously cured for 15 days led to a higher bacterial numbers in comparison with recently made inoculants. An additional curing time of solid inoculants up to 120 days had a beneficial effect on rhizobial survival on seeds. The performance of different formulations of two highly effective soybean rhizobia strains was assayed under field conditions. Soybean inoculated with cork compost, perlite and liquid formulations produced seed yields that were not significantly different to those produced by peat-based inoculants. (Source Albareda et al 2008 Soil Biology and Biochemistry 40, (11) : 2771-2779)

**Biological control of sunflower necrosis virus disease with powder**

**and liquid formulations of plant growth promoting microbial consortia under field conditions**

- Sunflower necrosis virus disease (SNVD) was reported to cause a significant damage to sunflower production in India. Based on the biocontrol efficacy against SNVD observed in the previous greenhouse experiments, two plant growth promoting microbial consortia (PGPMCs) viz., PGPMC-1 consisting of *Bacillus licheniformis* strain MML2501 + *Bacillus* sp. strain MML2551 + *Pseudomonas aeruginosa* strain MML2212 + *Streptomyces fradiae* strain MML1042 and PGPMC-2 consisting of *B. licheniformis* MML2501 + *Bacillus* sp. MML2551 + *P. aeruginosa* MML2212 were used in the present study. Powder and liquid formulations of the above plant growth promoting microorganisms (PGPMs) were evaluated along with farmers' practice (imidacloprid + mancozeb) and control in farmers' fields. Significant disease reduction, increase of seed germination, plant height and yield parameters with an additional seed yield of 840 kg/ha, an additional income of Rs. 10,920/ha and benefit cost ratio of 6.1 were recorded following treatment with a powder formulation of PGPMC-1 compared to control. Further, liquid formulation of PGPMC-1 significantly reduced SNVD up to 51.4% compared to control. Besides, this treatment improved the seed germination (24.4%), plant height (61.3%), yield parameters with an additional seed yield of 936 kg/ha and an additional income of Rs. 12,168/ha. Benefit cost ratio was calculated as 6.8 in this treatment compared to 4.6 and 3.7, respectively for PGPMC-2 liquid formulation and

farmers' practice compared to control. This is the first report of PGPMCs mediated biological control of SNVD under field conditions. (Source - Srinivasan and Mathivanan 2009 Biological Control 51(3) : 395-402)

### **Mycorrhizal Inoculants: Progress in Inoculant Production Technology**

Of the seven types of mycorrhizae, the symbiotic association of plants with arbuscular mycorrhizae (AM) and ectomycorrhizae (ECM) is the most abundant and widespread. Mycorrhizal inoculant technology, especially of AM and ECM, appears to be a promising avenue for sustainable agriculture and forestry because of their extensive and productive association with plants. Production of mycorrhizal inocula is a complex procedure that requires commercial enterprises to develop the necessary biotechnological skill and ability to respond to legal, ethical, educational, and commercial requirements. At present, commercial mycorrhizal inocula are produced in pots, nursery plots, containers with different substrates and plants, and aeroponic systems, and by nutrient film technique, or *in-vitro*. Different formulated products are now marketed, which creates the need for the establishment of standards for widely accepted quality control. Generally, preparation and formulation of mycorrhizal inocula are carried out by applying polymer materials with well-established characteristics and which are useful for agriculture and forestry. The most commonly used methods involve entrapment of fungal materials in natural polysaccharide gels, which includes immobilization of mycorrhizal root pieces, vesicles, and spores, in some cases co-entrapped with other

plant-beneficial microorganisms. Efforts should be devoted toward registration procedures of mycorrhizal inoculants to stimulate the development of mycorrhizal products industry. Biotechnology research and development in such activities must be encouraged, particularly with regard to interactions of mycorrhizal fungi with other rhizosphere microbes, and selection of new plant varieties with enhanced mycorrhizal traits to provide maximum benefits to agriculture and forestry. (Source - Siddiqui and Kataoka 2011 In Microbes and Microbial Technology 489-506)

### **Rhizosphere and Root Colonization by Bacterial Inoculants and Their Monitoring Methods: A Critical Area in PGPR Research**

