

Short Review

Biofertilizers as Nutrient Coextends of Other Sources for Rainfed Crops

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Abstract

This article deals with microbial inoculants of N_2 -fixing bacteria, vesicular arbuscular mycorrhizae (VAM), phosphate solubilizers and plant-growth promoting rhizobacteria (PGPR) and their role in production of crops. Factors determining the success of inoculant technology along with a survey on their field performance are discussed. The rhizobial inoculants are dealt in greater detail as a general example, and for the other inoculants the discussion is restricted to the points which are specific to a particular group of inoculants. To popularize biofertilizers amongst farmers a holistic approach covering production of quality inoculants, selecting areas which need inoculation, training and educating extension staff and appropriate crop management practices is suggested.

Introduction

Biofertilizers have an important role to play in improving the nutrient supplies and their crop-availability in dryland crop production. Although *Rhizobium* is the most researched and well known among these, there are a number of microbial inoculants with possible practical application in dryland crops where they can serve as useful

components of integrated plant nutrient supply systems. Such inoculants may help in increasing crop productivity by way of increased biological nitrogen fixation (BNF), increased availability or uptake of nutrients through solubilization or increased absorption, stimulation of plant growth through hormonal action, or antibiosis, or decomposition of organic residues.

Rhizobium

In the new classification the slow-growing rhizobia are grouped under the genus *Bradyrhizobium* and the fast growers under the genus *Rhizobium* (Jordan, 1984). Here, we have used the terms *Rhizobium* and *Bradyrhizobium* interchangeably. Much of the applied research on BNF has aimed at identifying efficient strains of bacterial inoculants. But, before considering which strain to use, it needs to be determined whether inoculation itself is necessary in the first place.

Most cultivated tropical soils are believed to have relatively large populations ($> 100 \text{ g}^{-1}$ dry soil) of rhizobia (Nambiar et al. 1988), however, surveys of farmers' grain and fodder legume crops have shown poor nodulation in large areas and good nodulation only in a few pockets (Fig. 1) (Tauro and Khurana, 1986; Wani et al. 1994). In a survey of farmers' chickpea fields around Gwalior, Madhya Pradesh (India), 39% fields had < 100 rhizobia g^{-1} soil, 17% had 10^2 - 10^3 g^{-1} soil, and 44% fields had a population $> 10^3 \text{ g}^{-1}$ soil (Rupela et al. 1987). In a similar survey conducted in 43-47 villages each in three districts of Madhya Pradesh, for nodulation of pigeonpea, black gram, green gram, and lentil, nodulation was poor (0-10 nodules plant^{-1}) in 64 to 100% of the surveyed area (Namdeo and Gupta, 1992). In a survey of groundnut in farmers' fields in southern India, 52 out of 95 fields showed inadequate

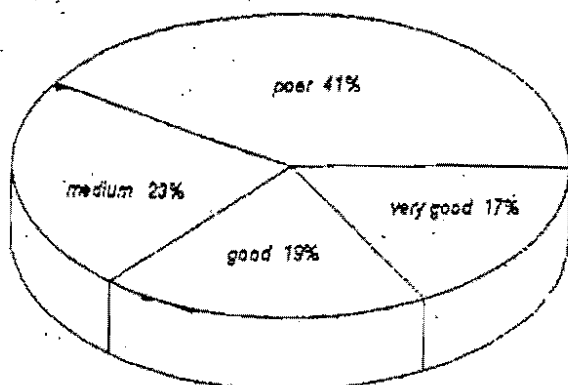


Fig. 1. Nodulation status of chickpea based on 314 fields (AICPIP data cited by Tauro and Khurana, 1986)

nodulation, with less than 10% ARA of what can be obtained under reasonable field conditions (Nambiar et al. 1982). Although adequate nodulation was observed in some parts, ineffective nodules exceeded the number of effective nodules. Field surveys have shown that the proportion of ineffective strains was as high as 40% in chickpea, 53% in green gram, and 63% in groundnut (Tauro and Khurana, 1986). Poor nodulation in farmers' fields could be due to inadequate soil moisture, lack of appropriate rhizobia in the soil, mineral deficiency/toxicity, soil water deficit, prolonged water logging, adverse pH, and pests and diseases. The need to inoculate legumes must be assessed by considering the three interacting factors, namely the soil, the host plant, and *Rhizobium*.

