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Editorial

Dear Readers,

The importance of microorganism in maintaining the fertility status of soil has been recognized since the discovery of microbial world and accordingly the efforts have been made to exploit them to the maximum possible. The microbes, as in hand technology, have been exploited to manage the nutrients or to manage the plant diseases. However, the soil environment and phyllosphere, the thrust media for use of microbes have always remained complex, critical and extremely heterogeneous to create the boundaries for their effectiveness, particularly in case of specific microbes used in Biofertilisers and Biopesticides. A number of methods have been employed to overcome the constraints faced, yet the effectiveness depends on many factors and a need for further improvement in this pathway has always been felt. Development of oil base formulations in this connection is a major breakthrough, promising hope for the farmers. The technology involved in development of oil base formulation, which ensures, longer shelf life with high viable count of microbes has been elaborated in this issue with the hope that it finds a place in manufacturing arena.

The plant Growth Promoting Rhizobacteria which have multiple effects on growth of plants and also act in many ways as bio-control agents against many bacterial and fungal diseases have not found due consideration in the research and development pathways. In the present issue, a unique assessment carried out by veteran workers on development of Plant Growth Promoting Rhizobacteria have been depicted for prospective exploitation by the industry.

In addition a case study of most popular Biofertiliser Rhizobium on Soybean in Madhya Pradesh has been given due consideration which is expected to fulfill the requirement of the soybean growers in the Madhya Pradesh.

The other standard columns of newsletter are there in their usual format with quantum of latest inputs in the relevant field. It is expected that the newsletter would prove a tool to enhance the set of information for the benefits of all attached to its utility.

Krishan Chandra
Editor

Oil Based Liquid Inoculant Technology for Biocontrol agents, Biopesticides and Biofertilizers

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Introduction

Key obstacle in the bio-efficacy of microbial inoculants is the extremely heterogeneous nature of the soil and harsh environment for introduced organisms. There are only a few unoccupied niches in the soil for the introduced species. A competitive war immediately erupts among the introduced and indigenous species for their ecological space. Introduced organisms are highly stressed, alien to the natural soil environment, and often physiologically not ready to compete in soil with the indigenous species that have adapted to the ecological niche over several generations. Many inoculant formulations specifically address these issues by incorporating microorganisms into carriers enriched with selective food sources, suppressants for indigenous species, buffers and other ingredients, which can transiently alter the microphysical environment of the soil to provide a temporary safe haven for the introduced species. These additives help organisms to adjust physiologically to the new environment, and propagate. A successful formulation allows the introduced species to establish itself on or inside host roots or, at least temporarily, to shift biotic dynamics in the soil to favour introduced organism's development in a timely manner.

To achieve a desirably long shelf life and the required ease of transport and storage, most organisms in commercial products for soil application are propagated in a rich medium, and later packed as concentrates with the organisms driven to a dormant or a semi-dormant physiological state. These products are able to survive 6 months to 2 years depending upon the formulation. Keeping in view of the growing market for long shelf life products with very high CFU counts, author had developed an oil based formulation for biofertilizers like *Azotobacter, Azospirillum, Rhizobium, Phosphate Solubilizer, Potash Mobilizer*, *Zinc Solubilizers*; biocontrol fungus like *Trichoderma, Beauveria, Metarhizium, Verticillium* etc and biocontrol bacteria like *Pseudomonas fluorescens, Bacillus subtilis and Bacillus thuringensis* etc. The formulation has been successfully tried in the field and laboratory conditions and has been found to promise a shelf life of more than 4 years with very high total viable count (TVC) in the range of 10^9 to 10^{10} cells/ml. Bio-efficacy tests on different crops have also been found to be superior over conventional carrier based products.

Types of inoculants

Market is flooded with different types and forms of inoculants in liquid and solid form ranging from wet or dry solid products (moist powdered, dusts, granules and briquettes) to liquid suspensions (oil or water-based and emulsions).

Powdered Fungal inoculants:

Commercially 40 products are being sold worldwide for biocontrol of plant pathogens. In addition, there are several other microbial products, for plant growth promotion and nutrient mobilization. Many of such products, although, not labeled for biocontrol, help plants not only in growth promotion but also in disease control. The fungus *Trichoderma* is most frequently used for control of plant pathogens. At least 12 commercial products containing *Trichoderma sp* as main active ingredient control variety of pathogens, including *Botrytis, Fusarium, Gaeumannomyces, Pythium, Rhizoctonia, Sclerotinia, Sclerotium, Verticillium* and wood-rot fungi. Formulations of *Trichoderma* vary considerably depending on their intended use. For example, a combination of *T. viride* and *T. harzianum* is formulated as liquid for soil incorporation, as dowels for insertion into wood, as a wettable powder in a syringe for injection into grape vines and as a wettable powder, which is formulated into a paste and applied with a paintbrush to wounds (Tricho seal). It is also available as granule, as a wettable powder, impregnated in sticks, and as 'crumbles' for mixing into soil. Liquid formulation have been introduced recently and has proved their superiority perhaps because their ease in use and longer shelf life

.

Wettable powder inoculants and their limitations

Most wettable powders contain 50-80% technical grade powder, 15-45% filler, 1-10% dispersant and 3-5% surfactant by weight. The filler is usually inert and hydrophilic in nature to mix well with water. Normally a mineral such as silica is added to prevent clumping and lump formation during grinding (grinding may not be possible with some organisms) and during storage. Usually, wettable powders tend to mix slowly into water and separate mixers may be needed before filling in spray tanks, since tank agitators are often not forceful enough. Powders form unwetted balls, a few of which may persist even after protracted mixing pose problems of nozzle blocking, therefore a filter, preferably of nylon mesh, is essential in the spray line. Dry blending of powder with a binder and forming the mix into water-dispersible granules can largely solve mixing problems. Such products offer many advantages such as, very high concentrations of organisms, flowing freely with little dust and can be accurately measured by volume like a liquid but their, production costs are high, require vigorous agitation for dispersion in cold water, and difficult to achieve in aerosol form. These formulations offer excellent results and longevity but the technology is not being used in India (Chandra and Greep 2005).

Liquid formulations:

Although liquid inoculants are picking up fast with the industry and end users, but many researchers still doubt about their usefulness and potency. In spite of some reservations by few, it is proven beyond doubt that the fungi like *Beauveria, Verticillium, and Trichoderma spp* etc survives well in liquid formulation for more than two year without losing their efficiency. The bacteria like *Bacillus subtilis, Pseudomonas fluorescens, Bacillus thuringiensis*, etc. can sustain more than 2 years of shelf life. Most notable feature of the liquid formulations is to avoid contaminations (Chandra and Greep 2005).

These liquid formulations use liquid as carrier, which is usually water, oil or some solvent and in the form of suspension, concentrates or emulsions. The liquid suspension developed by the author contain particular organisms broth 10-40%, suspender ingredient 1-3%, dispersant 1-5%, surfactant 3-8% and carrier liquid (oil or water) 35-65% by weight. Viscosity is adjusted at equal to the setting rate of the particles. This is achieved by the use of colloidal clays, polysaccharide gums, starch, cellulose or synthetic polymers.

Emergence of liquid technology while improved the prospects of better product with

higher efficiency, has also triggered the race among producers to launch products without any technology base. In some cases, the products are just the microbial broths with high level of contaminants and no control over shelf life. Such substandard products not only spoil the market but also cast doubt about the usefulness of the technology among users.