Roots serve a multitude of functions in plants including anchorage, acquisition of nutrients and water, and production of exudates with growth regulatory properties. The root-soil interface, or rhizosphere, is the site of greatest biological and chemical activity within the soil matrix. Plant growth-promoting rhizobacteria (PGPR) are known to influence plant health by controlling plant pathogens or via direct enhancement of plant development in the laboratory and in greenhouse experiments. Unfortunately, however, results in the field have been less consistent. The colonization of roots by inoculated bacteria is an important step in the interaction between beneficial bacteria and the host plant. However, colonization is a complex phenomenon influenced by many biotic and abiotic parameters, some of which are only now apparent. Monitoring fate and metabolic activity of microbial inoculants as well as their impact on

rhizosphere and soil microbial communities are needed to guarantee safe and reliable application, independent of whether they are genetically modified or not. The first and most crucial prerequisite for effective use of PGPRs is that strain identity and activity are continuously confirmed. A combination of both classical and molecular techniques must be perfected for more effective monitoring of inoculants strain (both genetically modified and unmodified) after release into the soil. Recent developments in techniques for studying rhizobacterial communities and detection and tracking systems of inoculated bacteria are important in future application and assessment of effectiveness and consistent performance of microbial inoculants in crop production and protection. (Source Ahmad et al 2011 *Microbes and Microbial Technology* pp 363-391)

**A Roadmap to the Successful Development and Commercialization of Microbial Pest Control Products for Control of Arthropods** - Several regulations apply to the handling and use of microbial pest control agents. Microorganisms used for control of pests are subject to registration as a "plant protection product". The history of the development of data requirements for entomopathogens is presented. Current registration requirements are reviewed, particularly for the European Union. For several topics, data requirements are unclear or even lacking; procedures are both long and expensive. Initiatives to ameliorate these impediments are reviewed. The EU project REBECA provided recommendations for

improvement of procedures as well for data requirements. The OECD BioPesticides Steering Group continues this work and is developing guidance for several data requirement issues. A general estimate of costs for the generation of a registration dossier is provided. Entomopathogenic nematodes are not covered by the legislation of plant protection products in the EU. Several countries, however, have a registration for macroorganisms in place. Regulations for import and for export, and for release of macroorganisms have become complex and an administrative burden for biocontrol companies. The current situation in the Netherlands is highlighted as an example. Various other regulations, such as laws on biodiversity, importation and release of exotic organisms, and safe handling apply to the use of microorganisms, and are briefly treated. Intellectual property rights are important for a company. The possibility to obtain a patent for a biopesticide and the value thereof will be discussed. Regulations form a major obstacle in the commercialization of biopesticides. Recommendations for improvements are presented for data requirements and for regulatory procedures. The role that the biocontrol industry should have in this political field is outlined. (Source - *Progress in Biological Control*, 2011, Volume 10, 171-233).

**Effect of Bio-fertilizer Inoculations on Growth and Yield of Dwarf Field Pea (*Pisum sativum* L.) in Conjunction with Different Doses of Chemical Fertilizers** - A field experiment was conducted during two consecutive Rabi seasons of 2007 and 2008 to study the effect of bio-

fertilizers in conjunction with inorganic fertilizers on growth and yield of dwarf field pea (cv. Jai) at Oil Seed Research Farm, Kalyanpur in C.S.A. University of Agriculture and Technology, Kanpur. The experiment was laid out in split plot design with three replications in sandy loam soil. The experiment comprised 32 treatment combinations of four levels of fertility (Control, 50, 75 and 100% RDF) and eight bio-fertilizer treatments (Control, *Rhizobium*, PSB, PGPR, *Rhizobium* + PSB, *Rhizobium* + PGPR, PSB+PGPR and *Rhizobium* +PSB+PGPR). Results indicated that the combined application of 100% RDF and seed inoculation with *Rhizobium*+PSB+PGPR improved the growth; yield attributes and yields of field pea. Fresh and dry weight plant<sup>-1</sup>, nodules number and dry weight plant<sup>-1</sup> were found significantly maximum. Number of grains pod<sup>-1</sup>, number and weight of pods plant<sup>-1</sup> at maturity attributed significantly to increasing the grain yield of field pea up to 31.00 q ha<sup>-1</sup> and net return up to Rs.26187 ha<sup>-1</sup> with the application of 100% RDF and seed inoculation of *Rhizobium* + PSB + PGPR, yield was 10.96 and 11.93% higher over co-inoculation of *Rhizobium* + PSB + PGPR (27.60 q ha<sup>-1</sup>) and 100% RDF (27.30 q ha<sup>-1</sup>) application. Thus, it can be recommended that to obtain the maximum grain yield and net profit from dwarf field pea, seed should be inoculated with *Rhizobium* + PSB + PGPR and crop should be fertilized with 100% recommended dose of fertilizer. (Source – Mishra et al J. Agronomy Year, 9 (4) : 163-168)