Using a network approach, NIFTAL initiated the Worldwide Rhizobial Ecology Network (WREN) and conducted standardized inoculation trials with extensive environmental data. Thies et al. (1991) developed a mathematical model using most probable number (MPN) of native rhizobia and soil mineral N data as inputs to predict the

inoculation responses at different sites. This model has been incorporated into an interactive computer program, called "RESPONSE", which reduces the need for costly, site-specific field inoculation trials to determine the need for inoculation with *Rhizobium*. Such an approach remains valid to determine the need for inoculation in most of the cases. However, Nambiar (1985) reported significant yield increases in groundnut from Cameroon, India, and China due to inoculation with the rhizobia strain NC 92 although the soils had large populations of native rhizobia. These results indicate that a simulation model using only MPN data and mineral N data cannot provide reliable answers in all the cases; the model needs to be fine-tuned.

Competitive and effective strains: The establishment and persistence of the inoculant strain decreased with increase in population of the native rhizobia with few exceptions e.g. NC 92 on groundnut (Nambiar et al. 1984). Little is known of the factors that control competitiveness, but host cultivar, soil factors, soil microflora, and the nature of the competing strains can influence the success of inoculant strains in nodule formation (Alexander, 1982). Repeated inoculation and higher inoculation rate of 10^6 - 10^8 cells per seed at the initial inoculation helped in early establishment and nodulation by the inoculated strain (Nambiar et al. 1984). Strains of vesicular arbuscular mycorrhizae (VAM) significantly influenced nodule formation by *Bradyrhizobia* strains. In the absence of any VAM, when a mixture of NC 92 and NC 43.3 was used as inoculant, strain NC 92 occupied more nodules (89%) than strain NC 43.3 (34%). In the presence of *Acaulospora laevis*, 86% nodules in the NC 92 + NC 43.3 mixture were formed by NC 92, but the presence of *Glomus fasciculatus* reduced the competitive ability of strain NC 92 (49% of the nodules were those of

NC 92) (Nambiar and Anjaiah, 1989). Competition between inoculated and native *Rhizobium* strains and response to inoculation were less pronounced in the presence of soil mineral N than under conditions where such N was unavailable (Somasegaran and Bohlool, 1990).

Factors affecting the performance of inoculant strains: Being biological agents, the survival and efficiency biofertilizers are governed by several factors. The seed coat of dicotyledonous plants is often carried on top of the cotyledons into the open air, so that only a part of the inoculum may be left to multiply within the rhizosphere. In crops grown on residual moisture, such as chickpea, the inoculated rhizobia cannot move downwards with the growing root, resulting in poor nodulation. Secondly, deep sowing results in a good crop stand but affects nodulation adversely (Nambiar et al. 1988). While all methods of inoculation were successful under favorable conditions, "liquid" and "solid" methods were superior to seed inoculation under adverse conditions (Brockwell et al. 1980). Soil properties like high acidity and alkalinity affect the survival of inoculated rhizobia (Nambiar, 1985). For such problem soils, specific strains with the ability to overcome such adverse conditions need to be selected as inoculants.

Carrier-based inoculants are usually coated on the seeds to introduce bacterial strains into the soil. However, alternative inoculation methods are necessary where seed is to be treated with fungicides and insecticides or where seed of crops such as groundnut and soybean can be damaged when inoculated with an adhesive. Contact with superphosphate, an acidic fertilizer, can be harmful to the inoculated *Rhizobium*. Often the soils themselves are acidic, and coating the seeds with lime has been a popular measure for additional protection only.