Basic Concept of oil based formulations:

For any inoculant to be successful and effective, it needs to be judged on the four important parameters (Chandra and Greep 2006) which ensure that:

- The target organism remains viable in quantity and quality during the entire process of production, distribution and storage
- It facilitates ease in handling and application till it is delivered to the target place in the most appropriate manner and form
- It protects the bio-agent from adverse environmental factors at the target site, thereby increasing persistence
- It enhance activity of the organism at the target site by increasing its activity, reproduction, contact and interaction with the target insect pest or disease organism

Limitations of Improvement through formulation

Every formulation has its own limitations, which may be procedural or scientific and such limitations need to be assessed based on science, practicability and economics to achieve a scientifically proven, commercially acceptable and economically affordable product. Therefore, to achieve an acceptable formulation a systematic approach is required, where organism's mode of action and target behavior are properly understood, and acceptable window for their effective use is ensured. Unidirectional improvements can change the goal in another direction at any stage, from organism production, to storage, to care of the organisms after application. In the recent years, there is a growing trend to adopt yield-optimized production to improve harvest and formulation by media modification to make a more friable product at harvest, and by use of nutrients with wetting properties, may improve ease in production and shelf life enhancement, but can affect the overall performance of the product at target place. Such innovations pay when the benefit outweighs both extra costs and any loss of yield. Shelf life, an important parameter with liquid formulations may be influenced by various production factors. For instance decreasing water potential in the culture medium results in conidia of *Trichoderma* with increased desiccation tolerance (Whipps and McQuilken, 1993), culturing *M. favoviride* at 30^0C rather than 26^0C results in increased temperature tolerance in storage. The addition of humectants during fermentation also influences conidial survival. Shelf life parameter plays key role on the commercial viability and economy of the product. A product with less than 6 months shelf life requires direct order service, 0.5-2 years is good enough for conventional off shelf sales, while longer shelf life is helpful in deeper and sustainable markets. Therefore before adopting any technology all benefits, advantages and limitations need to be assessed and addressed properly keeping the four important goals in mind.

Commercialization requirements for biocontrol products:

As biocontrol products are biological in nature and their production, processing, storage and use are subjected to biological variations, decision on their commercialization in particular form need to be taken with utmost care. Decisions purely on commercialized considerations away from science do not sustain the test of time and efficiency. In addition, decisions purely based on science do not result into commercially viable option. Therefore, to ensure a scientifically proven product with appropriate commercial value a balance is to be achieved in science,

technology and commerce. While deciding to go for commercial production, besides technology and efficiency of the product a company must also assess other commercial factors including demand for the product, potential market size, and existing competing products. Legal issues and statutory requirements of registration or licensing are also important factors, which may differ from country to country and sometimes between states in a country, and need to be addressed.

Achieving desirable Shelf life

What are the limits of shelf life? With the help of dormancy, some insect eggs and bacterial spores survive over 10 years in an inert condition, even at periodically high temperatures. Once a constitutive dormancy is broken, the prerequisite for staying dormant is that they remain sufficiently dry if not absolute. Although, water is necessary for life and organisms may die if they become too dry. Therefore, to ensure a desired shelf life, one has to have adequate knowledge on minimum water requirement of that organism for keeping it dormant and alive. As the moisture content of materials used in formulations fluctuates to some extent by equilibrating with ambient relative humidity; formulations need to factor in such eventualities vis-à-vis capability of organisms in use, e.g. many fungal conidia, can supplement their water reserves by absorption from moist air, in some cases moisture is partially replenished by water produced during respiration, albeit only slightly. While in contrast, by absorbing moisture from humid air, an organism can exceed the low moisture content needed to prolong shelf life, even to a level high enough for physiological spoilage or damage due to growth of fungi. Recent experiments have shown that prolonged shelf life in certain organisms can be achieved by keeping them very dry. Studies have also shown that, drying also could have a safe low limit, and there may be limit for optimum low moisture content. In the economy of life, energy is limited by finite food reserves. If the physiology of an organism is known well enough, this energy limit can be calculated from cumulative respiration. Auto-intoxication can also be a limiting factor, depending on how well the organism can excrete wastes or store them harmlessly. Fundamental studies on the mechanisms of inactivation, mentioned above, might lead to novel areas of formulation research to combat these mechanisms. An example from early formulation research is the use of oxygen sinks to mop up free radicals that may be formed by exposure to ultra violet light. Would these sinks generally improve storage characteristics? Information from the food industry on storage and stability of some foods (e.g. vegetable oils), as well as on the anti-oxidants used, proved useful here.

Role of additives

Each species/strain as we know today presumably has shelf-life limits controlled by its genetic make-up. These limits can be improved either by finding new strains or by using some shelf life enhancing additive. Additives also play an important role in helping the agent to spread over surface and reach desired niches on leaves, stem and fruits. In addition to the practical limit of cost, each type of additive has its own unique limits. Wetter's not only make a water spray stay on leaves but, like oil carriers, they also enable organisms to reach otherwise inaccessible places between leaf hairs, into depressions, even into stomata and between intersegment membranes on the insect body. This improves the chances of establishment of organisms for weed, plant-disease and insect control. In this respect, there is no upper limit to desired efficiency, provided activity is not extended to non-target biota. The new super wetter's (organosilicones) are likely to be superior over existing ones with added advantages of better shelf life, spreadability and retention. However, some times wetter's also reduce spray retention if the volume applied results in run off, acting as detergents during rainfall and increasing

wash-off of organisms, therefore, amounts used should be limited to those needed to give efficient wetting and organism dispersal. Stickers need to be as rain tolerant as possible without impairing the activity of organisms.

Best storage achievable with oil based formulations:

Recent studies with *M. flavoviride* showed that the future for long shelf life lies particularly with formulations to keep spores ultra dry. Before this was realized, studies have been made where conidia with various moisture contents were tried to achieve desirable storability. Such studies need repeated attempts under well defined conditions, using synchronized batches of well defined, high quality conidia of various species to decide the best moisture content(s), best oils, value of the absence of O_2, best desiccants and the effect of absorbent clays, pre-dried or not. The process of maturation in storage over a long period should be traced by regular measurements of moisture content, spore size, respiration, chemical composition, germination (including speed of germination) and potency in insects. A quality control should be developed form the easiest of these methods, e.g. microscopic measurement of spore size, or mean spore weight, if size and weight can be correlated with quality. The predictive value of accelerated tests, i.e. at high temperature, needs examination. Whether silica gel can acts as an absorbent of metabolic wastes, and as a desiccant, needs further study.

Production and Quality of Spores:

Oil based or liquid production systems will predominate the future or not is not clear, but the key advantages offered by these formulations are; that 3-year shelf life of conidia is realizable with oil base systems against the easy technical operation and scale-up of some strains in liquid systems with 2-year shelf life. The effect of moisture content on storability, with some organisms is very crucial and may be a necessity to maintain certain moisture levels for their biological activity, which is generally fulfilled in liquid inoculums but in case of carrier based inoculums bacteria get stressed, when carrier become dry during transport and storage. Antagonistic bacteria used against plant pathogens need the plant surface to be wet in order to establish them. Fungal spores normally need high humidity to germinate. These needs can be overcome by Oil formulation as these products contain humectants. However, in case of non-fungal inoculants, there is little or no direct effect of relative humidity on the activity of viruses and spore forming bacteria in Oil form (Bateman 1997).

When no other technical factor dominates, the choice narrows down to the cost. Critical cost analysis would be valuable to illustrate the cost of striving for spore quality and optimal viability possibly through sophisticated formulation. The consequences of compromise, particularly with formulation, to lower the cost must be studied in terms of performance. In case of oil based formulations although the cost is significantly high compared to liquid and carrier based formulations, but their capability of higher microbial load and better spreadability ensure lower volumes per unit area. For foliar application in one acre while 20ml oil based formulations will suffice, 200ml will be needed for liquid based and 2 kg will be required in case of carrier based formulations. Therefore, in spite of being costly the cost of application per unit area of such oil based formulations will be almost at par with other ones.

Basics of production technology

Fermentation method

Under this process (Chandra, 1994, 1995, 2009; Chandra et.al.2000) all types of Fungus can be cultured with jaggry, glucose as carbon source. Fortification with yeast extract is essential for multiplication of some fungus like

Beauveria, Verticillium, Trichoderma spp. *Metarhizium anisopliae* var. *acridum* etc,

Method 1 - After multiplication the broth is transferred to the mixing tank where 40-70% oil (soybean, Groundnut, light oil, vegetable oil etc) is mixed with emulsifier, cell protactant and packed in bottle. This provides 3-4 years shelf life.

Method 2 – In an alternative process the broth is mixed with water and then passed through filter to harvest spores and *mycelium* and the harvest mass is mixed with 60-80% oil (soybean, Groundnut, light oil, vegetable oil etc) emulsifier, cell protactant and packed in bottle. This provides more than 5 years shelf life.