#### ***In vitro* Studies on the Effects of Biofertilizers (*Azotobacter* and *Rhizobium*) on Seed Germination**

**and Development of *Trigonella foenum-graecum* L. using a Novel Glass Marble containing Liquid Medium** - Biofertilizers are the formulations of living microorganisms, which are capable of fixing atmospheric nitrogen in the soil and thereby, increasing the crop yield. *Trigonella foenum-graecum* L. is a medicinally important plant, possessing anti-diabetic, anti-cancerous, anti-microbial and hypocholesterolaemic properties. The present study was conducted to develop an *in vitro* method for studying the effects of biofertilizers (*Azotobacter* and *Rhizobium*) on the seed germination and development of *Trigonella foenum graecum* L. using a simple and cost-effective liquid culture medium containing glass marbles as reusable and biologically inert support matrix. Sucrose optimization studies revealed maximum development for the plantlets grown on 1X Murashige and Skoog liquid medium containing 4% sucrose and glass marbles. *Azotobacter* and *Rhizobium* were isolated from rhizosphere soil and root nodules of *Trigonella* plants, respectively and identified following the standard procedures. Mass cultivation of the bacteria carried out for 5 days reported counts of 2.3x10<sup>4</sup> cells mL<sup>-1</sup>. The harvested bacterial cells were used to coat the seeds in the presence and absence of charcoal. After 15 days of growth under *in vitro* conditions, the root length, shoot length, fresh weight, protein, carbohydrate and chlorophyll contents of the plantlets were determined. Maximum growth was observed for the plantlets grown on 1X MS medium with 4% sucrose and glass marbles, inoculated with 40% concentration of *Azotobacter*,

*Rhizobium* and their co-inoculum mixed with charcoal. Field trials, conducted under green house conditions, revealed that 10% biofertilizer co-inoculum supported maximum growth of the plants when the seeds were coated with charcoal (Source – Nagananda et al 2010, Int J. Botany 6(4) : 394-403).

**Effect of bio-fertilizers on growth, yield and quality of onion cv. Sukhsagar-**

A field experiment was carried out during the winter season of two consecutive years 2006-07 and 2007-08 to study the effect of six combinations of bio-fertilizers and two chemical fertilizers on onion cv. Sukhsagar. The treatments were Azotobacter+PSB, Azotobacter+VAM, Azotobacter+Azospirillum, Azospirillum+PSB, Azospirillum+VAM, PSB+VAM, NPK 100%, NPK 50% and Control. The height of the plant was maximum (43.46cm) with the application of Azotobacter+VAM. No. of leaves, no. of inflorescence / plot and bulb diameter were maximum of Azotobacter+Azospirillum. Azotobacter+Azospirillum and NPK 100% gave maximum length of bulbs (6.03cm). The maximum number of scale per bulb (9.81) was counted from NPK 50%. The plants raised under NPK 100% produced the maximum bulb weight 67.45g. TSS % was found maximum (12.29%) from NPK 100% but the highest reducing sugar (1.420%) and starch percentage (6.27%) were noted from NPK 50%. The total loss of weight (%) up to 60 days, was found minimum (11.5%) from Azotobacter+PSB followed by Azotobacter+Azospirillum (14.32%). It is therefore, concluded that Azotobacter+Azospirillum combination

is the best for onion as compared to others so far as the sustainability in production and environmental consideration are concerned. (Source - Ghanti and Sharangi 2009 Journal of Crop and Weed 5(1): 127-130).