Yield response to inoculation: Little on-farm data are available on the impact of inoculation on grain yields. In 12 trials with chickpea, inoculated plots gave on an average 116 kg ha^{-1} more grain than the non-inoculated plots. In another set of field demonstrations, inoculation increased grain yield by $112\text{-}227 \text{ kg ha}^{-1}$ (Chandra and Ali, 1986). Grain yield of pigeonpea inoculated with effective *Rhizobium* increased by 19% to 68% over non-inoculated controls (Nambiar et al. 1988). In 16 trials on research stations, inoculation of chickpea with *Rhizobium* increased grain yield by 342 kg ha^{-1} (range $30\text{-}610 \text{ kg ha}^{-1}$). The results of 1500 demonstrations on farmers' fields with pigeonpea conducted in Gulbarga district of Karnataka State showed 100% increase in yield ($1035 \text{ vs. } 516 \text{ kg ha}^{-1}$) due to balanced use of DAP and *Rhizobium* inoculation (Chinmulgund and Hegde, 1987). In groundnut, inoculation responses varied from yields lower than non-inoculated controls to significantly increased yields (Subba Rao, 1976; Nambiar et al. 1988). In 228 inoculation trials conducted under the International Network of Legumes Inoculation Trials (INLIT) by cooperating scientists in 28 countries over the years, inoculation with *Rhizobium* resulted in significant yield increases in approximately 52% of the trials (Davis et al. 1985). Yield responses to inoculation were highly variable and site-specific.

Azotobacter and Azospirillum

Although, many genera and species of N_2 -fixing bacteria are isolated from the rhizosphere soil of various cereals, mainly members of *Azotobacter* and *Azospirillum* genera have been widely tested for their ability to increase yields of cereals under field conditions.

Yield Responses to Inoculation: Recent reviews have evaluated the worldwide crop responses to

inoculation with azotobacters and azospirilla (Wani, 1990; Wani and Lee, 1992). In many cases inoculations increased plant yields and that such increases are variable. Wani (1990) evaluated the reported worldwide success of *Azotobacter* and *Azospirillum* inoculations and concluded that statistically significant yield increases were described in approximately 60% of the trials in USSR, Israel and India. The responses varied with crops, host cultivars, locations, seasons, agronomic practices, bacterial strains, soil fertility, and interaction with native soil microflora (Wani, 1990; Katyral et al. 1994).

Multilocal trials in India showed that seed inoculation with *Azospirillum brasilense* increased the mean grain yields significantly with pearl millet at 6 and with sorghum at 4 locations out of 9 locations tested. The yield increase in these trials with pearl millet varied from 10% to 17% and with sorghum 7% to 31% (Subba Rao, 1986). The average increases in grain yield of pearl millet with inoculation were higher (11%) in case of *Azospirillum lipoferum* and 8% with *Azotobacter chroococcum* (ICM 2001) over the uninoculated controls (Table 1).

Effect of Soil Nutrients: The experiments conducted at different locations with pearl millet showed that higher increases in grain and total plant biomass yield and also total plant N uptake were observed with zero N + inoculation treatments and the extent of response declined with the increasing levels of applied N (Table 2). In this data set the grain yields obtained from zero N treatments inoculated with N₂-fixing bacteria are similar to the yields from the noninoculated plots receiving 20 kg N ha⁻¹. It is not uncommon to observe yield increases equivalent to 20 kg N ha⁻¹ treatments depending on locations, soil fertility and

other factors (Wani, 1990). Such increased grain yields and N uptake could not be explained only in terms of BNF based on acetylene reduction assays and ¹⁵N based studies (Wani, 1990).

Frequency of Inoculation: Most trials have measured the effect of one time inoculation, but there are a few reports that assayed the residual benefits of continued inoculations. Three years of continued inoculation enabled the millet crops (3 main crops and one succeeding crop) to assimilate 26 kg extra N ha⁻¹ over the uninoculated plots. These increases were observed along with a 2-3 fold increase in the MPN and enzyme-linked immunosorbant assay (ELISA) counts of azospirilla and azotobacters. Such increased yields due to continued inoculation suggest that every year crop needs to be inoculated (Wani et al. 1988). Use of FYM, green manures or other organic amendments enhanced the benefits from inoculation (Wani, 1990). Continued inoculation of the same plot for three consecutive seasons showed that during the 4th year, earlier inoculations with *Azospirillum lipoferum* and *Azotobacter chroococcum* resulted in increased MPN and ELISA counts in the rhizosphere soil over the noninoculated control by 1.4 to 3 fold (Wani et al. 1988).