Method 3 - After multiplication, the broth is subject to high-speed centrifugation and biomass is harvested. Harvested biomass (30-70%) is mixed with 30-70% oil (soybean, Groundnut, light oil, vegetable oil) emulsifier, cell protactant and packed in bottle. This provides 3-4 years shelf life. The process is applicable for all type of bacteria like-*Bacillus subtilis, Pseudomonas, Bacillus thuringensis, Phosphate Solubilizing bacteria, and Potash mobilizer bacteria, Azospirillum, Azotobacter, and Rhizobium* and even in all types of fungus.

Spores harvesting

Under this process, the fungus is multiplied on half cooked rice, wheat bran media. Once full growth is achieved, the material is dried, spores are harvested mechanically or manually and then mixed in 70-80% oil (soybean, Groundnut, light oil, vegetable oil) emulsifier, cell protactant and packed in bottle. This provides more than 5 years shelf life. To ensure proper spore count it is important to ensure proper growth of spores. Poor production conditions may not be reducing the germination percentage after brief storage, but may stress spores viability in medium to long term. Media ingredients, light, temperature and humidity during growth, duration of culture, minimizing agitation during the sporulation period on solid substrates, and conditions maintained during drying and harvest are important process parameters and need to be controlled with utmost care.

Handling and Application of oil products:

Oil ensures that the product is easy to handle and apply **(**Bateman et al 1999**)**. For example, in suspensions where thickeners or suspenders are added by the authors it was observed that they all help in maintaining even distribution of the organism. Although the liquid formulations prevent clumping of the organism and ensures its ready resuspension even after prolonged storage, but they are not capable of providing that spreadability, which oil based formulations offer. Dusts and wettable powders are difficult not only in obtaining clumps free suspensions but are also have problems of proper spread over plant surfaces.

Effective and economic use of a product requires the active ingredient to reach the target; no matter how good the product, if it does not reach the target it will not perform the required function. With liquid bio-insecticides, this problem was partly solved with wetting and spreading agents but with oil, base formulation problem was completely solved due to its better spreadability and deeper reach.

Effect of storage on germination

Prolonged storage sometimes slows subsequent spore germination. In germination tests on agar plates, the germination of *Metarhizium* spp and *Beauveria spp* was complete in 24h, but after prolonged storage, it was delayed. Germination was observed to be slowed after storage of conidia in powder from, or in water. In powder or water, long survival of *Metarhizium* conidia depends on thorough drying, which minimizes metabolism. The delay in germination may be due to the extra time taken for recovery from

physiological dehebilitation in storage. In oil-based formulations, conidia are not dehydrated and operative factors probably recover faster due to grater metabolism facilitation in oil.

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Isolation and Characterization of Plant Growth Promoting Rhizobacteria for Multiple use as Composite Biofertilizer in Organic crop Production

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Introduction

Plant Growth Promoting Rhizobacteria (PGPR) is a group of bacteria that actively colonize plant roots and increase plant growth and yield through: (a) asymbiotic N_2 fixation (Mrkovacki and Milic, 2001; Salantur *et al*., 2006), (b) solubilisation of mineral phosphates and other nutrients (Cattelan *et al*., 1999), (c) production of phytohormones (Egamberdiyeva, 2007; Shaharoona, 2006) and (d) production of siderophores, antibiotics, enzymes and/or fungicidal compounds. Some PGPR may promote plant growth indirectly by affecting symbiotic N_2 fixation, nodulation or nodule occupancy (Fuhrmann and Wollum, 1989).

The ability of soil microorganisms to convert insoluble forms of phosphorus to a soluble form is an important trait of plant growth-promoting bacteria for increasing plant yields (Richardson, 2001). Many phosphate-solubilizing bacteria (PSB) belong to the *Pseudomonas*, *Bacillus*, *Enterobacter*, *Serratia*, *Pantoea*, *Rhizobium*, *Flavobacterium* and to the fungal genera of *Aspergillus* and *Penicillium* (Whitelaw 2000; Sulbaran *et al.,* 2009). The application of bacterial inoculants as biofertilizers have resulted improved growth and increased yield of cereal crops (Arcand and Schneider, 2006; Lucy *et al.,* 2004). Through the present study, efforts have been made for isolation of plant growth promoting bacteria from the soils of eastern Uttar Pradesh, India and their characterization based on morphological and physiological traits.

Material and Methods

Soil sampling:

Soil samples were collected from rhizospheric soil of different crops (chickpea, wheat and rice) grown in different district of eastern Uttar Pradesh, during 2006. Healthy chickpea root nodules were also collected from the same locations.

Isolation of endophytic PGPR: *Rhizobium* strains

Surface sterilized root nodules were crushed in sterile water and *Rhizobium* was isolated by serial dilution and plating on Yeast Extract Manitol Agar media (Vincent 1970). The pure cultures of *Rhizobium* were maintained on YEMA in test tubes and stored at 4°C.

Isolation of rhizospheric free living PGPR

Ten gram of rhizospheric soil sample was suspended in sterile water, serially diluted up to 10^{-3} to 10^{-5} and spread on the plates containing specified media (Nutrient Agar, Pikovaskaya Agar and King's B Agar). The plates were incubated for 2-5 days at 30 °C

for isolation of targeted organisms. Isolated strains were purified and sub culturing was done for storage at 4°C. For comparison a pure cultures of *Azotobacter chroococcum* strain MTCC-446 was obtained from Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India.

Isolation and collection of plant growth promoting fungi:

From the serial dilutions made as above, 0.1 ml aliquots from 10^3 and 10^4 dilutions were spread on plates containing potato dextrose agar (PDA) media, Czaper-Dox media and Pikovaskaya media separately. The culture plates were incubated for 2-5 days at 30 °C and colonies appearing on the medium were isolated and sub-cultured for further analysis.

Biochemical characterization of bacterial strains

Strains were assessed for morphology, physiology, Gram reaction and biochemical characteristic (Gram reaction, Catalase reactions, Methyl red, Voges-Proskauer test, hydrolysis of starch and utilization of glucose, sucrose and mannitol) using standard methods (Cappuccino and Sherman, 1992; Aneja, 2003) (Table 2).

Evaluation of plant growth promoting activities in-vitro

The quantitative analysis of indole-3-acetic acid was done following method of Bric *et al*. (1991). For qualitative estimation, bacterial isolate were tested for their phosphate solubilizing activity on Pikovskaya medium (Pikovskaya, 1948) and assessment of clear halo around them, indicating P solubilization. For estimation of ammonia production, bacterial isolates were grown in peptone water (g l^{-1}: peptone 10 g, NaCl 5 g, pH 7) and incubated at 30°C for four days. One ml of Nessler reagent was added into each tube and presence of yellow colour indicating ammonia production was recorded (Cappuccino and Sherman, 1992). The analysis of siderophore production by the bacterial isolates was performed following the chrome azurol S (CAS) method of Alexander and Zuberer (1991). HCN production by the bacterial isolates was detected by the method of Bakker and Schipper (1987). Antifungal activity was assessed by the method described by Ragendran *et al*., 2008.

Results and Discussion

Plant growth promotion activity

In present investigation, indigenous plant growth promoting rhizobacteria such as *Pseudomonas* and *Bacillus* were isolated from soils of chickpea rhizosphere and *Rhizobium* and *Mesorhizobium* from root nodules. On the basis of cultural, morphological and biochemical characteristics, soil isolates were grouped into *Bacillus*, and *Pseudomonas* as described in Bergey's Manual of Determinative Bacteriology (Holt et al., 1994, Aneja, 2003). Root nodule isolates of chickpea (*Cicer arietinum* L.) were characterized as *Rhizobium* groups (Aneja, 2003).