**Effect of biofertilizers, fertility level and weed management on weed growth and yield of late sown chickpea (*Cicer arietinum*)**

- A field experiment was conducted during winter (rabi) season of 2000-01 and 2001-02 at the Agriculture Research Farm of Institute of Agriculture Science, Banaras Hindu University, Varanasi, integrating biofertilizers, fertility levels and weed management to minimize weed interference for higher yield of late sown chickpea (*Cicer arietinum* L.). The fertility levels and biofertilizers applied to chickpea caused significant effect on the weed population, biomass and seed yield but no effect on seed protein content. Application of VAM alone was at par to inoculation of *Rhizobium* exhibited significantly lower weed population, dry matter accumulation and seed yield than the dual application of *Rhizobium* and VAM. Decreasing levels of fertility from 125 to 75% of recommended NPK dose exerted significantly marked reduction in population and dry matter of weeds and increased in seed yield by 0.43 tonnes/ha. Application of pendimethalin 0.5 kg ai/ha coupled with 1 hoeing at 40 days after sowing significantly reduced the density and dry matter of weeds which resulted in 16% higher seed yield over weedy check condition. (Source Singh et al 2006 The Indian Journal of Agricultural Sciences Vol 76, No 9 (2006))

**The nematicidal effect of some bacterial biofertilizers on *Meloidogyne incognita* in sandy soil**

- In a greenhouse experiment, the nematicidal effect of some bacterial biofertilizers including the nitrogen fixing bacteria (NFB) *Paenibacillus polymyxa* (four strains), the phosphate solubilizing bacteria (PSB) *Bacillus megaterium* (three strains) and the potassium solubilizing bacteria (KSB) *B. circulans* (three strains) were evaluated individually on tomato plants infested with the root-knot nematode *Meloidogyne incognita* in potted sandy soil. Comparing with the uninoculated nematode-infested control, the inoculation with *P. polymyxa* NFB7, *B. megaterium* PSB2 and *B. circulans* KSB2, increased the counts of total bacteria and total bacterial spores in plants potted in soil from 1.2 to 2.6 folds estimated at 60 days post-inoculation. Consequently, the inoculation with *P. polymyxa* NFB7 increased significantly the shoot length (cm), number of leaves / plant, shoot dry weight (g) / plant and root dry weight (g) / plant by 32.6 %, 30.8 %, 70.3 % and 14.2 %, respectively. Generally, the majority treatments significantly reduced the nematode multiplication which was more obvious after 60 days of inoculation. Among the applied strains, *P. polymyxa* NFB7, *B. megaterium* PSB2 and *B. circulans* KSB2 inoculations resulted in the highest reduction in nematode population comparing with the uninoculated nematode-infested control. They recorded the highest reduction in numbers of hatched juveniles/root by 95.8 %, females/root by 63.75 % and juveniles/1kg soil by 57.8 %. These results indicated that these bacterial biofertilizers are

promising double purpose microorganisms for mobilizing of soil nutrients (nitrogen, phosphate and potassium) and for the biological control of *M. incognita*. (Source - El-Hadad et al 2011, Braz. J. Microbiol. vol.42 (1))

***Rhizobium* bio-fertilisers for sustained Soybean productivity in Madhya Pradesh – Institutional and Policy Issues**

- Soybean is an important cash crop in India, and Madhya Pradesh (MP) dominates with largest area under Soybean cultivation. With initial exponential growth, its productivity is stagnating now and further scope for increase can hardly be done by expanding the area under cultivation. Negative environmental impacts and stagnation in productivity of major crops even with increased use of chemical fertilizers point toward the need for resource improvement to get sustained yield of crops. With ability to withstand erratic climatic conditions, Soybean holds lot of promise for the State economy. Application of *Rhizobium* bio-fertilisers for effective biological nitrogen fixation (BNF) and resource improvement is the key for sustained Soybean productivity. However, there are several constraints to the adoption of these bio-fertilisers despite being recommended as an essential packages of practice for Soybean by State Agricultural Universities. This paper presents the key policy and institutional requirements to promote strategies and capacity building needs of the sectoral players involved with *Rhizobium* bio-fertilisers to help achieve sustained Soybean productivity in the state. (Sunita Sangar 2010, Paper presented at 5th