Phosphate Solubilizing Microorganisms

A group of heterotrophic microorganisms, bacteria: *Bacillus megaterium*, *B. circulans*, *B. subtilis*, *Pseudomonas striata*, *P. rathonis*; fungi: *Aspergillus awamori*, *Penicillium digitatum*, *Trichoderma sp.* and yeast: *Schwanniomyces occidentals* (Wani and Lee, 1992) are known to have the ability to solubilize inorganic P from insoluble sources. In USSR, 5% to 10% increase in yield due to inoculation with *B. megaterium var. phosphaticum*, popularly known as

Table 1. Summary of pearl millet inoculation experiments conducted during 1982-86 at different locations

	Test culture	
	<i>Azospirillum lipoferum</i> (ICM 1001)	<i>Azotobacter chroococcum</i> (ICM 2001)
Total no. of field experiments conducted	24	24
No. of experiments which showed significant increase in grain yield (with an average increase of)	11 18.7%	8 13.6%
No. of experiments which showed increased grain yields over the control, but increases were not significant (with an average increase of)	10 9.3%	12 8.3%
No. of experiments which showed no response in terms of increased/ decreased grain yield	1	2
No. of experiments which showed reduction in grain yield (with average reduction of)	2 2.7%	2 4.5%
Average increase in grain yield due to inoculation	11%	8%

Source: Wani et al. 1988

'phosphobacterin' was observed in about one-third of the trials (Mishustin and Naumova, 1962). During the 1970s, out of 37 field trials conducted in India, only 10 trials showed significant increases in yields, with wheat, Egyptian clover, maize, chickpea, soybean, groundnut, pigeonpea and rice (Sundara Rao, 1968). Significant increase in soybean yield was obtained due to inoculation with *B. polyomyxa* or *P. striata* along with rock phosphate application over the control, whereas application of superphosphate (80 kg P₂O₅ ha⁻¹) did

not result in similar increase.

Vesicular-Arbuscular Mycorrhizae

The survival and performance of VAM fungi is affected by the host plant, soil fertility, cropping practices, biological and environmental factors. A maximum root colonization and sporulation occurs in low fertility soils (Hayman, 1970). Internal P concentration of roots rather than external P concentration in the soil controls root colonization

Table 2. Mean grain, total plant biomass yield and total plant N uptake by Pearl millet inoculated with N₂-fixing bacteria with different N levels

N levels (kg ha ⁻¹)	Bacterial culture		Non- inoculated control	Mean	SE±
	<i>Azospirillum lipoferum</i>	<i>Azotobacter chroococcum</i>			
Grain yield (t ha⁻¹)¹					
0	1.8 (16) ²	1.8 (16)	1.5	1.7	0.059 ^{NS}
20	2.0 (10)	1.9 (4)	1.8	1.9	
40	2.0 (6)	2.0 (3)	1.9	2.0	
Mean	1.93	1.88	1.76		
SE±			0.033 ^{**}	0.036 ^{**}	
CV(%)			20		
Total plant dry matter (t ha⁻¹)²					
0	5.4 (13)	5.2 (9)	4.8	5.1	0.141 ^{NS}
20	5.7 (4)	5.6 (4)	5.4	5.6	
40	6.1 (5)	5.8 (0.2)	5.7	5.9	
Mean	5.7	5.5	5.3		
SE±		1.72 ^{NS}		0.831 ^{**}	
CV(%)		24			
Total plant N uptake (kg ha⁻¹)³					
0	32.2 (27)	29.9 (18)	25.3	29.1	
20	37.0 (13)	36.6 (12)	32.6	35.4	
40	39.2 (8)	37.3 (3)	36.2	37.6	
Mean	36.1	34.6	31.4		

1. Mean across 7 locations, at each location four replications were grown
2. Figures in parentheses indicate percentage increase over respective control
3. Mean across three locations

** P = <0.01.

NS = Nonsignificant.

Source: ICRISAT trials

by VAM fungi (Wani et al. 1991). Application of FYM stimulated VAM (Harinikumar and Bagyaraj, 1988). Long fallows reduced mycorrhizal colonization of crops grown later (Thompson, 1987). Cereals grown in rotation with legumes showed higher root colonization than cereals grown in monocropping. Further, the number of VAM propagules in soil was higher following legumes than following monocropping with cereal (Harinikumar and Bagyaraj, 1988; Wani et al. 1991). Application of fungicides, soil fumigation, soil solarization or prolonged waterlogging can reduce number of VAM propagules in soil.