These strains were further screened *in vitro* for plant growth promoting activities (Table-1). IAA production was detected in all the test isolates. The production of IAA production was highest in *Pseudomonas* group isolates of *Burkolderia cepacia* BHUPSB03, *P. aeruginosa* BHUPSB01and *P. putida* BHUPSB04 as compared to other strains. In broth, IAA production in bacterial culture varies from 35.11% to 114.19% at 100 μgml^{-1} tryptophan after 72 h incubation. IAA production was more significant in *P. fluorescens* (114.19%), *Rhizobium* sp. (70.37%) and *A. chroococcum* (35.11%) as compared with *B. megaterium* at 100 μgml^{-1} tryptophan after 72 h incubation. The production of IAA was also significant in *Bacillus* group isolates *Bacillus megaterium* BHUPSB14 and *Paenibacillus polymyxa* BHUPSB17 as compared to BHUPSB16, BHUPSB19. In *Rhizobium* group isolates the production of IAA production was significant in

Rhizobium leguminosarum BHURC04 followed by BHURC03 and BHURC05. Synthesis of IAA by *Rhizobium* spp. in the presence and absence of tryptophan has also been demonstrated (Kittel *et al.,* 1989). The results on the production of growth promoting substances indicated that all the isolates of PGPR were able to produce IAA. PGPR were isolated from rhizospheric soil are known to produce growth regulating substances (Barea et al., 1978). Lal (2002) reported that PSB isolated from the soils of pearlmillet produce IAA, GA3 and cytokinin like substances, which ultimately enhanced plant metabolism. Production of IAA varies greatly among different strains and is influenced by culture condition, growth stage and availability of substrate (Vijila, 2000). The results of study, indicate that production of IAA by different PGPR also vary in nutrient broth supplemented with two different concentration of L-tryptophan (100 and 200 µg/ml). The production of IAA was increased as the concentration of L-tryptophan increase in the growth medium after 48 h. Substantial production of IAA by the different PGPR was observed during stationary phase. IAA production was found to be dependant upon bacterial isolates and concentration of tryptophan. Such findings may have direct practical application, although intrinsic ability of bacteria to produce IAA in the rhizosphere depends on the availability of precursors and uptake of microbial IAA by plant (Arshad and Frankenberger, 1993).

All the isolates of *Bacillus, Pseudomonas* and *Azotobacter* produced IAA, whereas only 85.7% of *Rhizobium* was able to produce IAA. Production of IAA in fluorescent *Pseudomonas* isolates increased with an increase in tryptophan concentration from 1 to 5 mg/ml in the majority of isolates.

Among fungal isolates under study, *Aspergillus niger* and *Trichoderma harzianum* showed more significant IAA production compare to *Penicillium citrinum* at 6 days of incubation at 30ºC. The production of IAA by *P. putida* and *Trichoderma atraviride* were stimulated by the addition (200 µg/ml) of L-tryptophan, tryptamine and tryptophol. *P. putida* produced the highest level of IAA (38.5µg/ml) in presence of tryptophol. *Trichoderma atraviride* produced the highest level of IAA (6.2, 9.8 and 38.55µg/ml) in presence of 200 µg/ml tryptophan, tryptamine and tryptophol, respectively (Gravel et al., 2007). *Trichoderma* spp. have been also shown to exhibit plant growth-promoting activity on numerous cultivated plants (Altomare et al., 1999; Harman, 2000; Yedidia et al., 2001). Tryptophan is naturally secreted in root exudates of tomato plants and most of the auxin found in the rhizosphere is believed to come from the biosynthesis by microorganism (Kamilova et al., 2006).

Phosphate solubilization

Phosphate solubilization was most frequently encountered by *P. aeruginosa* followed by *B. polymyxa*, *B. megaterium*, *P. putida* and least by *R. leguminosarum*. *P. aeruginosa* produced largest halos; approximate 20 mm around their colonies within 5 days of incubation. However, production of ammonia was a common trait in all isolates of bacteria. Qualitative estimation of phosphate solubilization was studied after 3 and 6 days of incubation. Maximum solubilization of phosphorus was found in BHUPSB05, BHUPSB06, BHUPSB03 and BHUPSB10 at 3 days of incubation in broth medium but after 6 days of incubation maximum solubilization of phosphate was found in BHUPSB04 (223.01 µg/ml), BHUPSB10 (213.98 µg/ml), BHUPSB14 (208.33 µg/ml) and BHUPSB01 (194.78 µg/ml) which is within the range as estimated by Pandey et al. (2006). The phosphorus efficiency of isolated PGPPR strain indicated that all the strains were solubilizing inorganic phosphate content effectively in the medium. Among different PGPR eg. BHUPSB05 was found best phosphate solubilizer after 3 days of incubation and strain BHUPSB04 was found

as highest solubilizer after 6 days of incubation. It was observed that the phosphate solubilizing efficiency showed a wide range of variation from 127.03 to 223.01 µg/ml.

There was no correlation between phosphorus solubilization efficiency on solid and liquid medium. The universal relationship was observed between the pH and soluble phosphorus concentration indicating that, organic acid production by these PGPR strains play significant role in acidification of the medium facilitating the phosphorus solubilization.

Similar results were observed in broth culture with fungal isolates with higher solubilization by *Aspergillus niger* followed by *Penicillium citrinum* and *Trichoderma harzianum* in broth cultures at 3 and 6 days incubation. Lowest pH values were recorded in *Aspergillus niger* broth culture followed by *Penicillium citrinum* compared to *Trichoderma harzianum.*

Antifungal activity

The indirect mechanism of plant growth also occurs when PGPR lessen or prevent the deleterious effects of plant pathogens on plants by production of inhibitory substances or by increasing the natural resistance of the host. Control of phytopathogenic microorganism by releasing siderophores, B-1,3-glucanase, chitinases, antibiotics, fluorescent pigment and cynide (Scher and Baker, 1982; Voisard *et al.,* 1989; Catellan et al, 1999; Pal *et al.,* 2001). *P. aeruginosa* was detected positive for siderophore and HCN production. *P. aeruginosa* strain BHUPSB01 prevents the wilting and root rot disease in chickpea plant by inhibiting the growth of soil pathogenic fungi *Fusarium oxysporum* f. sp. *ciceri* and *Rhizoctonia solani*. The antifungal activity of the test isolate indicated a close relationship between production of HCN and siderophores. In this study *P. aeruginosa* strain BHUPSB01 was found to produce HCN. HCN affects the respiratory system of pathogenic fungi and results in their growth inhibition. The antifungal activity of the test isolates might be due to the production of siderophore and HCN or synergistic interaction of these two or with other metabolites.

Conclusion

Considering all the plant growth promoting parameters, *Pseudomonas aeruginosa* (strain BHUPSB01), *Pseudomonas putida* (strain BHUPSB04), *Burkholderia cepacia* (strain BHUPSB03), *Bacillus megaterium* (strain BHUPSB14), *Paenibacillus polymyxa* (strain BHUPSB17) and *Azotobacter chroococcum* (MTCC-446) were selected as effective PGPR. The plant growth promoting fungi, *Aspergillus niger* and *Trichoderma harzianum* have shown synergistic relationship with above PGPR. After field testing, combined application of above PGPR and PGPF is advised to the farmers for multiple use as composite biofertilizer for cultivation of cereals (rice and wheat) and legumes (in combination with suitable strain of *Rhizobium*) in Indo-Gangatic plains of Uttar Pradesh.