International Nitrogen Conference  
2010 held in New Delhi)

**Efficacy of fly-ash based bio-fertilizers vs perfected chemical fertilizers in wheat (*Triticum aestivum*)** - Fly-ash was evaluated for possible use as carrier for *Azotobacter* and *Azospirillum* formulation. *Azotobacter* and *Azospirillum* strains were isolated from healthy wheat rhizosphere soil and bio-formulated in fly-ash (300 meshes). Fly-ash based *Azotobacter* and *Azospirillum* formulation alone and in combination with chemical fertilizer was evaluated for bio-efficacy on wheat. Population of *Azotobacter* and *Azospirillum* was also evaluated in treated soil. The results of the studies showed that, seed treatment with *Azotobacter* and *Azospirillum* and soil treated with chemical fertilizer alone and in combination significantly enhanced the seed germination, plant height, plant biomass and crop yield compared to control. Chemical fertilizer treated wheat plant observed more effective bio-efficacy than bio-fertilizers treated wheat but reduced (destroyed) the microbial population in soil. Whereas *Azotobacter* and *Azospirillum* treated soil observed significantly enhanced the microbial population with slightly lesser plant growth as compared to chemical fertilizer. In the present study it was showed that utilization of fly-ash as carrier in bio-fertilizer formulations emerged as safe and effective alternatives. Use of fly-ash as carrier in these formulations is an effective way of utilization of problematic fly-ash waste in a useful manner. (Source – Kumar et al 2010, International Journal of Engineering, Science and Technology Vol. 2(7) pp. 31-35)

**Co-inoculation of potassium solubilizing and nitrogen fixing bacteria on solubilization of waste mica and their effect on growth promotion and nutrient acquisition by a forage crop** - Waste mica, a potassium-bearing mineral, is a by-product of mica industry; however, its potassium (K)-supplying capacity for crop production is not well understood. A greenhouse trial was made to study the effect of co-inoculation of potassium solubilizing (*Bacillus mucilaginosus*) and nitrogen (N) fixing (*Azotobacter chroococcum* A-41) bacteria on solubilization of waste mica and their effects on growth promotion and nutrient uptake by a forage crop of sudan grass (*Sorghum vulgare* Pers.) in a Typic Haplustalf. Results revealed that significantly higher biomass accumulation and nutrient acquisition were obtained in all the pots treated with mica and/or bacterial strain as compared to control. Data indicated that co-inoculation of waste mica with *B. mucilaginosus* and *A. chroococcum* A-41 resulted in highest biomass production and nutrient acquisition. Co-inoculation of bacterial strains maintained consistently highest amounts of available K and N in soils even at 150 days of crop growth than other treatments. Thus, co-inoculation of potassium solubilizing and nitrogen fixing bacteria to waste mica could be a promising and alternative option for utilizing this potent source as K fertilizer to crops (Source – Basak and Biswas Biology and Fertility of Soils 46(6) : 641-648)



## Workshops/ Seminars/ Conferences/ National and International Events

**9th European Nitrogen Fixation Conference was held during September 6 - 10, 2010 at Geneva, Switzerland** - In September 2010, the 9<sup>th</sup> European Nitrogen Fixation Conference (ENFC) took place at the International Conference Centre of Geneva, Switzerland. Since 1994, when the ENFC series began in Szeged, Hungary, this biennial conference evolved into a key meeting for scientists who share an interest in biological nitrogen fixation and related topics. Traditionally, the ENFC conference brings together senior scientists and junior researchers not only from Europe, but also from all over the world. It provides a unique platform to share novel information about nitrogen fixation research, to discuss emerging fields, and to stimulate interactions and collaborations among different groups. Important topics discussed during the conference were:

- Evolution and biodiversity of diazotrophs and their hosts
- Global impact of biological nitrogen fixation
- Genomics and post-genomics of diazotrophs and their hosts
- Nitrogen-fixing associations with plants
- Novel tools and technologies to study diazotrophy
- Physiology of free-living and symbiotic diazotrophs
- Regulation of nitrogen fixation
- Structure, function and biosynthesis of nitrogenase

**21st North American Symbiotic Nitrogen Fixation Conference** - The 21st North American Symbiotic Nitrogen Fixation Conference was hosted by the University of Missouri-Columbia in cooperation with the MU Conference Office. The 21st North American Symbiotic Nitrogen Fixation Conference returned to the University of Missouri campus after many years. The conference hosted scientist from around the globe and discussed various constraints and challenge being faced by the symbiotic BNF research. For further details and proceedings contact: David W. Emerich, Professor & Associate Director, Department of Biochemistry 117 Schweitzer Hall, University of Missouri, Columbia, Missouri 65211

**Launching of "Biofertilizer Society of Pakistan"**- Biofertilizer society of Pakistan has been initiated on 10th of March, 2010, during a workshop on "Biofertilizer and Environment" organized by Center for Molecular Genetics, University of Karachi, Pakistan under the patronage of Dr. Sohail Hameed, Principal scientist at National Institute for Biotechnology and Genetic Engineering (NIBGE), Pakistan. The society has been initiated in collaboration with the major scientific institutes of Pakistan: National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad and Centre for Molecular Genetics (CMG), University of Karachi

The prevailing agricultural practices largely rely on high inputs of mineral fertilizers to achieve high yields and involve applications of chemical pesticides to protect crops against pathogens and pests. These practices are now being re-evaluated and are coming under increased scrutiny as the awareness of potential health and environmental consequences of excessive mineral fertilizer and chemical fertilizer usage improves. This has led to strong interest in alternative strategies to ensure competitive yields and protection of crops. The new approach to farming, often referred to as sustainable agriculture, seeks to introduce agricultural practices that are friendlier to the environment and that maintain the long-term ecological balance of the soil ecosystem. In this context, the use of microbial inoculants (biofertilizers, photostimulators and biopesticides) is considered as the alternate source to meet the nutrient requirement of crops and to bridge the future gaps. The concept of biofertilizers is to domesticate some of these microorganisms in our agricultural production systems, so that the vast natural reservoir of nitrogen in the atmosphere can be tapped as an additional source to meet the requirements and will augment

additional yields and monetary returns to farmers. For popularizing Biofertilizers among the farmers, scientific institutions are looking forward to tie up with interested entrepreneurs for transfer of production technology and proper training at their production site. The use of biofertilizer can significantly increase the crop yield in country. For example, rice is grown in about two million hectares in Pakistan and most of the farmers reported 500kg (or more) increase in the paddy yield per hectares. Similarly the increase in the yield of other crops such as wheat and legumes will also have a great impact on the economy of the country. The use of biofertilizer will minimize the dependence on the chemical fertilizers and save renewable energy resources. Pakistan for example, stands to gain a net saving of over Rs. 2 billion annually if we met only 10 percent fertilizer requirements through biofertilizers. Therefore, returns on the investment in this technology are quite well proven. Biofertilizer Society of Pakistan aims to improve the biofertilizer technology and enhance the biofertilizers use by actual farmers. The society provides a huge forum for scientists, researchers and students to work in collaboration in the field of biofertilizer.

## Book Reviews

**Microbes For Sustainable Agriculture** K.V.B.R. Tilak, K.K. Pal, Rinku Dey, I. K. International Pvt Ltd, 2010 - 212 pages - The present book comprising 16 chapters covers various aspects of microorganisms as natural resources for sustainable agriculture. The composition of media used for culturing the microorganisms and methods are also enlisted in this book. Figures with updated information is also a salient feature of this book. Selected references, suggested readings, glossary of terms used is given at the end of the book. The book is written as per the syllabi of the various universities. This will form a textbook for both UG and PG level of traditional universities, agricultural universities, industrial microbiology, microbial biotechnology, microbial ecology, applied microbiology and plant pathology.