Crop response to VAM inoculation is governed by soil type, host variety, VAM strains in addition to the biotic and abiotic factors mentioned earlier (Bagyaraj and Varma, 1988; Lee and Wani, 1991). In general, field experiments with VAM inoculation are few when compared to other organisms. The major constraint for field trials with VAM has been the inability to produce 'clean pure' inoculum on a large scale as the fungi are obligate symbionts and have to be maintained and multiplied on living host plants. The field trials conducted in India indicate that inoculation increases yields significantly in around 50% of the total trials (Wani and Lee, 1992); responses varied with soil type, soil fertility, and VAM cultures. Until suitable methods are evolved to multiply the fungus on a large scale for field inoculation of crops, the best strategy to utilize VAM fungi is to concentrate on plants grown in nursery beds and then transplanted.

Plant Growth Promoting Rhizobacteria

A group of rhizosphere bacteria rhizobacteria (Schroth and Hancock, 1981) that exerts a beneficial effect on plant growth is referred as

plant growth promoting rhizobacteria (PGPR). PGPR include several genera, e.g. *Actinoplanes*, *Agrobacterium*, *Alcaligenes*, *Amorphosporangium*, *Arthrobacter*, *Azotobacter*, *Bacillus*, *Cellulomonas*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Pseudomonas*, *Rhizobium* and *Bradyrhizobium*, *Serratia*, *Streptomyces* and *Xanthomonas* (Weller, 1988). Fluorescent pseudomonads are especially suited for rapid uptake or scavenging of nutrients, since they are nutritionally versatile and grow rapidly in the rhizosphere. Certain areas on the root, such as cell junctions and points of emergence of lateral roots, appear to be favoured for colonization by many microorganisms including pathogens because of the abundance of root exudates. Inoculating planting material with PGPR presumably prevents or reduces the establishment by pathogens at these sites (Suslow, 1982). In field trials with wheat, potato, sugarbeet and zinnia conducted on experimental and commercial scale indicated that only 40 out of 63 trials (63%) showed significant results with yield increases varying from 7% to 136% and with an average yield increase ranging from 7% to 35% in different crops over the control treatments (Weller, 1988). A multitude of factors could account for inconsistent results, given the complex interactions among host, inoculated organism, other rhizosphere organisms and the environment.

How to Popularize Biofertilizers

Most important constraints to effective exploitation of BNF technology in India are:

- inconsistent response to inoculation under field conditions
- poor quality of the inoculants
- inadequate knowledge about inoculation technology for the extension personnel and the farmers

- lack of effective inoculant delivery system
- formulation of the policy dictating the desire to exploit BNF successfully (Wani and Lee, 1992; Katyal et al. 1994).

For increasing crop yields through biofertilizers the following strategy is suggested keeping *Rhizobium* inoculants as an example. For success of biofertilizers in India, concerted efforts right from production, demonstration to distribution will be required. There is some hope of success with a mission-oriented approach under which at least production of mother cultures in lyophilised form must be centralised. The scientists involved in biofertilizers projects in the Universities and ICAR institutions can identify the target crops, areas to be covered and recommend strains for preparing biofertilizers. People involved in biofertilizer production should be trained microbiologists who are aware of the pitfalls in the processes involved. The non-government organisation, extension agencies along with the National Biofertilizer Development Centre can play an important role in popularising biofertilizers in India. To attract the farmers the pricing of the biofertilizers must be controlled if private agencies are involved. Biofertilizers should be used or considered for harnessing BNF to its maximum potential taking systems approach. Non-nodulating or low nodulated plants look similar in appearance to that of well nodulated plants but this is at the cost of soil or fertilizer N. We must take the view that in all we may derive benefit in terms of maintaining or improving the productivity of our soils and should not be disappointed by not seeing the direct benefits in some cases. As evident from the experience in Karnataka, nodulation in pigeonpea has improved substantially by addition of DAP rather than inoculating with *Rhizobium* alone. A holistic approach to improve production of legumes is needed and we must ensure that all

the constraints for good plant growth other than N nutrition must be alleviated for better performance of BNF technology.

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