Table 1. Biochemical characterization and plant growth promoting activities of PGPR isolates in *vitro* condition

Bacterial strains	Gram's test	Shape	Catalase	MR test	VP test	SH test	Carbohydrate fermentation			IAA (μgml^{-1})		NH_3	HCN	Sidrophore	Growth Inhibition*	
							G	S	M	150	300				F.o.	R.s.
BHUPSB01	-	rod	+	-	-	-	-	-	-	29.29^{d}	45.53^{d}	+	+	+	+	+
BHUPSB02	-	rod	+	-	-	-	-	-	-	19.35^{c}	35.36^{c}	+	+	+	+	+
BHUPSB10	-	rod	+	-	-	-	-	-	-	22.33^{c}	33.45^{c}	+	-	-	+	+
BHUPSB04	-	rod	-	-	-	-	+	-	-	25.65^{cd}	48.46^{dc}	+	+	-	+	+
BHUPSB06	-	rod	+	-	+	-	+	+	+	20.76^{c}	32.24^{c}	+	-	-	-	-
BHUPSB03	-	rod	-	+	-	-	+	-	-	35.51^{c}	53.23^{c}	+	+	+	+	+
BHUPSB15	-	rod	-	+	-	-	+	-	-	27.81^{d}	42.36^{d}	+	-		-	-
BHUPSB14	+	rod	+	-	-	+	+	+	+	29.12^{d}	44.32^{d}	+	-	-	-	-
BHUPSB19	+	rod	+	-	+	-	-	-	-	14.71^{b}	25.63^{b}	+	-	-	-	-
BHUPSB16	+	rod	-	+	+	+	+	+	+	13.03^{b}	23.84^{b}	+	+	-	+	+
BHUPSB17	+	rod	-	+	+	+	+	+	+	19.79^{c}	29.30^{bc}	+	+	-	+	+
BHURC01	-	rod	+	-	-	-	+	-	-	19.38^{c}	32.14^{c}	+	-	-	-	-
BHURC02	-	rod	+	-	-	-	+	-	-	18.56^{c}	26.54^{b}	+	-	-	-	-
BHURC03	-	rod	+	-	-	-	-	-	-	21.03^{c}	34.15^{c}	+	-	-	-	-
BHURC04	-	rod	+	-	-	-	+	+	+	29.35^{d}	41.65^{c}	+	-	-	-	-
BHURC05	-	rod	+	-	-	-	+	+	+	20.03^{c}	32.15^{c}	+	+	+	+	+
MTCC-446										19.24^{a}	19.24^{a}	+	-	-	-	-

- Values are the mean within column followed by same lette are not significantly different from each other according to Dunnett T3 test at $P \leq 0.05$.
- MR=Methyl red, VP = Voges – Proskauer, SH = Starch hydrolysis, IAA Indole acetic acid, NH_3 = Ammonia, HCN = Hydrogen cynide
- *Growth inhibition of fungal mycelium (F.o. + *Fusarium oxysporum, R.s. = Rhizoctonia solani)* on nutrient agar plate media.

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

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Background

Soybean has specific preference of *Bradyrhizobium japonicum* inoculants (*Rhizobium* biofertilizer) for nodulation and effective Biological Nitrogen Fixation (BNF). BNF research continues to be a priority area with expanding focus through various R&D schemes and programmes in India (Rao et al 2004). Investment in BNF is also justified by the opportunities to reduce or replace the growing cost of nitrogenous fertilisers. *Rhizobium* bio-fertilisers hold lot of promise as a source of nitrogen for Soybean production in Madhya Pradesh (MP). The success of Soybean depends, amongst other factors, on the presence of Rhizobium: *Bradyrhizobium Japonicum* in the soil, which infects, the roots and produce effective nodules capable of fixing Nitrogen. However, being crop specific Rhizobia that occurs naturally in the soil habitat of Soybean is often inadequate in nature to effect increased crop production by just supplying crop nutrients biologically . This necessitates inoculating artificially cultured microorganisms (*Rhizobium* bio-fertilisers) in adequate numbers for ensuring the good and effective nodulation. Seed inoculation with *rhizobium* is done every year to ensure optimum population of effective rhizobia in rhizosphere leading to enhanced nodulation, nitrogen fixation, and yield (Hajare et al 1994, 1995). For the obvious need to improve the availability, quality and delivery of these bio-fertilisers, organisations/institutions were set up. The adoption of *Rhizobium* bio-fertilisers for Soybean in MP has been principally due to public sector R& D arrangements for Soybean and its specific *Rhizobium* that was promoted as part of essential package of practice advocated by the state.

***Rhizobium* production scenario**

Commercial production of *Rhizobium* bio-fertilisers in India in late eighties marked the beginning of bio-fertiliser industry in India (Jauhri 2005). With initial domination, its production as a proportion of total bio-fertilisers production has been declining over the years, specially in the current decade with other bio-fertilisers taking its place. Whereas, it constituted around 45% of the production in 1992-93, it has now dropped down to 12% during 2009-10 (NCOF 2010). Despite an overall decline in the production of *Rhizobium* in the country, MP still leads in production accounting for almost 17% of the total *Rhizobium* bio-fertilizer production in the country (NCOF 2010). *Rhizobium* production as a percentage of total bio-fertilizer production in the state has remained consistent between 20-30% of the total bio-fertilizer produced in the state (Fig. 1).

Figure 1: ***Rhizobium*** **production as a percentage of total BF production in MP**

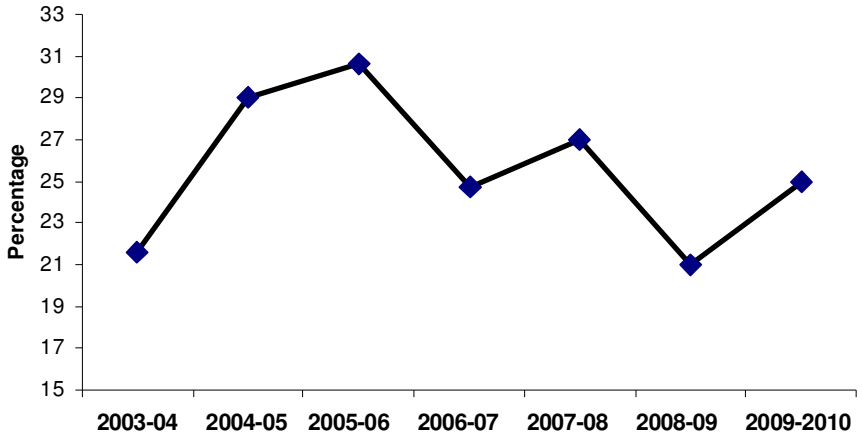
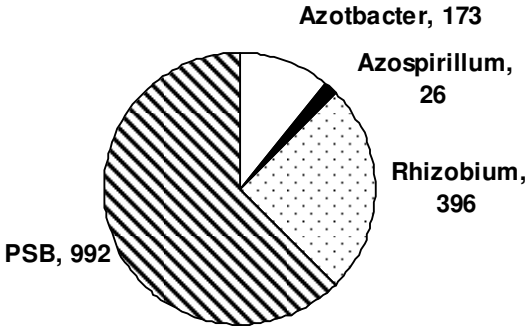


Fig. 2: Bio-fertilisers production in MP: 2009-10 (173 Azoto, 26 Azosp, 396 Rhizo, 992 PSB)



Source: NCOF, Annual Report, 2009-10.

Currently *Rhizobium* bio-fertilisers contribute to more that 25% of the total bio-fertiliser being produced within the State that is the largest among other nitrogenous bio-fertilisers (*Azospirillum* and *Azotobacter*). There is a big gap between the annual requirement of *Rhizobium* bio-fertilisers keeping in view the area under Soybean in the State and present production capacity for these bio-fertilisers (NCOF 2010). This indicates large potential for organized production, for example, considering *rhizobium* inoculation to be done in the entire cropped area (5.29 million hectares in 2007-08) for Soybean in Madhya Pradesh, the amount required would be 2252.075 tonnes,

whereas, the annual production from the seven manufacturing units in MP stands at about only 397 tonnes.

Production statistics from different manufacturing units reveal domination of public sector units in the states with little participation by the private units. Interaction with private unit professionals reveal that Government demand for these bio-fertilisers is largely being met through public sector units with private sector showing their inability to supply bio-fertilisers at the cheap prices supplied by the Government. These production statistics however, reveal only the production data and does not indicate their usage/adoption in the fields by the farmers. The technology is demand driven with farmers as mere recipients of these products. There is little data on the farm level usage or profitability of the production units. However, recent Soybean nodulation surveys in farmers' fields though, indicated a positive response of farmers towards inoculants (Rao and Sharma 2009). There has been lack of assessments on environmental & social benefits of these bio-fertilisers. Trajectory of *Rhizobium* bio-fertilisers, was explored through literature review and interviews with professionals and key stakeholders associated with the development and adoption of *Rhizobium* bio-fertilisers in the state. The analysis revealed that more than technological issues several institutional and policy issues impede the further progress of *Rhizobium* bio-fertilisers in the state.