**Soil Microbiology and Sustainable Crop Production** Geoffrey R. Dixon Springer, 2010 - Science - 436 pages - Soils into which crop plants root and from which they obtain essential minerals and water contain huge arrays of microbes. Many have highly beneficial effects on crop growth and productivity, others are pathogens causing diseases and losses to yield and quality, a few microbes offer protection from these pathogenic forms and others have little or no effect. These intimate and often complex inter-relationships are being explored with increasing success providing exciting opportunities for increasing crop yields and quality in sustainable harmony with the populations of

beneficial soil microbes and to the detriment of pathogens. This book explores current knowledge for each of these aspects of soil microbiology and indicates where future progress is most likely to aid in increasing crop productivity by means, which are environmentally benign and beneficial. Advanced first degree, post-graduate students, post-doctoral researchers, lecturers, research-leaders, consultants, advisors, crop practitioners and policy makers involved with crop agronomy, biological control, plant pathology, plant breeding, agriculture, soil science, environmental interactions and conservation, and others requiring up to date knowledge of the impact of soil microbiology on sustainable crop production will find this book invaluable

**Arbuscular Mycorrhizas: Physiology and Function**, Hinanit Koltai, Springer, 2010 - Science - 323 pages - In the years since the first edition of 'Arbuscular Mycorrhizas: Physiology and Function' was published, an exceptional proliferation of interest in mycorrhizal biology has developed. This has been associated with advances in different research disciplines such as genetics, genomics, proteomics, metabolomics and physiology, advances which have generated better insight into topics of mycorrhizal biology, including the mechanisms of host-mycorrhiza interactions pre- and post-penetration, the influence of the symbiosis on the host and its surroundings, and the evolution and diversity of mycorrhization.