Institutional Context of *Rhizobium* Bio-fertilisers

Initial success in its production and adoption came about largely due to institutional support (Government push through policy, subsidies, schemes, etc.) with little technological innovation. 40 years of research on *Rhizobium* inoculants has reached a plateau despite known benefits of the use of *Rhizobium* in integrated plant nutrient management. There have not been many improvements/changes in the technology that was developed in the intial phases in 1970s to 1980s due to efforts of various organization, programmes/projects and schemes (of ICAR, SAUs, State & Central Government) involved in the different stages of development and dissemination efforts taken up to popularize Soybean in India till date. Technology is largely supply driven with a great push from the state.

- Despite the empirical evidence that support the benefits of sustainable alternatives such as use of bio-fertilisers with their ability to integrate with the local resources and knowledge, mainstream R&D is still chemical fertilisers oriented justifying for food security needs. Knowing that overuse of chemical fertilisers has led to various problems there is a need to give equal thrust to bio-fertilisers through supportive Government policies for sustaining our resources.

- Since functional scenario of technology generation, production and distribution is largely done by the public sector domain, it has little participation by the farmers or local level organizations such as NGO's or farmers associations or private sector. Farmers are seen as mere passive recipients of these inputs.

- There had been good interactions among these ICAR researchers all over India which were linked through All India Coordinated Project on Bio-fertilisers. However, lack of interdisciplinary research was visible with breeders/agronomists concentrating more on the Soybean crop production (commodity bias) with neglect of improvements required in *Rhizobium* bio-fertilisers, which formed an essential part of package of practices. There was lack of trust among the agricultural research community and bio-technologists that has led to poor product development.

- There have not been regular impact assessment/evaluations or socioeconomic studies done to know the impact of this technology on farmers. Most studies prove its utility for increasing the yield and not how *Rhizobium* bio-fertilisers have/have not helped improve soil quality (resource improvement) or life of the farmers (socioeconomic conditions). A notable exception has been a recent survey report in MP coordinated and conducted by Dr. D.L.N Rao and his team as part of All India Coordinated Network Project, which was largely prompted by the stagnating Soybean yields and farmers becoming less keen about its cultivation (Rawat et al 2008). Participation and setting up of small units for production of *Rhizobium* inoculants by small entrepreneurs as an employment opportunity is almost absent. There were projects funded by Department of Biotechnology (DBT) in the state to help entrepreneurs set up small units for mass production of these inoculants, but they also have gone to State cooperative organizations already involved in the production of *Rhizobium* inoculants.

Conclusions

In India, where 70% lands are under dry farming, fertilizer nitrogen usage is very low, bio-fertilizer technologies need to be given priority. With intial exponential growth, productivity of Soybean in MP 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 (such as Rice, Wheat) even with increased use of chemical fertilizers point towards 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 BNF and resource improvement is the key for sustained Soybean productivity.

- Since regular need and application of *Rhizobium* bio-fertilisers for Soybean is essential for sustained yield and resource improvement, there is a need to facilitate institutions that would bring more participation, more networking, emergence of more stakeholders/actors especially the private sector, strengthening of weak actors and increased farmer participation.

- While *Rhizobium* bio-fertilisers are potentially beneficial to poor smallholder farming system in MP, without adequate policy support to create a suitable environment for active participation of private entrepreneurs at the local level its wider production and application are seriously limited.

- There is a need for policy attention, for shunning some of the instituional rigidities for promoting the *Rhizobium* bio-fertilisers, for example by bringing together both state and non-state actors for a larger impact on the poor farmers.

- These non-state actors would largely be local actors such as private entrepreneurs, NGO's, farmers cooperatives, local stockist and trade associations which are absent now but may form more sustainable interventions for Soybean *Rhizobium* distribution than total reliance on Government agencies. There is also need to detail the nature of constraints to effective partnership in the prevailing institutional environment.

- R &D efforts related to improvement in bio-fertilisers need to be more participative including all stakeholders specially farmers. This would require identification of capacity building needs of both extension personnel and farmers while creating awareness about these microbial inoculants. While training of researchers and extention workers in the region are essential to concentrate on research and

quality control aspects of *Rhizobium*, this should also lead to identification of entrepreneurs who can take over the distribution, marketing and sales of these inoculants.

Larger impact could be brought about through active collaborations or becomimg a component into the ongoing rural livelihoods related projects in that State such as, Madhya Pradesh District poverty initiative project, (MPDPIP), Madhya Pradesh Rural Livelihoods Project and Rainfed farming projects. Enabling institutional changes should be based on the learning capacities of the actors associated with *Rhizobium* bio-fertilisers with adequate policy support. Bio-fertilisers become especially crucial in the current scenario of climate change with increasing environmental and resources (soil & water) degradation. Though the need of *Rhizobium* bio-fertilisers for sustainable Soybean production is well established by the scientists, This analysis reveals some key institutional issues which must be acknowledged and addressed.

Acknowledgement

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Research Notes

Biofertilizer Technology and Pulse Production - Plant–microbe interactions are central feature of the life of not only plants but determine the soil health and plant health, thereby leading to a healthy ecosystem. These interactions range from advantageous symbiotic associations to pathogenic disease states. Numerous rhizobial strains have been identified that show nitrogen-fixing ability, with their target host legume Chickpea (*Cicer arietinum* L.) being one of the most important legume crop of the arid zones of India, and different cultivars of chickpea have been grown under field conditions since centuries. Most of the chickpea grown soils harbor diverse group of rhizobial populations. These rhizobial populations in the soil are capable of interacting and nodulating all chickpea cultivars to varying extent depending upon the soil, site, and management practices. The achievements of biofertilizer technology in enhancing chickpea productivity in India have been discussed in this chapter. (Source Dudeja et al 2011, Bioaugmentation, Biostimulation and Biocontrol, Soil Biology, 2011, Volume 28, (1), 43-63)

Comparative nitrogen fixation by mesophilic (HTS) vis-à-vis thermotolerant mutants (HTR) of *Azotobacter chroococcum* at high temperature and their effect on cotton biomass - The amount of nitrogen fixed by *Azotobacter* varied from 0.02-0.25 KgN/ha/d and has been reported to be variable with various physiological and environmental factors. The nitrogen fixation by mesophilic soil isolates and their analogue resistant mutants of *Azotobacter chroococcum* was compared with their thermotolerant mutants at three different temperatures (30°C, 37°C, 42°C). Mutants spontaneously resistant to methylammonium chloride were derived originally from a soil isolate of *A. chroococcum*. Ammonia excretion ability of different strains of *A. chroococcum* was determined. Nitrogenase activity of different strains of *A. chroococcum* was assayed by growing the cultures in the Burk medium. Effect of Azotobacter inoculation on cotton biomass was followed. Growth and ammonia excretion were related in both mesophilic as well as thermotolerant mutants at 30°C but not at elevated temperature (42°C). A thermotolerant strain (HTR71) has excreted as much as 24.1µg ammonia/ml of culture broth in a sucrose supplemented synthetic Jensen's medium at elevated temperature of 37°C under stationary conditions of growth while a thermotolerant mutant (HTR54) showed nitrogenase activity of 46.13 nmoles C_2H_4/h/mg protein at 37°C while Mac68, a mesophilic strain from which HTR54 is derived by mutation has nitrogenase activity of 35.07 nmoles C_2H_4/h/mg protein. Thermotolerant mutants showed better performance over mesophilic strains on the cotton biomass under pot house conditions. (Source Mittal et al Jundishapur J Microbiol. 2011; 4(2): 105-14)

Use of Plant-Associated *Bacillus* Strains as Biofertilizers and Biocontrol Agents in Agriculture - Plant-growth-promoting rhizobacteria (PGPRs) offer an environment-friendly and efficient alternative to chemical pesticides and fertilizers. Among them, endospore-forming bacilli are especially attractive because their long-term stability is comparable with that of agrochemicals. Although their use is steadily increasing, exploiting of these biologicals is still limited by insufficient knowledge about the mechanisms underlying plant growth promotion and biological control. However, in recent years, some progress has been made in uncovering molecular mechanisms responsible for beneficial interactions between PGP bacilli and plants. Authors describe here some

aspects of the plant–PGP bacilli relationship in light of the genomic data recently obtained from *Bacillus amyloliquefaciens*, and propose to choose *B. amyloliquefaciens* FZB42 as a paradigm for further research on PGP bacilli. (Source – Borriss 2011 Bacteria in Agrobiology : Plant Growth Responses 2011, 41-76)

Effects of *Rhizobium*, arbuscular mycorrhiza and whey applications on some properties in chickpea (*Cicer arietinum* L.) under irrigated and rainfed conditions - The study aimed to determine the effect of whey application, the inoculation of *Glomus intraradices* Shench & Shimith and *Mesorhizobium ciceri* on root colonization, nodulation, yield and the components of yield in chickpea (*Cicer arietinum* L. cv. Aziziye-94) under rain-fed and irrigation management. Experiments were carried out in a split plot design with four replications in 2003 and 2004. The above-mentioned factors were all applied to plants in single, double and triple combinations. The effect of irrigation was significant, with the heaviest yields being obtained under this treatment. Arbuscular mycorrhizal fungus (AMF) inoculation, alone or in combination with other treatments, was very effective under rain-fed conditions, resulting in large increases in yield, root colonization and phosphorus content of the seed and shoot. On the other hand, rhizobial inoculation increased significantly all traits examined, particularly root nodulation and the nitrogen content of seeds and shoots under irrigated conditions. Whey combined with AMF significantly increased root colonization while its combination with *Rhizobium* increased the number of nodules. Combinations of two or three treatments were more effective than individual applications. The greatest yield, root colonization and nodulation were obtained from the combination of all three treatments under irrigation. (Source – Erman et al 2011, Field Crops Research Volume 122 (1) : 14-24)

Insecticide-tolerant and plant-growth-promoting *Rhizobium* improves the growth of lentil (*Lens esculentus*) in insecticide-stressed soil - Application of insecticides in modern agriculture in order to enhance legume production has led to their accumulation in soils to levels that adversely affect soil microflora such as rhizobia and exert a negative impact on the physiological activities associated with them. This study was designed to identify rhizobial strains expressing higher tolerance to insecticides fipronil and pyriproxyfen and synthesising plant growth regulators even amid insecticide stress. The fipronil- and pyriproxyfen-tolerant *Rhizobium* sp. strain MRL3 produced plant-growth-promoting substances in substantial amounts, both in the presence and in the absence of the insecticides. In general, both insecticides at recommended and higher rates reduced plant dry biomass, symbiotic properties, nutrient uptake and seed yield of lentil plants. Interestingly, when applied with any concentration of the two insecticides, *Rhizobium* sp. strain MRL3 significantly increased the measured parameters compared with plants grown in soils treated solely with the same concentration of each insecticide but without inoculant. This study suggests that *Rhizobium* strain MRL3 may be exploited as a bioinoculant to augment the efficiency of lentil exposed to insecticide-stressed soils. (Source Ahmed and Khan 2011 Pest Management Science Volume 67(4), : 423–429)

Beneficial Endophytic Rhizobia as Biofertilizer Inoculants for Rice and the Spatial Ecology of This Bacteria–Plant Association – In previous studies authors have indicated that the clover root-nodule occupant *Rhizobium leguminosarum* bv. trifolii participates in a natural, beneficial association with rice roots independent of nodule formation and biological N_2-fixation, and can significantly improve rice growth, grain productivity and the agronomic fertilizer-use

efficiency with less dependence on additional chemical fertilizer inputs. Authors describe here the recent work on performance of selected endophytic strains in 24 large-scale field inoculation trials in 11 counties at the central and northern regions of the Nile delta, using seven inoculant strains tested on five rice varieties during five growing seasons (2000, 2002–2005), and the autecological biogeography of a high-performing rhizobial inoculant strain. Inoculation significantly increased paddy yield in 19 of these trials, including in salt-affected soils and in certain locations where chemical N-fertilization was less profitable. In seven experiments, inoculation increased the yield to levels that exceeded the world's highest national mean recorded in 2008. For the spatial ecology studies, authors are developing CMEIAS image analysis software (http://www.cme.msu.edu/cmeias/) to examine the biogeography of endophytic rhizobia at the km spatial scale in the Nile delta and the micrometer spatial scale during bacterial colonization of rice roots. (Source – Yanni et al 2011 Bacteria in Agrobiology: Crop Ecosystems 2011, 265-294)

Importance of Biofilm Formation in Plant Growth Promoting Rhizobacterial Action - Among the diverse soil microflora, plant growth promoting rhizobacteria (PGPR) mark an important role in enhancing plant growth through a range of beneficial effects. This is often achieved by forming biofilms in the rhizosphere, which has advantages over planktonic mode of bacterial existence. This chapter focuses on new insights and concepts with reference to improved PGPR effects caused by the biofilm formation by PGPR and its impact on overall plant growth promotion, compared with the planktonic lifestyle of PGPR. Beneficial PGPR play a key role in agricultural approaches through quorum sensing in their biofilm mode. The *in-vitro* production of biofilmed PGPR can be used to give increased crop yields through a range of plant growth mechanisms. They can be used as biofertilizers through improved N_2 fixation and micro- and macronutrient uptake. Further, higher levels of plant growth with PGPR have been observed due to their production of plant growth regulators and their abilities to act as biocontrol agents, which are carried out by the production of antibiotics and other antimicrobial compounds. The microbial inoculant industry would also benefit greatly by developing biofilmed PGPR with N_2 fixing microbes. Biofilmed PGPR can be manipulated to achieve results in novel agricultural endeavors and hence is as an area, which needs a deeper probing into its potential. (Source - Seneviratne et al 2011 Microbiology Monographs, 2011, Volume 18 : 81-95)

***Rhizobium sphaerophysae* sp. nov., a novel species isolated from root nodules of *Sphaerophysa salsula* in China** - Four gram-negative, aerobic, motile, nonspore, forming rods with a wide pH and temperature range for growth (pH 7.0–11.0, optimum pH 8.0; 20–45°C, optimum 28°C) strains were isolated from root nodules of Sphaerophysa salsula and characterized by means of a polyphasic approach. Phylogenetic analysis based on 16S rRNA gene sequences revealed that the four strains formed a new lineage related to the genus *Rhizobium* and the sequence similarities between the isolate and the most related type strain *Rhizobium giardinii* was 96.5%. These strains also formed a distinctive group from the reference strains for defined *Rhizobium* species based on housekeeping gene sequences (atpD and recA), BOX-PCR fingerprinting, phenotypic features and symbiotic properties. The representative strain CCNWGS0238 T has DNA-DNA relatedness of less than 33.4% with the most closely related species *R. giardinii*. It is therefore proposed as a new species, *Rhizobium sphaerophysae* sp. nov., with isolate CCNWGS0238 T (=ACCC17498 T= HAMBI3074T) as the type strain. (Source – Xu et al 2011, Antonie Von Leeuwenhoek Volume 99, (4), 845-854)

Plant Growth Promoting Rhizobacteria Improving the Legume–Rhizobia Symbiosis - The legume–rhizobia symbiosis is considered the most important nitrogen-fixing interaction from an agricultural point of view. However, biotic and abiotic factors can modify critical parameters of both the legumes and the rhizobia. These changes may lead to differences in the molecular dialogue, consequently reducing the symbiotic effectiveness. Therefore, optimal performance of the N-fixing symbiosis will be guaranteed by selection of both symbiotic partners for adaptation to the target environment. The symbiotic process can be negatively affected by many other rhizosphere interactions, resulting in important ecological, economic, and nutritional losses. The application of agricultural techniques that are friendly with the environment, based on the use of plant growth promoting rhizobacteria (PGPR), can increase the efficiency of the symbiotic process. The use of these beneficial microorganisms could reduce the use of polluting chemicals allowing sustainable production of legumes. Co-inoculations of appropriate rhizobia together with PGPR may profoundly increase the crop yield by different mechanisms. The negative effects of environmental stresses on the legume–rhizobia symbiosis may further be significantly diminished by applying mixtures of rhizobia and PGPR.(Source – Medeot et al 2010 Microbes for Legume improvement pp473-494)

Plant Growth-Promoting Bacteria Associated with Sugarcane - Sugarcane crop consumes heavy amount of nitrogen fertilizer and get affected by bacterial and fungal diseases for which chemical treatments are not recommended. Most of the countries use approximately 200–400 kg N ha^{-1} which is costly and hazardous for environment. For fungal disease control, farmers are advised to use disease-free seeds which is impractical due to the difficulty in diagnosing the dormant fungal infection in seed canes. Use of plant growth-promoting rhizobacteria (PGPR) can minimize the cost of fertilizer, environmental hazard, and suppress the diseases as well. PGPR are very well known for their role in plant growth promotion mainly for biological nitrogen fixation, phytohormone production, and acting as biocontrol agent. Several PGPR like *Azospirillum*, *Pseudomonas*, *Enterobacte*, *Klebsiella*,*Gluconacetobacter*, *Herbaspirillum*, etc., have been isolated from sugarcane. In this paper, a brief description of the genera, isolated up till now from sugarcane is provided. The role of these PGPR in sugarcane growth promotion and as a biocontrol agent has also been discussed.