## List of some Biofertilizer-Biopesticide Production Units Producing above 100 MT/annum

1. International Panaacea Limited, E-34, Connaught Circus, New Delhi- 110 001
2. Gujarat State Cooperative Marketing Fed. Ltd., Ahmedabad
3. Krishak Bharati Cooperative Limited (KRIBHCO), Kribhco Nagar, Surat- 395 515, Gujarat
4. CORDET Kalol, Gandhi Nagar
5. Kadur Agro, R.v. Vidyaniketan Post, Mylasandra, Bangalore- 560 059
6. Multiplex Biotech Pvt. Ltd., # 420-A, Peenya Indl. Area, Peenya Ist Stage, Bangalore- 58
7. M/s Agro Tech Agro India Ltd, Plot No. 1-D (Part-1), KIADB Industrial Area, Lokikere Road, Davanagere- 577 006
8. Madras Fertilizers Ltd, Bio Unit, Jigani, Bangalore
9. Biopest Management Pvt, Ltd, Bantanala Village, Maralawadi Hobli, Kanakapura Tq. Bangalore Rural DT, Bangalore
10. Karnataka Biofertilizers, Near Railway Bridge, Sindagi Road, Bijapur- 586 104
11. Anshul Agro Chemicals, Bangalore
12. S.S. Bio Gold, # 374, 4th Main, P.J. Extension, Davanagere- 577 002
13. Chaitra Fertilizers & Chemicals (P) Ltd., No. E-1, Sri Krishna Complex, D. Banumaiah Circle, Mysore
14. Nisarga Agri Technologies Inc., # 358, 4th Cross, Talakaveri Lyt., Post- Sahakarnagar, Amrutha Halli, Bangalore- 560 092
15. M & S., Charminar Bio- Tech, Mtisi Neem Cate, Organic Manure & Vermi- Compost, Bangalore Road, Challakere- 577 522
16. Karnataka Agro Chemicals No. 180, 1st Main, Mahalakshmi Layout, Bangalore
17. Managing Director, High Range Bio Pesticides Pvt. Ltd. Udumbanoor, Thodupuzha, Idukki Dist. Kerala
18. Plantrich Chemicals & Fertilizers Ltd., Industrial Estate, Manarcad P.O. Kottayam- 686 019, Kerala
19. The Managing Director Poabs Environtech (P) Ltd., Vilapisala (PO), Peyad, Trivendrum- 695 573, Kerala
20. High Range Fertilizers Biotech & Research Centre, Puliyanmala, Kattappana- 685 515, Kerala
21. Bio Fertilizer Unit, Ramnad
22. National Fertilizers Limited, Vijapur- 473 111 Dist.- Guna (M.P.)
23. M.P. Agro. Ind. Bhopal
24. Sai Agrotech, MIDC, Yavatmal
25. Krishi Vigyan Kendra, Sardanagar, Baramati, Pune
26. OM Agro Organic, A-76 MIDC, Yavatmal
27. Institute of Natural Organic Agriculture (INORA) , 11 B, Kulkarani Bungalow, Shikshak Nagar, Paud Road, Pune
28. Microplex India, 36 Mohata Market, Main Road, Wardha, Maharashtra
29. Rashtriya Chemicals & Fertilizer Ltd., Mumbai
30. M/s K.S. Agrochemicals, 84-Bapuji Nagar, Bhubaneshwar
31. East Cost Biotech Project Plot No. 99, Surya Nagar, Bhubaneshwar
32. Nitrofix Laboratories, 25, Bansdroni Avenue, Kolkata
33. Jaipur Biofertilizer, J-71, Ashok Chowk, Adarsh Nagar, Jaipur- 302 004
34. United biofertilizers, H -79 , Hirawala Industrial Area, Kanota, Jaipur- 303012
35. Monarch Biofertilizers & Res. Centre, No. 12, SIDCO Indl. Area, Thiramazhisai Chennai- 602 107
36. Esvin Advance Technologies Limited, Biotech Lab, SPB Premises, Palarpalayam, Erode- 638 007, Tamil Nadu
37. M/s Elbitech Innovations Ltd, 46 & 48, 2nd Floor, Masilamani Road, Balajinagar, Chennai- 600 014
38. The Agricultural Chemist Biofertilizer Prodn. Unit Seelanaickenpatty Salem- 636 201, Tamilnadu
39. The Agril. Chemist Biofertilizer Prodn. Unit Kudumianmalai- 622 104, Pudukottai Dist. (Tamilnadu)
40. M/s T. Stanes & Co. Ltd. 8/23-24, Race Course Rd, Coimbatore- 641 018
41. Krishi Care Bio Inputs Plot No. 51, Madha Nagar, Main Raod, Madhanagar, Mouliwakkam, Chennai- 16
42. M/s Jeyppee Biotech 25, Chinniah School Street, Virudhunagar- 626 001, Tamilnadu
43. Shritti Bioproducts Pvt. Ltd., 48, Ramar Koil Street, Ramnagar, Coimbatore- 641 009
44. Srivivasa Marine Chemicals, 19, Veeran Street Tirumangalam, Madurai, DT
45. Pasumai Agro Industries, #2, New Street, Valavachanur (PO), Chengam TK, Thiruvannamalai DT
46. Agriculture Chemist Biofertilizer Prodn. Unit, Dept. of Agriculture, Govt. of TamilNadu, Sakkottai, Thanjavur- 612 401
47. Tamil Nadu Cooperative Sugar Federation Ltd., 474, Anna Salai, Periyar EVR Bldg., 5th Floor Nandanam, Chennai- 600 035
48. R. Sundar, 25, Chinniah School Street, Virudhunagar- 626 001
49. Krishicare Bioinputs Kamatch Nagar, Kundrathur Rd., Madhanandhapuram, Porur, Chennai
50. Lakshmi Bio Techs, Tottapattu, Cuddalore
51. Bio Lab, Bannari Amman Sugars Ltd., Alathukombai, Satyamangalam, Erode, TN
52. M/s Arvee Biotech, 134, East Car Street, Chidambaram, Cuddalore DT, TN- 608 001
53. Senior Agricultural Officer, Biofertilizer Production Unit, Selam
54. Regional Biofertilizer Production Unit, Datta, A.D. Nagar, Tripura
55. Moti Lal Nehru Farmers Training Institute, CORDET, IFFCO, Ghiyanagar, Phulpur, Allahabad- 212 404
56. Krishak Bharati Cooperative Ltd., Biofertilizer Plant, Varanasi- 220 016
57. ROM Vijay Biotech No. 5, Cuddalore Main Road, Kanniakoil Pondicherry