Plant Growth Promoting Rhizobacteria: Constraints in Bioformulation, Commercialization, and Future Strategies - Bioformulations for plant growth promotion continue to inspire research and development in many fields. Increase in soil fertility, plant growth promotion, and suppression of phytopathogens are the targets of the bioformulation industry that leads to the development of ecofriendly environment. The synthetic chemicals used in the agriculture to increase yields, kill pathogens, pests, and weeds, have a big harmful impact on the ecosystem. But still the chemicals rule the agroindustry. The aim of the review is to assess the constraints associated with the effective development of bioinoculant industry particularly in developing countries. Another objective of the review is to evaluate what should be explored in the future to uplift the stature of the bioinoculants. Bioformulations offer an environmentally sustainable approach to increase crop production and health, contributing substantially in making the twenty-first century the age of biotechnology. (Source – Arora et al 2011 in Plant Growth Promoting and health Promoting Bacteria Microbiology Monographs, 2011, Volume 18, 97-116).

Workshop, Seminar, Conferences

Brainstorming session on "Microorganisms in Sustainable Agriculture 13th September 2011 at INSA, New Delhi - Sustainability in agricultural production has emerged as one of the most significant concerns in the present century. Organic wastes and microorganisms are alternate sources to meet the nutrient requirement and to control pests and diseases of crop plants to bridge the future needs. Thus, microorganisms can be harnessed to produce more biofertilizers, to decompose organic wastes more efficiently and combat plant diseases and pests with greater efficiency than ever before. Though considerable work has been done on these aspects in our country, with the new information available on microbial biodiversity and biotechnology many newer areas of research can emerge. Hence, the National Committee for International Union of Microbiological Societies (IUMS) in its meeting held at INSA, New Delhi under the Chairmanship of Prof. Anupam Varma proposes to have a brainstorming session on "Microorganisms in sustainable agriculture" under the convenership of Drs. D J Bagyaraj & Tapan Adhya. The brainstorming session is scheduled for September 13, 2011 at 10.00 A.M at INSA premises. Important topics to be discussed during the programme are: (1) Recent advances in nitrogen-fixing microorganisms-organisms (2) Recent advances in -cyanobacteria (3) Recent advances in phosphate solubilizing microorganisms (4) Mycorrhizal fungi (5) PGPRs (6) Management of plant pathogens by microorganisms (7) Management of insect pests and weeds by microorganisms (8) Degradation of pesticides in soil (9) Microorganisms in the conversion of agricultural wastes to compost and (10) Mass production and quality control of microbial inoculants.

ISCB Formulation Workshop at PTTC, ICRISAT, Hyderabad - Indo-Swiss Collaboration in Biotechnology (ISCB) is a well established bilateral research and development programme jointly funded and steered by SDC (Swiss Agency for Development and Cooperation, Government of Switzerland) and Department of Biotechnology, Government of India. Since last 10 years the programme has been focused on innovative biotechnologies in agriculture and environmental research, which have an impact on poverty reduction, food security and adaptation to climate change. On 25th April 2011, ISCB organized a Formulation Workshop at ICRISAT, Hyderabad. The main objective of the workshop was to showcase the excellent achievements of the Biofertilizer Network under the ISCB programme and also brainstorming ideas around modern technologies of formulation science for better and efficient delivery of biological agents in agriculture in Indian field conditions. Important issues discussed during the workshop were: (a) BFNet overview, shelf life and lyophilization method (b) Field efficacy and fluccolation method (c) Mass production and spray drying method (d) Bio-pesticide research at PCI with respect to mass production and formulation (e) Custom Nutrient management based on soil health assessment and (f) IP strategies and regulation

14th African Association of Biological Nitrogen Fixation Mali, 23 December 2010- 14th AABNF conference was held during 13-17th December 2010 in Bamako, Mali. The major constraints to implementation of nitrogen fixing technologies identified by N_2Africa were highlighted in the final communiqué from the conference.

Book Reviews

Bioaugmentation, Biostimulation and Biocontrol edited by Ajay Singh, Nagina Parmar, Ramesh C. Kuhad, Springer, 2011 - Science - 280 pages - Bioaugmentation, biostimulation and biocontrol approaches using microbial inoculants, biofertilizers, biochemicals and organic amendments improve soil biology, fertility and crop productivity by providing plant growth-promoting nutrients and suppressing soil-borne diseases and plant-parasitic nematodes. Our knowledge of microbial diversity and its function in soils has been increased tremendously due to the availability of a wealth of data gained through recent advances in the development of molecular methods and metagenomics for the evaluation of microbial diversity and functions in the rhizosphere environment of soil. Chapters dealing with the application of biofertilizers and organic amendments are contributed by experts; authorities in the area of soil science including microbiology and molecular biology; from academic institutions and the industry.

Microbes for Legume Improvement Edited by Khan, Mohammad Saghir; Zaidi, Almas; Musarrat, Javed Springer June 2010 535 pp ISBN 9783211997529 £153.00 - Microbes for Legume Improvement comprises 21 chapters and provides comprehensive information on concepts of microbial technology for the improvement of legumes grown in different agro-ecosystems. The role of microbes including symbiotic nitrogen fixers, asymbiotic nitrogen fixing bacteria (like Azospirillum), plant growth promoting rhizobacteria (PGPR), phosphate-solubilizing microbes, arbuscular mycorrhizal fungi and biocontrol agents in the improvement of both conventional and forage legumes growth is discussed. The role of bacterial biofilms in legume-Rhizobium interactions and metal tolerant microbes in the improvement of legumes is dealt separately. Furthermore, recent findings on the taxonomic status of rhizobia, various signal molecules affecting symbiosis, legume-pathogen and legume-rhizobial interactions and proteomic analysis of legume€microbe interactions are addressed. This volume gives a broad view of legume disease management using microbes and presents strategies for the management of cultivated legumes. It is therefore of special interest to both academics and professionals working in the field of microbiology, soil microbiology, environment microbiology, biotechnology and agronomy as well as plant protection sciences

Positive Plant Microbe Interaction - An agronomic study of wheat and soybean interaction with *Rhizobium* and Phosphate solubilizing bacteria Aftab Afzal and Asghari Bano VDM Verlag Dr. Müller, ISBN-13: 978-3639250503 Pages 176 - This book is basically an effort for sustainable agriculture through biofertilization, written for agronomists, soil scientists, plant physiologists and soil microbiologists. This writing has an equal importance for researchers, students and modern farmers thinking for cost effective, environment friendly and sustainable farming. It's an explanation of introduction to beneficial microbes with special focus on N-fixing and P-solubilizing microbes, methodology for testing of these microbes with a legume (Soybean) and a non-legume (Wheat) (Method of biofertilizer preparation and its application), Results of some experiments (Pot as well as field) conducted in natural and axenic conditions and finally logical reasoning in the form of discussion on these results. The authors hope that reading of this book will provide answers to many questions and will generate new areas of research for scientific community as well.

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